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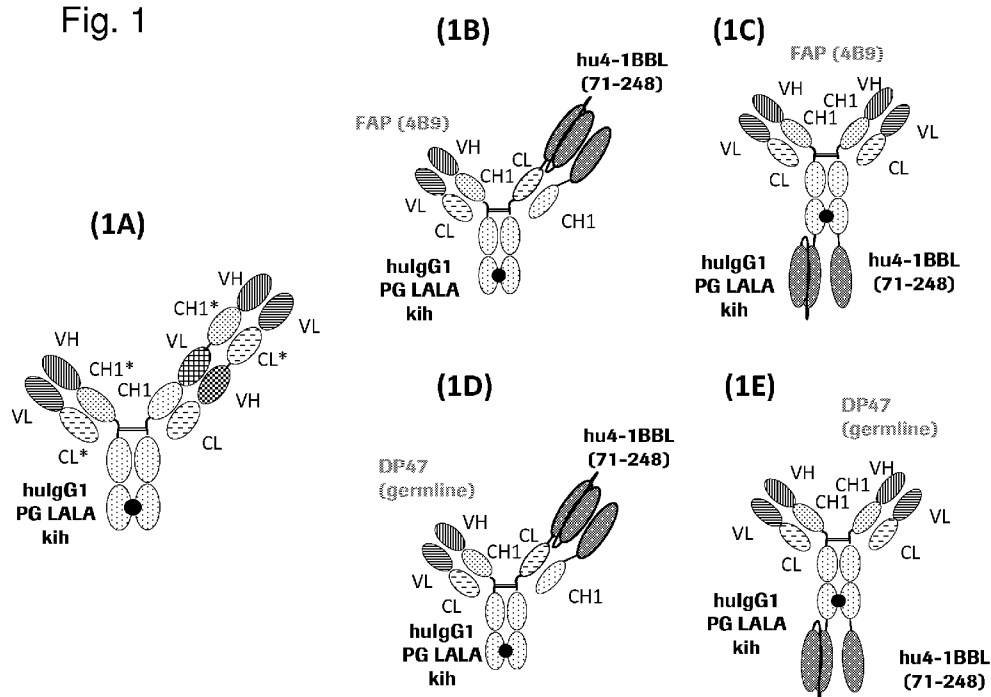
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(54) Title: COMBINATION THERAPY WITH TARGETED 4-1BB (CD137) AGONISTS

Fig. 1



(57) Abstract: The present application relates to combination therapies employing tumor targeted anti-CD3 bispecific antibodies and 4-1BB (CD137) agonists, in particular 4-1BBL trimer containing antigen binding molecules, the use of these combination therapies for the treatment of cancer and methods of using the combination therapies. The trimer CEA-4-1BBL (Figure 1B) provides the highest tumor regression when combined with CEA CD3 TCB (See table 18).



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Combination therapy with targeted 4-1BB (CD137) agonists

FIELD OF THE INVENTION

The present invention relates to combination therapies employing tumor targeted anti-CD3 bispecific antibodies and 4-1BB (CD137) agonists, in particular 4-1BBL trimer containing antigen binding molecules, the use of these combination therapies for the treatment of cancer and methods of using the combination therapies. Included are also combination therapies employing 4-1BB agonists comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular 4-1BBL trimer containing antigen binding molecules, with an agent blocking PD-L1/PD-1 interaction, in particular a PD-L1 antibody and/or with a tumor targeted anti-CD3 bispecific antibody.

BACKGROUND

Cancer is one of the leading causes of death worldwide. Despite advances in treatment options, prognosis of patients with advanced cancer remains poor. Consequently, there is a persisting and urgent medical need for optimal therapies to increase survival of cancer patients without causing unacceptable toxicity. Recent results from clinical trials have shown that immune therapies, particularly immune checkpoint inhibitors, can extend the overall survival of cancer patients and lead to durable responses. Despite these promising results, current immune-based therapies are only effective in a proportion of patients and combination strategies are needed to improve therapeutic benefit.

One way to recruit the patient's own immune system to fight cancer are T cell bispecific antibodies (TCB). An anti-CEA/anti-CD3 bispecific antibody is a molecule that targets CEA expressed on tumor cells and CD3 epsilon chain (CD3ε) present on T cells. Simultaneous binding leads to T-cell activation and T-cell mediated killing of B cells. In the presence of CEA positive cancer cells, whether circulating or tissue resident, pharmacologically active doses will trigger T-cell activation and associated cytokine release. Parallel to tumor cell depletion anti-CEA/anti-CD3 bispecific antibody leads to a transient decrease of T cells in the peripheral blood within 24 hours after the first administration and to a peak in cytokine release, followed by rapid T-cell recovery and return of cytokine levels to baseline within 72 hours. Thus, in order to achieve complete elimination of tumor cells, there is a need of an additional agent that conserves T-cell activation and immune response to cancer cells.

4-1BB (CD137), a member of the TNF receptor superfamily, was first identified as an inducible molecule expressed by activated T cells (Kwon and Weissman, 1989, *Proc Natl Acad Sci USA* 86, 1963-1967). Subsequent studies demonstrated that many other immune cells also express 4-1BB, including NK cells, B cells, NKT cells, monocytes, neutrophils, mast cells, dendritic cells (DCs) and cells of non-hematopoietic origin such as endothelial and smooth muscle cells (Vinay and Kwon, 2011, *Cell Mol Immunol* 8, 281-284). Expression of 4-1BB in different cell types is mostly inducible and driven by various stimulatory signals, such as T-cell receptor (TCR) or B-cell receptor triggering, as well as signaling induced through co-stimulatory molecules or receptors of pro-inflammatory cytokines (Diehl et al., 2002, *J Immunol* 168, 3755-3762; Zhang et al., 2010, *Clin Cancer Res* 13, 2758-2767).

4-1BB ligand (4-1BBL or CD137L) was identified in 1993 (Goodwin et al., 1993, *Eur J Immunol* 23, 2631-2641). It has been shown that expression of 4-1BBL was restricted on professional antigen presenting cells (APC) such as B-cells, DCs and macrophages. Inducible expression of 4-1BBL is characteristic for T-cells, including both $\alpha\beta$ and $\gamma\delta$ T-cell subsets, and endothelial cells (Shao and Schwarz, 2011, *J Leukoc Biol* 89, 21-29).

Co-stimulation through the 4-1BB receptor (for example by 4-1BBL ligation) activates multiple signaling cascades within the T cell (both CD4⁺ and CD8⁺ subsets), powerfully augmenting T cell activation (Bartkowiak and Curran, 2015). In combination with TCR triggering, agonistic 4-1BB-specific antibodies enhance proliferation of T-cells, stimulate lymphokine secretion and decrease sensitivity of T-lymphocytes to activation-induced cell death (Snell et al., 2011, *Immunol Rev* 244, 197-217). This mechanism was further advanced as the first proof of concept in cancer immunotherapy. In a preclinical model administration of an agonistic antibody against 4-1BB in tumor bearing mice led to potent anti-tumor effect (Melero et al., 1997, *Nat Med* 3, 682-685). Later, accumulating evidence indicated that 4-1BB usually exhibits its potency as an anti-tumor agent only when administered in combination with other immunomodulatory compounds, chemotherapeutic reagents, tumor-specific vaccination or radiotherapy (Bartkowiak and Curran, 2015, *Front Oncol* 5, 117).

Signaling of the TNFR-superfamily needs cross-linking of the trimerized ligands to engage with the receptors, so does the 4-1BB agonistic antibodies which require wild type Fc-binding (Li and Ravetch, 2011, *Science* 333, 1030-1034). However, systemic administration of 4-1BB-specific agonistic antibodies with the functionally active Fc domain resulted in influx of CD8⁺ T-cells associated with liver toxicity (Dubrot et al., 2010, *Cancer Immunol Immunother* 59, 1223-1233) that is diminished or significantly ameliorated in the absence of functional Fc-receptors in mice. In the clinic, an Fc-competent 4-1BB agonistic Ab (BMS-663513) (NCT00612664) caused a grade 4 hepatitis leading to termination of the trial

(Simeone and Ascierto, 2012, *J Immunotoxicol* 9, 241-247). Therefore, there is a need for effective and safer 4-1BB agonists.

Fusion proteins composed of one extracellular domain of a 4-1BB ligand and a single chain antibody fragment (Hornig et al., 2012, *J Immunother* 35, 418-429; Müller et al., 2008, *J Immunother* 31, 714-722) or a single 4-1BB ligand fused to the C-terminus of a heavy chain (Zhang et al., 2007, *Clin Cancer Res* 13, 2758-2767) have been made. WO 2010/010051 discloses the generation of fusion proteins that consist of three TNF ligand ectodomains linked to each other and fused to an antibody part. In the present invention, antigen binding molecules composed of a trimeric and thus biologically active 4-1BB ligand and an antigen binding domain specific for the tumor-associated antigen FAP and an Fc inactive domain, are shown particularly stable and robust (herein named as FAP-4-1BBL). The FAP antigen binding domain replaces the unspecific Fc γ R-mediated crosslinking that is responsible for Fc-mediated toxicity in particular in the liver, by a FAP-targeted specific crosslinking. The crosslinking by a tumor (stroma) antigen makes it possible to administer the 4-1BB agonist

It has been found that a better anti-tumor effect of 4-1BB agonism is achieved when the targeted 4-1BBL antigen binding molecule is combined with an anti-CEA/anti-CD3 bispecific antibody, i.e. a CEA TCB. The T-cell bispecific antibody provides the initial TCR activating signalling to T cells, and then the combination with FAP-4-1BBL leads to a further boost of anti-tumor T cell immunity. Thus, we herein describe a novel combination therapy for tumors expressing CEA (CEA-positive cancer). Furthermore, it has been shown that the combination of the targeted 4-1BB agonist FAP-4-1BBL with an agent blocking PD-L1/PD-1 interaction induced a strong tumor regression that cannot be observed with the agent blocking PD-L1/PD-1 interaction alone.

SUMMARY OF THE INVENTION

The present invention relates to anti-CD3 bispecific antibodies and their use in combination with 4-1BB (CD137) agonists, in particular 4-1BBL trimer containing antigen binding molecules, in particular to their use in a method for treating or delaying progression of cancer, more particularly for treating or delaying progression of advanced and/or metastatic solid tumors. It has been found that the combination therapy described herein is more effective in inhibiting tumor growth and eliminating tumor cells than treatment with the anti-CEA/anti-CD3 bispecific antibodies alone.

In one aspect, the invention provides a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method for treating or delaying progression of cancer, wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is used in combination with a 4-1BB (CD137) agonist. In one

aspect, the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is an anti-CEA/anti-CD3 bispecific antibody or an anti-FolR1/anti-CD3 bispecific antibody or an anti-MCSP/anti-CD3 bispecific antibody. Particularly, the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is an anti-CEA/anti-CD3 bispecific antibody or an anti-FolR1/anti-CD3 bispecific antibody. In a further aspect, the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is an anti-CEA/anti-CD3 bispecific antibody. Thus, the invention provides an anti-CEA/anti-CD3 bispecific antibody for use in a method for treating or delaying progression of cancer, wherein the anti-CEA/anti-CD3 bispecific antibody is used in combination with a 4-1BB (CD137) agonist.

In particular, the 4-1BB agonist is an antigen binding molecule comprising a tumor-associated antigen. In one aspect, the 4-1BB agonist is an antigen binding molecule comprising a Fc domain. In a particular aspect, the 4-1BB agonist is an antigen binding molecule comprising a Fc domain with modifications reducing Fc γ receptor binding and/or effector function. The crosslinking by a tumor associated antigen makes it possible to avoid unspecific Fc γ R-mediated crosslinking and thus higher and more efficacious doses of the 4-1BB agonists may be administered in comparison to common 4-1BB antibodies.

In a further aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen and the 4-1BB agonist are administered together in a single composition or administered separately in two or more different compositions.

In a further aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is used in combination with a 4-1BB (CD137) agonist, and wherein the 4-1BB agonist acts synergistically with the T-cell activating anti-CD3 bispecific antibody.

In another aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the anti-CEA/anti-CD3 bispecific antibody is administered concurrently with, prior to, or subsequently to the 4-1BB agonist.

In one aspect, the invention provides a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist comprises three ectodomains of 4-1BBL or fragments thereof. In a further aspect, provided is an anti-CEA/anti-CD3 bispecific antibody for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is a molecule comprising three ectodomains of 4-1BBL or fragments thereof and wherein the ectodomains of 4-1BBL comprise an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8, particularly the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:5. More particularly, the ectodomains of 4-1BBL comprise an amino acid sequence of SEQ ID NO:5.

In another aspect, the invention provides a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen. In one aspect, the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to a tumor-associated antigen. In a further aspect, the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain.

In a further aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular to FAP. In one aspect, provided is an anti-CEA/anti-CD3 bispecific antibody for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one moiety capable of specific binding to FAP, wherein 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: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_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, or

(b) a heavy chain variable region (V_{HFAP}) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:15, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:16, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:17, and a light chain variable region (V_{LFAP}) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:18, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:19, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:20.

In a particular aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP, wherein 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:21 and a light chain variable region (V_{LFAP}) comprising an amino acid sequence of SEQ ID NO:22 or wherein 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:23 and a light chain variable region (V_{LFAP}) comprising an amino acid sequence of SEQ ID NO:24.

In one aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule further comprising a Fc domain composed of a first and a second subunit capable of stable association. In particular, the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain. More particularly, the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function. In a particular aspect, the 4-1BB agonist comprises an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

In another aspect of the invention, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising (a) at least one antigen binding domain capable of specific binding to FAP, (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

In another aspect, the invention provides a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising

- 5 (a) at least one Fab domain capable of specific binding to FAP, and
- (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that
 - (i) the first polypeptide contains a CH1 or CL domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or
 - (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
 - (iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

In one aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising

- (a) at least one Fab domain capable of specific binding to FAP comprising a heavy chain variable region ($V_{\text{H}}\text{FAP}$) comprising the amino acid sequence of SEQ ID NO:21 and a light chain variable region ($V_{\text{L}}\text{FAP}$) comprising the amino acid sequence of SEQ ID NO:22 or a heavy chain variable region ($V_{\text{H}}\text{FAP}$) comprising the amino acid sequence of SEQ ID NO:23

and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:24, and

- (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

- 10 In a particular aspect, there is provided a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid
15 sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:67 and a second light chain comprising the amino acid sequence of SEQ ID NO:68.

- In another aspect, the invention provides a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3
20 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising

- (a) at least one antigen binding domain capable of specific binding to FAP,
(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers.

- 25 In one aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising

- (a) at least one antigen binding domain capable of specific binding to FAP,
30 (b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers, and
(c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the polypeptide comprising the three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers is fused to the N- or C-terminal amino acid
35 of one of the two subunits of the Fc domain, optionally through a peptide linker.

In another aspect, the invention provides a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an anti-FAP/anti-4-1BB bispecific antibody.

5 In a further aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA. In one aspect,
10 provided is an anti-CEA/anti-CD3 bispecific antibody for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises a heavy chain variable region (V_H CEA)
15 comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:49, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:50, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:51, and a light chain variable region (V_L CEA) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and (vi) CDR-L3 comprising the
20 amino acid sequence of SEQ ID NO:54.

In a particular aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or
25 fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises a heavy chain variable region (V_H CEA) comprising an amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_L CEA) comprising an amino acid sequence of SEQ ID NO:56.

In one aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a
30 tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule further comprising a Fc domain composed of a first and a second subunit capable of stable association. In particular, the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc
35 domain. More particularly, the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc

receptor and/or effector function. In a particular aspect, the 4-1BB agonist comprises an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

In another aspect of the invention, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3
5 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one antigen binding domain capable of specific binding to CEA,
(b) a first and a second polypeptide that are linked to each other by a disulfide bond,
wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that
10 are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

In another aspect, the invention provides a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer,
15 wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one Fab domain capable of specific binding to CEA, and
(b) a first and a second polypeptide that are linked to each other by a disulfide bond,
wherein the antigen binding molecule is characterized in that

- (i) the first polypeptide contains a CH1 or CL domain and the second polypeptide
20 contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or
25 a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or
(ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to
30 the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
(iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein
35 the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two

ectodomains of 4-1BBL or fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

- 5 In one aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising
- (a) at least one Fab domain capable of specific binding to CEA comprising a heavy chain variable region (V_{HCEA}) comprising the amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_{LCEA}) comprising the amino acid sequence of SEQ ID NO:56, and
- 10 (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID
- 15 NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

- In another aspect, the invention provides a T-cell activating anti-CD3 bispecific
- 20 antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising
- (a) at least one antigen binding domain capable of specific binding to CEA,
- (b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are
- 25 connected to each other by peptide linkers.

- In one aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an antigen binding molecule comprising
- 30 (a) at least one antigen binding domain capable of specific binding to CEA,
- (b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers, and
- (c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the polypeptide comprising the three ectodomains of 4-1BBL or fragments thereof
- 35 that are connected to each other by peptide linkers is fused to the N- or C-terminal amino acid of one of the two subunits of the Fc domain, optionally through a peptide linker.

In another aspect, the invention provides a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist is an anti-CEA/anti-4-1BB bispecific antibody.

5 In all these aspects, the invention further provides an anti-CEA/anti-CD3 bispecific antibody for use in a method for treating or delaying progression of cancer, wherein the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds to CD3, and a second antigen binding domain that binds to CEA. In particular, the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a
10 heavy chain variable region (V_H CD3) and a light chain variable region (V_L CD3), and a second antigen binding domain comprising a heavy chain variable region (V_H CEA) and a light chain variable region (V_L CEA). In one aspect, the first antigen binding domain comprises a heavy chain variable region (V_H CD3) comprising CDR-H1 sequence of SEQ ID NO:33, CDR-H2 sequence of SEQ ID NO:34, and CDR-H3 sequence of SEQ ID NO:35;
15 and/or a light chain variable region (V_L CD3) comprising CDR-L1 sequence of SEQ ID NO:36, CDR-L2 sequence of SEQ ID NO:37, and CDR-L3 sequence of SEQ ID NO:38. More particularly, the first antigen binding domain comprises a heavy chain variable region (V_H CD3) comprising the amino acid sequence of SEQ ID NO:39 and/or a light chain variable region (V_L CD3) comprising the amino acid sequence of SEQ ID NO:40. In another aspect,
20 the second antigen binding domain comprises a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43, and/or a light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:44, CDR-L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46. In another aspect, the second antigen binding domain
25 comprises a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:49, CDR-H2 sequence of SEQ ID NO:50, and CDR-H3 sequence of SEQ ID NO:51, and/or a light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54.

More particularly, the second antigen binding domain comprises a heavy chain variable
30 region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:48. More particularly, the second antigen binding domain comprises a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:56.

35 In another aspect, the invention further provides an anti-CEA/anti-CD3 bispecific antibody for use in a method for treating or delaying progression of cancer, wherein the anti-

CEA/anti-CD3 bispecific antibody further comprises a third antigen binding domain that binds to CEA. In particular, the third antigen binding domain comprises (a) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43, and/or a light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:44, CDR-L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46, or (b) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:49, CDR-H2 sequence of SEQ ID NO:50, and CDR-H3 sequence of SEQ ID NO:51, and/or a light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54. More particularly, the third antigen binding domain comprises a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:48 or wherein the second antigen binding domain comprises a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:56.

In a further aspect, provided is an anti-CEA/anti-CD3 bispecific antibody for use in a method for treating or delaying progression of cancer, wherein the first antigen binding domain is a cross-Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged, and the second and third, if present, antigen binding domain is a conventional Fab molecule.

In a further aspect, provided is an anti-CEA/anti-CD3 bispecific antibody for use in a method for treating or delaying progression of cancer, wherein (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

In a further aspect, the invention provides an anti-CEA/anti-CD3 bispecific antibody for use in a method for treating or delaying progression of cancer, wherein the anti-CEA/anti-CD3 bispecific antibody comprises an Fc domain comprising one or more amino acid

substitutions that reduce binding to an Fc receptor and/or effector function. More particularly, the anti-CEA/anti-CD3 bispecific antibody comprises an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

In a further aspect, the invention provides a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, wherein the tumor-associated antigen is FolR1. Thus, the invention provides an anti-FolR1/anti-CD3 bispecific antibody for use in a method for treating or delaying progression of cancer, wherein the anti-FolR1/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds to CD3, and a second antigen binding domain that binds to FolR1.

In one aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method for treating or delaying progression of cancer, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_H CD3), a second antigen binding domain comprising a heavy chain variable region (V_H FolR1) and a common light chain variable region.

In one aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method for treating or delaying progression of cancer, wherein the first antigen binding domain comprises a heavy chain variable region (V_H CD3) comprising CDR-H1 sequence of SEQ ID NO:121, CDR-H2 sequence of SEQ ID NO:122, and CDR-H3 sequence of SEQ ID NO:123; the second antigen binding domain comprises a heavy chain variable region (V_H FolR1) comprising CDR-H1 sequence of SEQ ID NO:124, CDR-H2 sequence of SEQ ID NO:125, and CDR-H3 sequence of SEQ ID NO:126; and wherein the common light chain comprises a CDR-L1 sequence of SEQ ID NO:127, CDR-L2 sequence of SEQ ID NO:128, and CDR-L3 sequence of SEQ ID NO:129. In a further aspect, the first antigen binding domain comprises a heavy chain variable region (V_H CD3) comprising the sequence of SEQ ID NO:130; the second antigen binding domain comprises a heavy chain variable region (V_H FolR1) comprising the sequence of SEQ ID NO:131; and wherein the common light chain comprises the sequence of SEQ ID NO:132.

In a further aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method for treating or delaying progression of cancer, wherein the anti-FolR1/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to FolR1. In particular, provided is an anti-FolR1/anti-CD3 bispecific antibody comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:133, a second heavy chain comprising the amino acid sequence of SEQ ID NO:134 and a common light chain of SEQ ID NO: 135.

In a further aspect, the invention provides a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, wherein the tumor-associated antigen is MCSP. Thus, the invention provides an anti-MCSP/anti-CD3 bispecific antibody for use in a method for treating or delaying progression of cancer, wherein the anti-MCSP/anti-CD3
5 bispecific antibody comprises a first antigen binding domain that binds to CD3, and a second antigen binding domain that binds to MCSP.

In particular, the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_H CD3) and a light chain variable region (V_L CD3), and a second antigen binding domain comprising a heavy chain variable
10 region (V_H MCSP) and a light chain variable region (V_L MCSP). In one aspect, the first antigen binding domain comprises a heavy chain variable region (V_H CD3) comprising CDR-H1 sequence of SEQ ID NO:33, CDR-H2 sequence of SEQ ID NO:34, and CDR-H3 sequence of SEQ ID NO:35; and/or a light chain variable region (V_L CD3) comprising CDR-L1 sequence of SEQ ID NO:36, CDR-L2 sequence of SEQ ID NO:37, and CDR-L3 sequence of SEQ ID
15 NO:38. More particularly, the first antigen binding domain comprises a heavy chain variable region (V_H CD3) comprising the amino acid sequence of SEQ ID NO:39 and/or a light chain variable region (V_L CD3) comprising the amino acid sequence of SEQ ID NO:40. In another aspect, the second antigen binding domain comprises a heavy chain variable region (V_H MCSP) comprising CDR-H1 sequence of SEQ ID NO:151, CDR-H2 sequence of SEQ ID NO:152, and CDR-H3 sequence of SEQ ID NO:153, and/or a light chain variable region (V_L MCSP) comprising CDR-L1 sequence of SEQ ID NO:154, CDR-L2 sequence of SEQ ID NO:155, and CDR-L3 sequence of SEQ ID NO:156. More particularly, the second antigen binding domain comprises a heavy chain variable region (V_H MCSP) comprising the amino acid
20 sequence of SEQ ID NO:157 and/or a light chain variable region (V_L MCSP) comprising the amino acid sequence of SEQ ID NO:158.

In another aspect, the invention further provides an anti-MCSP/anti-CD3 bispecific antibody for use in a method for treating or delaying progression of cancer, wherein the anti-MCSP/anti-CD3 bispecific antibody further comprises a third antigen binding domain that binds to MCSP. In particular, the third antigen binding domain comprises (a) a heavy chain
30 variable region (V_H MCSP) comprising CDR-H1 sequence of SEQ ID NO:151, CDR-H2 sequence of SEQ ID NO:152, and CDR-H3 sequence of SEQ ID NO:153, and/or a light chain variable region (V_L MCSP) comprising CDR-L1 sequence of SEQ ID NO:154, CDR-L2 sequence of SEQ ID NO:155, and CDR-L3 sequence of SEQ ID NO:156. More particularly, the third antigen binding domain comprises a heavy chain variable region (V_H MCSP) comprising the amino acid sequence of SEQ ID NO:157 and/or a light chain variable region (V_L MCSP) comprising the amino acid sequence of SEQ ID NO:158.
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In another aspect, provided is a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is used in combination
5 with a 4-1BB (CD137) agonist and wherein the combination is administered at intervals from about about one week to three weeks.

In yet another aspect, the invention provides a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer,
10 wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is used in combination with a 4-1BB (CD137) agonist and in combination with an agent blocking PD-L1/PD-1 interaction. In particular, the agent blocking PD-L1/PD-1 interaction is an anti-PD-L1 antibody or an anti-PD1 antibody. More particularly, the agent blocking PD-L1/PD-1 interaction is selected from the group consisting of atezolizumab,
15 durvalumab, pembrolizumab and nivolumab. In a specific aspect, the agent blocking PD-L1/PD-1 interaction is atezolizumab.

In a further aspect, the invention provides a pharmaceutical product comprising (A) a first composition comprising as active ingredient a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3
20 bispecific antibody, and a pharmaceutically acceptable carrier; and (B) a second composition comprising as active ingredient a 4-1BB agonist and a pharmaceutically acceptable carrier, for use in the combined, sequential or simultaneous treatment of a disease, in particular for the treatment of cancer.

In a further aspect, the T-cell activating anti-CD3 bispecific antibody specific for a
25 tumor-associated antigen, in particular the anti-CEA/anti-CD3 bispecific antibody, is for use in a method for treating or delaying progression of cancer, wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is used in combination with a 4-1BB agonist and wherein a pretreatment with an Type II anti-CD20 antibody, preferably obinutuzumab, is performed prior to the combination treatment, wherein the period of time
30 between the pretreatment and the combination treatment is sufficient for the reduction of B-cells in the individual in response to the Type II anti-CD20 antibody, preferably obinutuzumab. In another aspect, provided is a pharmaceutical composition comprising a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, and a 4-1BB agonist. In one aspect, the
35 pharmaceutical composition is for use in treating or delaying progression of cancer, in particular for the treatment of solid tumors. In a further aspect, the pharmaceutical

composition is for use in treating a disease selected from the group consisting of colon cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, breast cancer, kidney cancer, esophageal cancer, and prostate cancer.

5 In an additional aspect, the invention provides a kit for treating or delaying progression of cancer in a subject, comprising a package comprising (A) a first composition comprising as active ingredient a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, and a pharmaceutically acceptable carrier; (B) a second composition comprising as active ingredient a 4-1BB agonist and a pharmaceutically acceptable carrier, and (C) instructions for using the
10 compositions in a combination therapy.

In a further aspect, the invention relates to the use of a combination of a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, and a 4-1BB agonist in the manufacture of a medicament for treating or delaying progression of a proliferative disease, in particular cancer.

15 In particular, provided is the use of a combination of a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, and a 4-1BB agonist in the manufacture of a medicament for treating a disease selected from the group consisting of colon cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, breast cancer,
20 kidney cancer, esophageal cancer, and prostate cancer.

In another aspect, the invention provides a method for treating or delaying progression of cancer in a subject comprising administering to the subject an effective amount of a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, and a 4-1BB agonist. In particular, the invention
25 relates to a method for treating or delaying progression of cancer in a subject, wherein the 4-1BB agonist is an antigen binding molecule. In one aspect, the 4-1BB agonist is an antigen binding molecule comprising a Fc domain. In a particular aspect, the 4-1BB agonist is an antigen binding molecule comprising a Fc domain with modifications reducing Fc γ receptor binding and/or effector function. In a particular aspect, the 4-1BB agonist is an antigen
30 binding molecule comprising three ectodomains of 4-1BBL or fragments thereof. In one aspect, the 4-1BB agonist is a molecule comprising three ectodomains of 4-1BBL or fragments thereof and wherein the ectodomains of 4-1BBL comprise an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8, particularly the
35 amino acid sequence of SEQ ID NO:1 or SEQ ID NO:5. In one aspect, the 4-1BB agonist is

an antigen binding molecule comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen. In particular, the the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to a tumor-associated antigen.

5 More particularly, the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and an antigen binding domain capable of specific binding to FAP. In another particular aspect, the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and an antigen binding domain capable of specific binding to CEA.

10 In a further aspect, provided is a 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is used in combination with an agent blocking PD-L1/PD-1 interaction. In particular, the agent blocking
15 PD-L1/PD-1 interaction is an anti-PD-L1 antibody or an anti-PD1 antibody. More particularly, the agent blocking PD-L1/PD-1 interaction is selected from the group consisting of atezolizumab, durvalumab, pembrolizumab and nivolumab. In a specific aspect, the agent blocking PD-L1/PD-1 interaction is atezolizumab. Furthermore, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-
20 associated antigen for use in the method described herein is one, wherein the tumor-associated antigen is selected from Fibroblast activation protein (FAP) or CEA. More particularly, the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to Fibroblast activation protein (FAP).

25 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows particular FAP-4-1BBL antigen binding molecules and a particular anti-CEA/anti-CD3 bispecific antibody as used in the Examples. These molecules are described in more detail in Examples 1 and 2, respectively. The thick black point stands for the knob-into-hole modification. * symbolizes amino acid modifications in the CH1 and CL domain (so-called charged residues). In Figure 1A an exemplary bispecific anti-CEA/anti-CD3 antibody in 2+1 format is shown (named CEA CD3 TCB or CEACAM5 CD3 TCB, respectively). Figure 1B shows a monovalent FAP 4-1BBL-trimer containing antigen binding molecule with modifications in the CH1 and CL domain adjacent to the 4-1BBL dimer and 4-1BBL monomer. As it comprised the FAP binder 4B9, it was named mono FAP(4B9)-4-
30 1BBL herein. Figure 1C shows the bivalent construct with binder FAP(4B9), termed bi
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FAP(4B9)-4-1BBL. Figures 1D and 1E show untargeted control molecules (the FAP binder has been replaced by a non-binding DP47 Fab).

Figure 2 shows that the groups treated with combinations of CEA CD3 TCB with monovalent FAP(4B9)-4-1BBL showed improved efficacy in terms of tumor growth inhibition compared to the other groups.

Figures 3A to 3F show that treatment with combinations of CEA CD3 TCB with monovalent or bivalent FAP(4B9)-4-1BBL led to increased infiltration of CD8 and CD4 positive T-cells in the tumor compared to treatment with the single agents. The letters A to J refer to the treatment groups as defined in Figure 2.

Figures 4A and 4B show the histological analysis at the end of the study, i.e. the amount of CD8 positive (Figure 4A) and CD3 positive cells per mm² (Figure 4B).

Figure 5 shows that the groups treated with combinations of CEA CD3 TCB with monovalent FAP(28H1)-4-1BBL showed improved efficacy in terms of tumor growth inhibition compared to groups treated with the single agents.

Figures 6A and 6B show that treatment with combinations of CEA CD3 TCB with monovalent or bivalent FAP(4B9)-4-1BBL led to increased infiltration of CD8 and CD4 positive T-cells in the tumor (Figure 6A) and blood (Figure 6B) compared to treatment with the single agents.

Figure 7 shows that treatment with the combinations of CEA CD3 TCB + FAP(4B9)-4-1BBL at different doses mediates superior efficacy in terms of tumor growth inhibition compared to treatment with single agents. Mice were inoculated with MKN45 and 3T3 cells in a ratio of 1:1. At day 17 after tumor cell injection mice were randomized and treated with vehicle, CEA CD3 TCB, FAP-4-1BBL or the combinations. CEA CD3 TCB + FAP-4-1BBL at a dose of 3 and 1 mg/kg mediated superior efficacy in terms of tumor growth inhibition compared to the other group, whereas the combination in the group with 1 mg/kg is more efficacious than the combination with 3 mg/kg.

Figures 8A and 8B show the pharmacokinetic profile of injected compounds during the first week. 2 mice per Group were bled 10 min, 6h, 24h, 96h and 7d after the first therapy and injected compounds were analysed by ELISA. 4-1BBL was detected via 4-1BB binding (Figure 8A), whereas CEA CD3 TCB was detected via binding to anti-CD3 CDR antibody (Figure 8B). All groups injected with compounds show comparable exposure of the molecules between the different groups (dose dependent), either FAP-4-1BBL or CEA CD3 TCB.

In **Figure 9** is shown the T cell infiltration in tumor and spleen at study termination. Spleen and tumor from 2-5 mice/group were analysed by flow cytometry at termination. Single cell suspensions were stained for CD45, CD3, CD4 and CD8 and the amount of cells was analysed. Combining FAP-4-1BBL with CEA CD3 TCB leads to a statistically increased infiltration of CD3, CD8 and CD4 positive T-cells in the tumor at the end of the experiment compared to all other groups. Also in the spleen the combination leads to an increase of CD3 positive T cells compared to vehicle and CEA CD3 TCB monotherapy. (statistics One Way ANOVA, Tukey's multiple comparison test, $p < 0.05$). Figure 9A shows the increase of CD3 positive cells in the tumor, in Figure 9B is shown the increase of CD8 positive cells in the tumor, in Figure 9C the increase of CD4 positive cells in the tumor and in Figure 9D the increase of CD3 positive cells in the spleen.

The histological analysis at the end of the study is illustrated in **Figures 10A and 10B**. Immunohistochemical images of human MKN45 gastric subcutaneous tumors cografed with 3T3 murine fibroblasts derived from the indicated treatment groups in humanized NOG mice were generated. Tissue samples were prepared for immunohistochemical staining. Subcutaneous tumors were harvested from animals at day 52 and during the experiment, fixed in formalin 10% (Sigma, Germany) and later processed for FFPET (Leica 1020, Germany). 4 μ m paraffin sections were subsequently cut in a microtome (Leica RM2235, Germany). HuCD8 and HuCD3 immunohistochemistry was performed with anti-human CD8 (Cell Marque Corporation, California) and anti-human CD3 (ThermoFischer Scientific, USA) in the Leica autostainer (Leica ST5010, Germany) following the manufacture's protocols. Quantification of huCD3 (Figure 10A) and huCD8 positive T cells (Figure 10B) was performed with Definiens software (Definiens, Germany). Statistics were analyzed by one way ANOVA with multiple comparison tests. Results showed very low number of T cells in the MNK45/3T3 sc tumors from untreated mice. There is a significant increase of positive CD3 (A) and CD8 (B) T cell number in the CEA CD3 TCB + FAP-4-1BBL 3mg/kg group compared to vehicle and CEA CD3 TCB monotherapy. (statistics: One Way ANOVA, Tukey's multiple comparison test, $p < 0.05$). Animals analysed in histology are from different experiment days to increase the number of samples. (Vehicle: 5x day52; CEA CD3 TCB: 5x day52; CEA CD3 TCB + FAP-4-1BBL 3mg/kg: 2xday52, 1x day32, 2xday29; CEA CD3 TCB + FAP-4-1BBL 1mg/kg: 1xday52, 2xday50, 1xday37, 1xday35).

In **Figure 11** it is shown that the combinations of CEACAM5 CD3 TCB + FAP(4B9)-4-1BBL 10mg/kg mediate slightly improved efficacy in terms of tumor growth inhibition compared to the other groups.

Figures 12A and 12B show the pharmacokinetic profile of injected compounds during the first week. 2 mice per Group were bled 1h and 72h after 1st and 2nd therapy. Injected compounds were analysed by ELISA as described in Example 5.

Figures 13A and 13B show the T cell infiltration in tumor at study termination as analysed by FACS. The increase of CD8 positive T cells in the tumor is shown in Figure 13A and the increase of CD4 positive T cells in the tumor is illustrated in Figure 13B.

Figures 14A and 14B relate to the histological analysis at day 33 and the end of the study day 50 and **Figures 15A, 15B and 15C** refer to the cytokine analysis at the end of the study (see Example 5).

In **Figure 16** it is shown that combinations of CEA CD3 TCB + CEA-4-1BBL in the doses of 10 mg/kg and 3 mg/kg mediate improved efficacy in terms of tumor growth inhibition compared to CEA CD3 TCB monotherapy (see Example 7).

Figures 17A and 17B show the pharmacokinetic profile of injected compounds CEA CD3 TCB and CEA-4-1BBL during the first week. 2 mice per Group were bled 1h and 72h after 1st and 2nd therapy. Injected compounds were analysed by ELISA as described in Example 7. In Figure 17A 4-1BBL was detected via 4-1BB binding whereas in Figure 17B the concentration of CEA CD3 TCB was detected via binding to anti-CD3 CDR antibody.

T cell infiltration in tumor at study termination as analysed by FACS is shown in **Figures 18A** (CD3 positive T cells), **18B** (CD8 positive T cells) and **18C** (CD4 positive T cells).

Figures 19A and 19B relate to the histological analysis at day 44 of the study (see Example 7). There is a significant increase of CD3 positive T cells (**Figure 19A**) and CD8 positive T cells (**Figure 19B**) positive T cells in the groups treated with CEA CD3 TCB + CEA-4-1BBL 10mg/kg and 3 mg/kg group compared to CEA CD3 TCB monotherapy and vehicle.

In **Figure 20** TCB mediated lysis of MKN45 NucLight red tumor cells by various human immune cell preparations is shown (Example 8). Different human immune effector cell preparations (resting PBMC, CD4 or CD8 T cells, NLV specific CD8 T effector memory cells) were cocultured with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEACAM5 CD3 TCB for 48 hours. The amount of living tumor cells was quantified by fluorescence microscopy high content life imaging using the Incucyte Zoom System (Essenbioscience, HD phase-contrast, green fluorescence and red fluorescence, 10x objective) in a 3 hours interval for 48 hours at 37 °C and 5% CO₂. The

integrated red fluorescence of healthy tumor cells ($\text{RCU} \times \mu\text{m}^2/\text{image}$) of triplicates (median) was used to calculate the specific lysis which was plotted against the used TCB concentration to show the cytolytic potential of T cells.

Figures 21A-21D show the expression of 4-1BB on T cells upon TCB stimulation.

5 Different human immune effector cell preparations (resting PBMC, CD4 or CD8 T cells, NLV specific CD8 T effector memory cells) were cocultured with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEACAM5 CD3 TCB for 48 hours. The expression of 4-1BB was determined on CD4^+ and CD8^+ T cells by flow cytometry. The percentage of positive cells (**Figures 21A and 21C**) and MFI
10 (**Figures 21B and 21D**) of triplicates (median) was plotted against the used TCB concentration for CD4 positive T cells (**Figures 21A and 21B**) and CD8 positive (**Figures 21C and 21D**) T cells. Error bars indicate the SEM. TCB mediate a dose dependent cell surface expression of 4-1BB on CD4^+ T cells and on CD8^+ T cell, albeit at a lower extent on CD4^+ T cells.

15 **Figures 22A-22D** demonstrate that 4-1BB costimulation did not influence the cytolytic potential of CEACAM5 CD3 TCB. Different human immune effector cell preparations (resting PBMC in **Figure 22B**, CD4 T cells in **Figure 22A**, CD8 T cells in **Figure 22C**, NLV specific CD8 T effector memory cells in **Figure 22D**) were cocultured for 48 hours with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial
20 dilution row of CEACAM5 CD3 TCB with or without a fixed concentration of FAP 4-1BBL. The amount of living tumor cells was quantified by fluorescence microscopy high content life imaging using the Incucyte Zoom System (Essenbioscience, HD phase-contrast, green fluorescence and red fluorescence, 10x objective) in a 3 hours interval for 48 hours at 37 °C and 5% CO_2 . The integrated red fluorescence of healthy tumor cells ($\text{RCU} \times \mu\text{m}^2/\text{image}$) of
25 triplicates (median) was used to calculate the specific lysis which was plotted against the used TCB concentration to show the cytolytic potential of T cells. Here, the 42 hours timepoint is shown exemplary. Error bars indicate the SEM.

In **Figures 23A-23D** it is shown that 4-1BB costimulation did increase CEA CD3 TCB and CEACAM5 CD3 TCB mediated $\text{TNF-}\alpha$ release. Resting CD4 T cells were cocultured for
30 48 hrs with MKN45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEACAM5 CD3 TCB or CEA CD3 TCB with or without a fixed concentration of FAP 4-1BBL, respectively. The amount of $\text{TNF-}\alpha$ was quantified as GFP induction in $\text{TNF-}\alpha$ sensor cells by fluorescence microscopy high content life imaging using the Incucyte Zoom System (Essenbioscience, HD phase-contrast, green fluorescence and red
35 fluorescence, 10x objective) in a 3 hours interval for 42 hours at 37 °C and 5% CO_2 . The integrated green fluorescence of $\text{TNF-}\alpha$ sensor cells ($\text{GCU} \times \mu\text{m}^2/\text{image}$) of triplicates

(median) was plotted against the used TCB concentration to quantify TNF- α secretion of T cells. Error bars indicate the SEM. **Figure 23A** shows the results for CEACAM5 CD3 TCB without the presence of FAP-4-1BBL, the increase with the addition of FAP-4-1BBL is shown in **Figure 23B**. **Figures 23C** and **23D** show the TNF- α release mediated by CEA CD3 TCB without (**Figure 23C**) and with (**Figure 23D**) costimulation with FAP-4-1BBL.

In **Figures 24A** and **24B** the area under the curve (AUC) values are plotted against each timepoint. The amount of TNF- α was quantified as GFP induction in TNF- α sensor cells by fluorescence microscopy high content life imaging. The AUC of GFP was calculated for each condition and time point and was plotted against each timepoint to quantify TNF- α secretion of T cells. It can be seen that the TNF- α release mediated by CEACAM5 CD3 TCB (**Figure 24A**) and CEA CD3 TCB (**Figure 24B**) did increase with 4-1BB costimulation through the presence of FAP-4-1BBL.

In **Figures 25A-25D** it is shown how 4-1BB costimulation did modulate cytokine secretion by CEACAM5 CD3 TCB (called CEA CD3 TCB(2) in the graphs). Resting CD4 T cells were cocultured for 48 hrs with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEA CD3 TCB (2) with or without a fixed concentration of FAP 4-1BBL. The secreted amount of TNF- α , IFN- γ , IL-2 and IL-10 was quantified at the 48 hours end point using cytometric bead array technology. The respective cytokine concentrations were plotted against the TCB concentration. Off note – secretion of proinflammatory cytokine TNF- α (**Figure 25A**), IFN- γ (**Figure 25B**) and IL-2 (**Figure 25C**) was enhanced by 4-1BB costimulation, whereas that of immunosuppressive IL-10 (**Figure 25D**) was decreased.

In **Figures 26A-26D** similar data are shown for CEA CD3 TCB mediated cytokine secretion. Resting CD4 T cells were cocultured for 48 hrs with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEA CD3 TCB with or without a fixed concentration of FAP 4-1BBL. The secreted amount of TNF- α (**Figure 26A**), IFN- γ (**Figure 26B**), IL-2 (**Figure 26C**) and IL-10 (**Figure 26D**) was quantified at the 48h end point using cytometric bead array technology. The respective cytokine concentrations were plotted against the TCB concentration.

In **Figure 27** a comparison of FAP 4-1BBL costimulation mediated changes [%] in cytokine concentration in the presence of a high concentration of CEA CD3 TCB [50 nM] or CEACAM5 CD3 TCB [2 nM] (in the graph CEA CD3 TCB(2)) with or without a fixed concentration of FAP 4-1BBL are shown. The secreted amount of TNF- α , IFN- γ , IL-2, IL-10, IL-9 and IL-17A was quantified at the 48 hours end point using cytometric bead array

technology. The changes of cytokine concentration were calculated in percent, whereby the respective sample w/o FAP 4-1BBL costimulation was considered 100%.

Figures 28A-28H illustrate that 4-1BB costimulation did also modulate CEACAM5 CD3 TCB mediated cytokine secretion of resting CD8 T cells. Resting CD8 T cells were cocultured for 72 hrs with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEACAM5 CD3 TCB (called CEA TCB2 in the graphs) with or without a fixed concentration of FAP 4-1BBL. The secreted amount of IL-2 (**Figure 28A**), TNF- α (**Figure 28B**), IFN- γ (**Figure 28C**), IL-4 (**Figure 28D**), IL-9 (**Figure 28E**), IL-17a (**Figure 28F**), MIP-1 α (**Figure 28G**) and IL-10 (**Figure 28H**) was quantified at the 72 hours end point using cytometric bead array technology. The respective cytokine concentrations were plotted against the TCB concentration.

In **Figures 29A-29H** it is shown that 4-1BB costimulation did modulate CEACAM5 CD3 TCB (CEA TCB2) mediated cytokine secretion of NLV specific effector memory CD8 T cells. NLV specific effector memory CD8 T cells were cocultured for 72 hours with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEA TCB2 with or without a fixed concentration of FAP 4-1BBL. The secreted amount of IL-2 (**Figure 29A**), TNF- α (**Figure 29B**), IFN- γ (**Figure 29C**), IL-4 (**Figure 29D**), IL-9 (**Figure 29E**), IL-17a (**Figure 29F**), MIP-1 α (**Figure 29G**) and IL-10 (**Figure 29H**) was quantified at the 72 hours end point using cytometric bead array technology. The respective cytokine concentrations were plotted against the TCB concentration.

In **Figure 30** a comparison of FAP 4-1BBL costimulation mediated changes [%] in cytokine concentration in the presence of a high concentration of CEA CD3 TCB [50 nM] or CEACAM5 CD3 TCB [2 nM] (in the graph CEA CD3 TCB(2)) with or without a fixed concentration of FAP 4-1BBL are shown. The secreted amount of TNF- α , IFN- γ , IL-2, IL-10, IL-9 and IL-17A was quantified at the 48 hours end point using cytometric bead array technology. The changes of cytokine concentration were calculated in percent, whereby the respective sample w/o FAP 4-1BBL costimulation was considered 100%.

The results of *in vitro* co-culture assays of PBMCs with MKN45 cells expressing CEA and PDL-1 and NIH/3T3-FAP cells as described in Example 9 are shown in **Figures 31** (for CEA CD3 TCB) and **32** (for CEACAM5 CD3 TCB). As can be seen in **Figure 31**, a small increase of proliferating CD4 T cells and high increase of proliferating CD8 T cells was observed for the combination of CEA CD3 TCB and FAP-4-1BBL. As shown in **Figure 32**, an even clearer increase of proliferating CD4 T cells and proliferating CD8 T cells was caused by the combination of CEACAM5 CD3 TCB and FAP-4-1BBL.

The results of an *in vitro* assay testing the efficacy of the combination of CEA CD3 TCB and FAP-4-1BBL as well as the triple combination of CEA CD3 TCB and FAP-4-1BBL with a PD-L1 antibody (Atezolizumab) are shown in **Figures 33 to 40**. PBMCs were incubated for four days in the presence of MKN45-PD-L1 and NIH/3T3-huFAP cells and different combinations of T cell activator CEA CD3 TCB, checkpoint inhibitor PD-L1 (Atezolizumab) and immunomodulator FAP-4-1BBL or control molecule DP47-4-1BBL. Each symbol indicate one well (each group were tested in triplicate), each color/pattern indicate a specific treatment combination, the bar indicates the mean with SD. Because different donors showed high diversity of frequencies, a graph for each donor is shown. The effect of the combinations compared to the single components and an untargeted 4-1BBL (DP47-4-1BBL) on proliferation (**Figure 33**) and surface expression of CD25 (**Figure 34**) and CD137 (4-1BB, **Figure 35**) on CD8 T cells is shown for 5 different donors.

Secreted cytokines IFN γ , GM-CSF and TNF α were analyzed in the supernatant after 4 days of incubation using Bio-RAD Bio-Plex Pro Human Cytokine 8 plex. The increase in IFN γ release is shown in **Figure 36**, **Figure 37** shows the increase in GM-CSF release and **Figure 38** shows the release of TNF α . Each symbol indicates one well (each group tested in duplicate), each color/pattern indicates a specific treatment combination, the bar indicates the mean with SD. Because different donors showed high diversity of frequencies, a graph for each donor is shown.

In **Figure 39** a comparison between the treatment with 100 nM CEA CD3 TCB and the the combination of 100 nM CEA CD3 TCB and 1 nM FAP-4-1BBL is shown. To analyze the improvement of T cell activation obtained by the combination and in consideration of the donor diversity the fold increase was calculated for each donor and each tested parameter. For each donor the mean is shown, whereby each donor is represented by a different symbol. A significant change is defined as a fold increase of 2 (grey area). No change (e.g. fold increase =1) is indicated as dotted line.

In **Figure 40** the combination treatment of CEA CD3 TCB with FAP-4-1BBL is compared versus the triple combination treatment of CEA CD3 TCB, FAP-4-1BBL and PD-L1 antibody (Atezolizumab). To analyze the improvement of T cell activation by combining CEA CD3TCB with FAP-4-1BBL and PD-L1 antibody (Atezolizumab) and in consideration of the donor diversity the fold increase was calculated for each donor and each tested parameter. For each donor the mean is shown, whereby each donor is represented by a different symbol. A significant change is defined as a fold increase of 2 (grey area). No change (e.g. fold increase =1) is indicated as dotted line.

Figures 41A and 41B show the simultaneous binding of hybrid surrogate FAP-mu4-1BBL (Analyte 1) to immobilized murine 4-1BB and human FAP or murine FAP (Analyte 2), respectively. In **Figure 41C** is shown the simultaneous binding of murine bispecific FAP-4-1BB antibody muFAP-4-1BB (Analyte 1) to immobilized murine 4-1BB and murine FAP (Analyte 2).

Figure 42 shows the pharmacokinetic profile of muFAP-4-1BB after single injection in C57BL/6 mice as described in Example 13. A stable PK-behavior was observed which suggests that the compound can be administered in a once weekly schedule. This led to the treatment schedule of the efficacy study with muFAP-4-1BB and anti-PD-L1 antibody in the MC38-CEA model as shown in **Figure 43** and described in Example 14.

Figure 44A shows the tumor growth kinetics (Mean +/- SEM) as observed in mice treated with muFAP-4-1BB alone, anti-PD-L1 antibody alone or with the combination of both. **Figure 44B** shows a waterfall plot indicating the tumor growth change of any animal from start of treatment at day 15 until day 37. The individual tumor growth kinetics of each animal for all treatment groups is furthermore shown in more detail in **Figure 44C**. Monotherapy of muFAP-4-1BB did not reveal any tumor growth inhibition. Treatment with anti-PD-L1 alone induced tumor growth inhibition with one mouse being tumor free at day 37. However, the combination of muFAP-4-1BB and a-PD-L1 induced strong tumor regression in 5 out of 10 mice resulting in 50% tumor free mice by day 37.

Figures 45A and 45B show that the groups treated with combinations of FolR1 CD3 TCB with monovalent FAP(28H1)-4-1BBL showed improved efficacy in terms of tumor growth inhibition compared to the other groups receiving FolR1 CD3 TCB alone or monovalent FAP(28H1)-4-1BBL as single agent (**Fig. 45B**). In **Fig. 45A** it can be seen that monovalent FAP(28H1)-4-1BBL is much more potent in inhibiting tumor growth as untargted 4-1BBL, but less potent than in combination with FolR1 CD3 TCB.

Figure 46 shows the pharmacokinetic profile of injected compounds during the first week. 2-3 mice per Group were bled 10 min, 1h, 8h, 24h and 7d after the first therapy and injected compounds were analysed by ELISA. 4-1BBL was detected via 4-1BB binding. All groups injected with compounds show comparable exposure of the molecules between the different groups (dose dependent).

Figures 47A to 47D show that treatment with FolR1 CD3 TCB alone or combinations of FolR1 CD3 TCB with monovalent FAP(28H1)-4-1BBL led to increased infiltration of CD3⁺ T-cells (Fig. 47A), CD8⁺ T cells (Fig. 47C) and CD4⁺ T-cells (Fig 47B) in the tumor compared to treatment with 4-1BBL alone, however the ratio of CD8⁺/CD4⁺ T-cells is much

higher in the combination groups compared to the group receiving FolR1 CD3 TCB alone (Fig. 47D).

Figures 48A to 48C relate to the histological analysis at the end of the study, in particular to the quantification of CD3⁺ (Fig. 48A) and CD8⁺ T cells (Fig. 48B) in subcutaneous SKOV3 ovarian tumors collected at day 44, 1 day after last administration. Immunohistochemistry staining of CD3 and CD8 T cells was performed on human SKOV3 ovarian subcutaneous tumors derived from the indicated treatment groups in PBMC transfer NOG mice. Tissue samples were prepared for immunohistochemical staining: subcutaneous tumors were harvested from animals at day 44, fixed in formalin 10% (Sigma, Germany) and later processed for FFPET (Leica 1020, Germany). 4 µm paraffin sections were subsequently cut in a microtome (Leica RM2235, Germany). HuCD8 and HuCD3 immunohistochemistry was performed with anti-human CD8 (Cell Marque Corporation, California) and anti-human CD3 (ThermoFischer Scientific, USA) in the Leica autostainer (Leica ST5010, Germany) following the manufacturer's protocols. Quantification of huCD3 and huCD8 positive T cells was performed with Definiens software (Definiens, Germany). Statistics were analyzed by t-test. There is a significant increase of positive CD3 and CD8 T cell number in the FolR1-TCB treated groups. The treatment of mono FAP-4-1BBL changes the ratio of CD8 to CD3.

Figure 49 shows that the groups treated with combinations of FolR1 CD3 TCB with monovalent or bivalent FAP(28H1)-4-1BBL showed improved efficacy in terms of tumor growth inhibition compared to groups treated with the single agents.

Figure 50 shows the pharmacokinetic profile of injected compounds during the first week. 2 mice per Group were bled 10 min, 1h, 8h, 24h and 7d after the first therapy and injected compounds were analysed by ELISA. 4-1BBL was detected via 4-1BB binding. All groups injected with compounds show comparable exposure of the molecules between the different groups.

Figures 51A and 51B show that treatment with combinations of FolR1 CD3 TCB with monovalent or bivalent FAP(4B9)-4-1BBL led to increased infiltration of CD8 and CD4 positive T-cells in the tumor and blood compared to treatment with the single agents. **Figure 51C** shows that the ratio of CD8⁺/CD4⁺ T-cells is much higher in the combination groups compared to the group receiving FolR1 CD3 TCB alone.

Figure 52 shows a comparison of MCSP CD3 TCB-mediated killing of MV3 melanoma cells without or in the presence of FAP-4-1BBL with CD8⁺ T cells of 3 donors (see Example 16). For all three donors a significantly increased tumor target cell killing was observed. **Figure 53** shows the comparison of TCB-mediated killing of MV3 melanoma cells

without or in the presence of FAP-4-1BBL with pan T cells of 3 donors. Significantly increased tumor target cell killing was observed for all 3 donors.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

5 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.

10 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 "**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
15 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
20 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
25 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. Typically, a bispecific antigen binding molecule comprises 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
30 simultaneously binding two antigenic determinants, particularly two antigenic determinants expressed on two distinct cells.

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”, “tetravalent”, and “hexavalent” denote the presence of two binding sites, four binding sites, and six binding sites, respectively, in an antigen binding molecule.

5 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
10 heterotetrameric glycoproteins of about 150,000 daltons, composed of two light chains and two heavy chains that 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
15 constant domain (CL), also called a light 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
20 constant domain.

 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
25 antibody molecules (e.g. scFv); and single domain antibodies. For a review of certain antibody fragments, 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, Rosenburg 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
30 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.,
35 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.

5 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 domain and a constant domain of a light chain (CL),
10 and a VH domain 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 in which 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
15 Fab fragments) and a part of the Fc region.

 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 crossover Fab molecule are possible and comprised in the bispecific antibodies of the invention: On the one hand, the
20 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
25 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
30 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
35 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).

5 A “crossover single chain Fab fragment” or “**x-scFab**” is a polypeptide consisting of an antibody heavy chain variable domain (V_H), an antibody constant domain 1 (CH1), an antibody light chain variable domain (V_L), 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) V_H-CL-linker-V_L-CH1 and b) V_L-CH1-linker-V_H-CL; 10 wherein V_H and V_L 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).

15 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, 20 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 V_H domain, namely being able to assemble together with a V_L domain, or of a V_L domain, namely being able to assemble together with a V_H domain to a functional antigen binding site and thereby 25 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 (DARPin) 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., 30 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 35 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).

5 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:
10 337-350 (2000), US7250297B1 and US20070224633.

Designed Ankyrin Repeat Proteins (DARPs) 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
15 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
20 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.

An **"antigen binding molecule that binds to the same epitope"** as a reference
25 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.

The term **"antigen binding domain"** refers to the part of an antigen binding molecule
30 that comprises the area which specifically binds to and is complementary to part or all of an antigen. Where an antigen is large, an antigen binding molecule may only bind to a particular part of the antigen, which part is termed an epitope. An antigen binding domain may be provided by, for example, one or more variable domains (also called variable regions). Preferably, an antigen binding domain comprises an antibody light chain variable region (VL)
35 and an antibody heavy chain variable region (VH).

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.

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 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, an molecule that binds to the antigen 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^{-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 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 methods known in the art, including those described herein. A particular method for measuring affinity is Surface Plasmon Resonance (SPR).

The term “**tumor-associated antigen**” means any antigen that is highly expressed by tumor cells or in the tumor stroma. Particular tumor-associated antigens are CEA or FAP, but also other targets such as Folate Receptor.

The term “**Fibroblast activation protein (FAP)**”, also known as Prolyl endopeptidase 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 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:80), 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 a His-tagged human FAP ECD is shown in SEQ ID NO: 81. The amino acid sequence of mouse FAP is shown in UniProt accession no. P97321 (version 126, SEQ ID NO:82), or NCBI RefSeq NP_032012.1. The extracellular domain (ECD) of mouse FAP extends from amino acid position 26 to 761. SEQ ID NO: 83 shows the amino acid sequence of a His-tagged mouse FAP ECD. SEQ ID NO: 84 shows the amino acid sequence of a His-tagged cynomolgus FAP ECD. 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.

The term “**Carcinoembryonic antigen (CEA)**”, also known as Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5), refers to any native CEA 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 amino acid sequence of human CEA is shown in UniProt accession no. P06731 (version 151, SEQ ID NO:85). CEA has long been identified as a tumor-associated antigen (Gold and Freedman, J Exp Med., 121:439-462, 1965; Berinstein N. L., J Clin Oncol., 20:2197-2207, 2002). Originally classified as a protein expressed only in fetal tissue, CEA has now been identified in several normal adult tissues. These tissues are primarily epithelial in origin, including cells of the gastrointestinal, respiratory, and urogenital tracts, and cells of colon, cervix, sweat glands, and prostate (Nap et al., Tumour Biol., 9(2-3):145-53, 1988; Nap et al., Cancer Res., 52(8):2329-23339, 1992). Tumors of epithelial origin, as well as their metastases, contain CEA as a tumor associated antigen. While the presence of CEA itself does not indicate transformation to a cancerous cell, the distribution of CEA is indicative. In normal tissue, CEA is generally expressed on the apical surface of the cell (Hammarström S., Semin

Cancer Biol. 9(2):67-81 (1999)), making it inaccessible to antibody in the blood stream. In contrast to normal tissue, CEA tends to be expressed over the entire surface of cancerous cells (Hammarström S., Semin Cancer Biol. 9(2):67-81 (1999)). This change of expression pattern makes CEA accessible to antibody binding in cancerous cells. In addition, CEA expression
5 increases in cancerous cells. Furthermore, increased CEA expression promotes increased intercellular adhesions, which may lead to metastasis (Marshall J., Semin Oncol., 30(a Suppl. 8):30-6, 2003). The prevalence of CEA expression in various tumor entities is generally very high. In concordance with published data, own analyses performed in tissue samples confirmed its high prevalence, with approximately 95% in colorectal carcinoma (CRC), 90%
10 in pancreatic cancer, 80% in gastric cancer, 60% in non-small cell lung cancer (NSCLC, where it is co-expressed with HER3), and 40% in breast cancer; low expression was found in small cell lung cancer and glioblastoma.

CEA is readily cleaved from the cell surface and shed into the blood stream from tumors, either directly or via the lymphatics. Because of this property, the level of serum CEA
15 has been used as a clinical marker for diagnosis of cancers and screening for recurrence of cancers, particularly colorectal cancer (Goldenberg D M., The International Journal of Biological Markers, 7:183-188, 1992; Chau I., et al., J Clin Oncol., 22:1420-1429, 2004; Flamini et al., Clin Cancer Res; 12(23):6985-6988, 2006).

The term “**FolR1**” refers to Folate receptor alpha and has been identified as a potential
20 prognostic and therapeutic target in a number of cancers. It refers to any native FolR1 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 amino acid sequence of human FolR1 is shown in UniProt accession no. P15328, murine FolR1 has the amino acid sequence of UniProt accession no. P35846 and
25 cynomolgus FolR1 has the amino acid sequence as shown in UniProt accession no. G7PR14. FolR1 is an N-glycosylated protein expressed on plasma membrane of cells. FolR1 has a high affinity for folic acid and for several reduced folic acid derivatives and mediates delivery of the physiological folate, 5-methyltetrahydrofolate, to the interior of cells. FOLR1 is a desirable target for FOLR1-directed cancer therapy as it is overexpressed in vast majority of
30 ovarian cancers, as well as in many uterine, endometrial, pancreatic, renal, lung, and breast cancers, while the expression of FOLR1 on normal tissues is restricted to the apical membrane of epithelial cells in the kidney proximal tubules, alveolar pneumocytes of the lung, bladder, testes, choroid plexus, and thyroid. Recent studies have identified that FolR1 expression is particularly high in triple negative breast cancers (Necela et al. PloS One 2015,
35 10(3), e0127133).

The term “**MCSP**” refers to Melanoma-associated Chondroitin Sulfate Proteoglycan, also known as Chondroitin Sulfate Proteoglycan 4 (CSPG4). It refers to any native FolR1 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 amino acid sequence of human MCSP is shown in UniProt accession no. Q6UVK1). MCSP is a highly glycosylated integral membrane chondroitin sulfate proteoglycan consisting of an N-linked 280 kDa glycoprotein component and a 450-kDa chondroitin sulfate proteoglycan component expressed on the cell membrane (Ross et al., *Arch. Biochem. Biophys.* 1983, 225:370-38). MCSP is more broadly distributed in a number of normal and transformed cells. In particular, MCSP is found in almost all basal cells of the epidermis. MCSP is differentially expressed in melanoma cells, and was found to be expressed in more than 90% of benign nevi and melanoma lesions analyzed. MCSP has also been found to be expressed in tumors of nonmelanocytic origin, including basal cell carcinoma, various tumors of neural crest origin, and in breast carcinomas.

A “**T-cell antigen**” as used herein refers to an antigenic determinant presented on the surface of a T lymphocyte, particularly a cytotoxic T lymphocyte.

A “**T cell activating therapeutic agent**” as used herein refers to a therapeutic agent capable of inducing T cell activation in a subject, particularly a therapeutic agent designed for inducing T-cell activation in a subject. Examples of T cell activating therapeutic agents include bispecific antibodies that specifically bind an activating T cell antigen, such as CD3, and a target cell antigen, such as CEA or Folate Receptor.

An “**activating T cell antigen**” as used herein refers to an antigenic determinant expressed by a T lymphocyte, particularly a cytotoxic T lymphocyte, which is capable of inducing or enhancing T cell activation upon interaction with an antigen binding molecule. Specifically, interaction of an antigen binding molecule with an activating T cell antigen may induce T cell activation by triggering the signaling cascade of the T cell receptor complex. An exemplary activating T cell antigen is CD3.

The term “**CD3**” refers to any native CD3 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 CD3 as well as any form of CD3 that results from processing in the cell. The term also encompasses naturally occurring variants of CD3, e.g., splice variants or allelic variants. In one embodiment, CD3 is human CD3, particularly the epsilon subunit of human CD3 (CD3ε). The amino acid sequence of human CD3ε is shown in UniProt (www.uniprot.org) accession no. P07766 (version 144), or NCBI (www.ncbi.nlm.nih.gov/)

RefSeq NP_000724.1. See also SEQ ID NO: 106. The amino acid sequence of cynomolgus [Macaca fascicularis] CD3ε is shown in NCBI GenBank no. BAB71849.1. See also SEQ ID NO: 107.

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 domain may be 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 and/or form structurally defined loops (“hypervariable loops”). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the “complementarity determining regions” (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. Exemplary hypervariable loops occur 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).) Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3. (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).) Hypervariable regions (HVRs) are also referred to as complementarity determining regions (CDRs), and these terms are used herein interchangeably in reference to portions of the variable region that form the antigen binding regions. This particular region has been described by Kabat et al., U.S. Dept. of Health and Human Services, “Sequences of Proteins of Immunological Interest” (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table B as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

TABLE A. CDR Definitions¹

CDR	Kabat	Chothia	AbM ²
V _H CDR1	31-35	26-32	26-35
V _H CDR2	50-65	52-58	50-58
V _H CDR3	95-102	95-102	95-102
V _L CDR1	24-34	26-32	24-34
V _L CDR2	50-56	50-52	50-56
V _L CDR3	89-97	91-96	89-97

¹ Numbering of all CDR definitions in Table A is according to the numbering conventions set forth by Kabat et al. (see below).

² "AbM" with a lowercase "b" as used in Table A refers to the CDRs as defined by Oxford Molecular's "AbM" antibody modeling software.

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 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.

With the exception of CDR1 in V_H, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise "specificity determining residues," or "SDRs," which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3. (See Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008).) Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

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 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 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 “Fc domain” or “**Fc region**” herein is used to define a C-terminal region of an antibody heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. An IgG Fc region comprises an IgG CH2 and an IgG CH3 domain. The “CH2 domain” of a human IgG Fc region usually extends from an amino acid residue at about position 231 to an amino acid residue at about position 340. In one embodiment, a carbohydrate chain is attached to the CH2 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 (i.e. from an amino acid residue at about position 341 to an amino acid residue at about position 447 of an IgG). 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 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 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). 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 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

5 comprises the amino acid substitution T366W in one of the two subunits of the Fc domain, and the hole modification comprises the amino acid 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

10 additionally comprises the amino acid substitution Y349C. Introduction of these two cysteine residues results in the formation of a disulfide bridge between the two subunits of the Fc region, thus further stabilizing the dimer (Carter, J Immunol Methods 248, 7-15 (2001)).

A "region equivalent to the Fc region of an immunoglobulin" is intended to include naturally occurring allelic variants of the Fc region of an immunoglobulin as well as variants

15 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 biological function. Such variants can be selected according to general

20 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 "**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

25 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.

An "**activating Fc receptor**" is an Fc receptor that following engagement by an Fc

30 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).

An "**ectodomain**" is the domain of a membrane protein that extends into the

35 extracellular space (i.e. the space outside the target cell). Ectodomains are usually the parts of

proteins that initiate contact with surfaces, which leads to signal transduction. The ectodomain of 4-1BBL as defined herein thus refers to the part of the 4-1BBL that extends into the extracellular space (the extracellular domain), but also includes shorter parts or fragments thereof that are responsible for the trimerization and for the binding to the corresponding
5 receptor 4-1BB. The term “ectodomain of 4-1BBL or a fragment thereof” thus refers to the extracellular domain of 4-1BBL that forms the extracellular domain or to parts thereof that are still able to bind to the receptor (receptor binding domain).

“**4-1BBL**” or “**4-1BB ligand**” or “**CD137L**” is a costimulatory TNF ligand family member, which is able to costimulate proliferation and cytokine production of T-cells.
10 Costimulatory TNF family ligands can costimulate TCR signals upon interaction with their corresponding TNF receptors and the interaction with their receptors leads to recruitment of TNFR-associated factors (TRAF), which initiate signalling cascades that result in T-cell activation. 4-1BBL is a type II transmembrane protein. Complete or full length 4-1BBL having the amino acid sequence of SEQ ID NO:86 has been described to form trimers on the
15 surface of cells. The formation of trimers is enabled by specific motives of the ectodomain of 4-1BBL. Said motives are designated herein as “trimerization region”. The amino acids 50-254 of the human 4-1BBL sequence (SEQ ID NO:87) form the extracellular domain of 4-1BBL, but even fragments thereof are able to form the trimers. In specific embodiments of the invention, the term “ectodomain of 4-1BBL or a fragment thereof” refers to a polypeptide
20 having an amino acid sequence selected from SEQ ID NO:4 (amino acids 52-254 of human 4-1BBL), SEQ ID NO:1 (amino acids 71-254 of human 4-1BBL), SEQ ID NO:3 (amino acids 80-254 of human 4-1BBL), SEQ ID NO:2 (amino acids 85-254 of human 4-1BBL), SEQ ID NO:5 (amino acids 71-248 of human 4-1BBL), SEQ ID NO:6 (amino acids 85-248 of human 4-1BBL), SEQ ID NO:7 (amino acids 80-248 of human 4-1BBL) and SEQ ID NO:8 (amino
25 acids 52-248 of human 4-1BBL), but also other fragments of the ectodomain capable of trimerization are included herein.

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-
30 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: 88 (Uniprot accession no. Q07011), the amino acid sequence of an exemplary murine 4-1BB is shown in SEQ ID NO: 89 (Uniprot accession no. P20334) and the amino acid sequence of an
35 exemplary cynomolgous 4-1BB (from *Macaca mulatta*) is shown in SEQ ID NO:90 (Uniprot accession no. F6W5G6).

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
 5 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).

10 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 2 and 4, in particular 2, i.e. the peptides selected from the group consisting of
 15 GGGGS (SEQ ID NO: 91) GGGGSGGGGS (SEQ ID NO:92), SGGGSGGGG (SEQ ID NO:93) and GGGGSGGGGSGGGG (SEQ ID NO:94), but also include the sequences GSPGSSSSGS (SEQ ID NO:95), $(G_4S)_3$ (SEQ ID NO:96), $(G_4S)_4$ (SEQ ID NO:97), GSGSGSGS (SEQ ID NO:98), GSGSGNGS (SEQ ID NO:99), GGSGSGSG (SEQ ID NO:100), GGSGSG (SEQ ID NO:101), GGSG (SEQ ID NO:102), GGSGNGSG (SEQ ID
 20 NO:103), GGNGSGSG (SEQ ID NO:104) and GGNGSG (SEQ ID NO:105). Peptide linkers of particular interest are (G_4S) (SEQ ID NO:91), $(G_4S)_2$ and GGGGSGGGGS (SEQ ID NO:92).

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:
 25 A), 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 polypeptide and an
 30 ectodomain of 4-1BBL) 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 that are identical with the amino acid residues in the reference polypeptide sequence, after
 35 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 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-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 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 C 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 B

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
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;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- 5 (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

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 antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more CDR 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 CDRs 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 CDRs. 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.

35 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 antigen binding molecules 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 antigen binding molecules.

5 In certain embodiments, the 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 antigen binding molecule comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies
10 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
15 structure. In some embodiments, modifications of the oligosaccharide in the antigen binding molecules may be made in order to create variants with certain improved properties. In one aspect, variants of antigen binding molecules 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 Publication Nos. US 2003/0157108
20 (Presta, L.) or US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Further variants of the antigen binding molecules of the invention include those 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 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US
25 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.).

In certain embodiments, it may be desirable to create **cysteine engineered variants** of
30 the 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 embodiments, 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 be used to conjugate the antibody to other moieties, such as drug moieties or linker-
35 drug moieties, to create an immunoconjugate. In certain embodiments, 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. Patent No. 7,521,541.

In certain aspects, the antigen binding molecules 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, 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 copolymers, 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 4-1BBL-containing antigen binding molecules 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 “**polynucleotide**” 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

5 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

10 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.

15 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

20 having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides 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

25 anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or 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

30 above for polypeptides (e.g. ALIGN-2).

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

35 acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among 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 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 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 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 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 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.

The combination therapies in accordance with the invention have a synergistic effect. A “**synergistic effect**” of two compounds is one in which the effect of the combination of the two agents is greater than the sum of their individual effects and is statistically different from the controls and the single drugs. In another embodiment, the combination therapies disclosed herein have an additive effect. An “**additive effect**” of two compounds is one in which the effect of the combination of the two agents is the sum of their individual effects and is statistically different from either the controls and/or the single drugs.

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 therapeutic or prophylactic result. A therapeutically effective amount of an agent for example eliminates, decreases, delays, minimizes or prevents adverse effects of a disease.

5 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 individual or subject is a human.

10 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.

15 A "**pharmaceutically acceptable carrier**" 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.

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

25 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 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.

30 The term "**cancer**" as used herein refers to proliferative diseases, such as solid tumors, or melanoma.

"**CD20**" refers to B-lymphocyte antigen CD20, also known as B-lymphocyte surface antigen B1 or Leukocyte surface antigen Leu-16, and includes any native CD20 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 amino acid sequence of human CD20 is shown in Uniprot accession no. P11836 (version 149, SEQ ID NO:159). CD20 is a hydrophobic transmembrane protein with a molecular weight of approximately 35 kD expressed on pre-B and mature B lymphocytes. The corresponding human gene is membrane-spanning 4-domains, subfamily A, member 1, also known as MS4A1. This gene encodes a member of the membrane-spanning 4A gene family. Members of this nascent protein family are characterized by common structural features and similar intron/exon splice boundaries and display unique expression patterns among hematopoietic cells and nonlymphoid tissues. This gene encodes the B-lymphocyte surface molecule which plays a role in the development and differentiation of B-cells into plasma cells. This family member is localized to 11q12, among a cluster of family members. Alternative splicing of this gene results in two transcript variants which encode the same protein. The term “CD20” encompasses “full-length,” unprocessed CD20 as well as any form of CD20 that results from processing in the cell. The term also encompasses naturally occurring variants of CD20, e.g., splice variants or allelic variants.

The terms “**anti-CD20 antibody**” and “an antibody that binds to CD20” refer to an antibody that is capable of binding CD20 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting CD20. In one embodiment, the extent of binding of an anti-CD20 antibody to an unrelated, non-CD20 protein is less than about 10% of the binding of the antibody to CD20 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to CD20 has a dissociation constant (Kd) 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). In certain embodiments, an anti-CD20 antibody binds to an epitope of CD20 that is conserved among CD20 from different species.

By “**Type II anti-CD20 antibody**” is meant an anti-CD20 antibody having binding properties and biological activities of Type II anti-CD20 antibodies as described in Cragg et al., Blood 103 (2004) 2738-2743; Cragg et al., Blood 101 (2003) 1045-1052, Klein et al., mAbs 5 (2013), 22-33. A type II anti-CD20 antibody binds to class II epitope on CD20, it does not localize CD20 to lipid rafts, shows ADCC activity, but low CDC if it is a IgG1 isotype antibody, has less binding capacity to B cells compared with antibodies binding to the Class I CD20 epitope, shows homotypic aggregation and strong death induction. Examples of type II anti-CD20 antibodies include e.g. obinutuzumab (GA101), tositumumab (B1), humanized B-Ly1 antibody IgG1 (a chimeric humanized IgG1 antibody as disclosed in WO 2005/044859), 11B8 IgG1 (as disclosed in WO 2004/035607) and AT80 IgG1. In a particular aspect, the Type II anti-CD20 antibody is obinutuzumab (recommended INN, WHO

Drug Information, Vol. 26, No. 4, 2012, p. 453). As used herein, obinutuzumab is synonymous for GA101. The tradename is GAZYVA® or GAZYVARO®. This replaces all previous versions (e.g. Vol. 25, No. 1, 2011, p.75-76), and is formerly known as afutuzumab (recommended INN, WHO Drug Information, Vol. 23, No. 2, 2009, p. 176; Vol. 22, No. 2, 2008, p. 124). In one aspect, the Type II anti-CD20 antibody is tositumomab.

Exemplary anti-CEA/anti-CD3 bispecific antibodies for use in the invention

The present invention relates to anti-CEA/anti-CD3 bispecific antibodies and their use in combination with 4-1BB (CD137) agonists, in particular to their use in a method for treating or delaying progression of cancer, more particularly for treating or delaying progression of solid tumors. The anti-CEA/anti-CD3 bispecific antibodies as used herein are bispecific antibodies comprising a first antigen binding domain that binds to CD3, and a second antigen binding domain that binds to CEA.

Thus, the anti-CEA/anti-CD3 bispecific antibody as used herein comprises a first antigen binding domain comprising a heavy chain variable region (V_H CD3) and a light chain variable region (V_L CD3), and a second antigen binding domain comprising a heavy chain variable region (V_H CEA) and a light chain variable region (V_L CEA).

In a particular aspect, the anti-CEA/anti-CD3 bispecific antibody for use in the combination comprises a first antigen binding domain comprising a heavy chain variable region (V_H CD3) comprising CDR-H1 sequence of SEQ ID NO:33, CDR-H2 sequence of SEQ ID NO:34, and CDR-H3 sequence of SEQ ID NO:35; and/or a light chain variable region (V_L CD3) comprising CDR-L1 sequence of SEQ ID NO:36, CDR-L2 sequence of SEQ ID NO:37, and CDR-L3 sequence of SEQ ID NO:38. More particularly, the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_H CD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:39 and/or a light chain variable region (V_L CD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:40. In a further aspect, the anti-CEA/anti-CD3 bispecific antibody comprises a heavy chain variable region (V_H CD3) comprising the amino acid sequence of SEQ ID NO:39 and/or a light chain variable region (V_L CD3) comprising the amino acid sequence of SEQ ID NO:40.

In one aspect, the antibody that specifically binds to CD3 is a full-length antibody. In one aspect, the antibody that specifically binds to CD3 is an antibody of the human IgG class, particularly an antibody of the human IgG₁ class. In one aspect, the antibody that specifically binds to CD3 is an antibody fragment, particularly a Fab molecule or a scFv molecule, more particularly a Fab molecule. In a particular aspect, the antibody that specifically binds to CD3 is a crossover Fab molecule wherein the variable domains or the constant domains of the Fab

heavy and light chain are exchanged (i.e. replaced by each other). In one aspect, the antibody that specifically binds to CD3 is a humanized antibody.

In another aspect, the anti-CEA/anti-CD3 bispecific antibody comprises a second antigen binding domain comprising

- 5 (a) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43, and/or a light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:44, CDR-L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46, or
- (b) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:49, 10 CDR-H2 sequence of SEQ ID NO:50, and CDR-H3 sequence of SEQ ID NO:51, and/or a light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54.

- More particularly, the anti-CEA/anti-CD3 bispecific comprises a second antigen binding domain comprising a heavy chain variable region (V_H CEA) that is at least 90%, 95%, 15 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_L CEA) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:48. In a further aspect, the anti-CEA/anti-CD3 bispecific comprises a second antigen binding domain comprising a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain 20 variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:48. In another aspect, the anti-CEA/anti-CD3 bispecific comprises a second antigen binding domain comprising a heavy chain variable region (V_H CEA) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_L CEA) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the 25 amino acid sequence of SEQ ID NO:56. In a further aspect, the anti-CEA/anti-CD3 bispecific comprises a second antigen binding domain comprising a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:56.

- In another particular aspect, the anti-CEA/anti-CD3 bispecific antibody comprises a 30 third antigen binding domain that binds to CEA. In particular, the anti-CEA/anti-CD3 bispecific antibody comprises a third antigen binding domain comprising
- (a) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43, and/or a light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:44, CDR- 35 L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46, or
- (b) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:49, CDR-H2 sequence of SEQ ID NO:50, and CDR-H3 sequence of SEQ ID NO:51, and/or a

light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54.

More particularly, the anti-CEA/anti-CD3 bispecific comprises a third antigen binding domain comprising a heavy chain variable region (V_H CEA) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_L CEA) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:48. In a further aspect, the anti-CEA/anti-CD3 bispecific comprises a third antigen binding domain comprising a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:48. In another particular aspect, the anti-CEA/anti-CD3 bispecific comprises a third antigen binding domain comprising a heavy chain variable region (V_H CEA) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_L CEA) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:56. In a further aspect, the anti-CEA/anti-CD3 bispecific comprises a third antigen binding domain comprising a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:56.

In a further aspect, the anti-CEA/anti-CD3 bispecific antibody is bispecific antibody, wherein the first antigen binding domain is a cross-Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged, and the second and third, if present, antigen binding domain is a conventional Fab molecule.

In another aspect, the anti-CEA/anti-CD3 bispecific antibody is bispecific antibody, wherein (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

The Fab molecules may be fused to the Fc domain or to each other directly or through a peptide linker, comprising one or more amino acids, typically about 2-20 amino acids. Peptide linkers are known in the art and are described herein. Suitable, non-immunogenic peptide linkers include, for example, $(G_4S)_n$, $(SG_4)_n$, $(G_4S)_n$ or $G_4(SG_4)_n$ peptide linkers. "n" is

generally an integer from 1 to 10, typically from 2 to 4. In one embodiment said peptide linker has a length of at least 5 amino acids, in one embodiment a length of 5 to 100, in a further embodiment of 10 to 50 amino acids. In one embodiment said peptide linker is $(GxS)_n$ or $(GxS)_nG_m$ with G=glycine, S=serine, and $(x=3, n=3, 4, 5 \text{ or } 6, \text{ and } m=0, 1, 2 \text{ or } 3)$ or $(x=4, n=2, 3, 4 \text{ or } 5 \text{ and } m=0, 1, 2 \text{ or } 3)$, in one embodiment $x=4$ and $n=2$ or 3, in a further embodiment $x=4$ and $n=2$. In one embodiment said peptide linker is $(G_4S)_2$. A particularly suitable peptide linker for fusing the Fab light chains of the first and the second Fab molecule to each other is $(G_4S)_2$. An exemplary peptide linker suitable for connecting the Fab heavy chains of the first and the second Fab fragments comprises the sequence (D)-(G₄S)₂. Another suitable such linker comprises the sequence $(G_4S)_4$. Additionally, linkers may comprise (a portion of) an immunoglobulin hinge region. Particularly where a Fab molecule is fused to the N-terminus of an Fc domain subunit, it may be fused via an immunoglobulin hinge region or a portion thereof, with or without an additional peptide linker.

In a further aspect, the anti-CEA/anti-CD3 bispecific antibody comprises an Fc domain comprising one or more amino acid substitutions that reduce binding to an Fc receptor and/or effector function. In particular, the anti-CEA/anti-CD3 bispecific antibody comprises an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

In a particular aspect, the anti-CEA/anti-CD3 bispecific antibody comprises a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 61, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 62, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 63, and a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 64. In a further particular embodiment, the bispecific antibody comprises a polypeptide sequence of SEQ ID NO: 61, a polypeptide sequence of SEQ ID NO: 62, a polypeptide sequence of SEQ ID NO: 63 and a polypeptide sequence of SEQ ID NO: 64 (CEA CD3 TCB).

In a further particular aspect, the anti-CEA/anti-CD3 bispecific antibody comprises a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO:57, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO:58, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO:59, and a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO:60. In a further particular embodiment, the bispecific antibody comprises a polypeptide sequence of SEQ ID NO:57, a polypeptide sequence of SEQ ID NO:58, a polypeptide sequence of SEQ ID NO:59 and a polypeptide sequence of SEQ ID NO:60 (CEACAM5 CD3 TCB).

Particular bispecific antibodies are described in PCT publication no. WO 2014/131712 A1.

In a further aspect, the anti-CEA/anti-CD3 bispecific antibody may also comprise a bispecific T cell engager (BiTE®). In a further aspect, the anti-CEA/anti-CD3 bispecific antibody is a bispecific antibody as described in WO 2007/071426 or WO 2014/131712. In another aspect, the bispecific antibody is MEDI565.

5 Exemplary anti-FolR1/anti-CD3 bispecific antibodies for use in the invention

The present invention also relates to anti-FolR1/anti-CD3 bispecific antibodies and their use in combination with 4-1BB (CD137) agonists, in particular to their use in a method for treating or delaying progression of cancer, more particularly for treating or delaying progression of solid tumors. The anti-FolR1/anti-CD3 bispecific antibodies as used herein are
10 bispecific antibodies comprising a first antigen binding domain that binds to CD3, and a second antigen binding domain that binds to FolR1. In a particular, the anti-FolR1/anti-CD3 bispecific antibodies as used herein comprise a third antigen binding domain that binds to FolR1.

In one aspect, the T-cell activating anti-CD3 bispecific antibody comprises a first
15 antigen binding domain comprising a heavy chain variable region (V_H CD3), a second antigen binding domain comprising a heavy chain variable region (V_H FolR1), a third antigen binding domain comprising a heavy chain variable region (V_H FolR1) and three times a common light chain variable region.

In another aspect, the first antigen binding domain comprises a heavy chain variable
20 region (V_H CD3) comprising CDR-H1 sequence of SEQ ID NO:121, CDR-H2 sequence of SEQ ID NO:122, and CDR-H3 sequence of SEQ ID NO:123; the second antigen binding domain comprises a heavy chain variable region (V_H FolR1) comprising CDR-H1 sequence of SEQ ID NO:124, CDR-H2 sequence of SEQ ID NO:125, and CDR-H3 sequence of SEQ ID NO:126; the third antigen binding domain comprises a heavy chain variable region (V_H FolR1)
25 comprising CDR-H1 sequence of SEQ ID NO:124, CDR-H2 sequence of SEQ ID NO:125, and CDR-H3 sequence of SEQ ID NO:126; and the common light chains comprise a CDR-L1 sequence of SEQ ID NO:127, CDR-L2 sequence of SEQ ID NO:128, and CDR-L3 sequence of SEQ ID NO:129. In another aspect, the first antigen binding domain comprises a heavy chain variable region (V_H CD3) comprising the sequence of SEQ ID NO:130; the second
30 antigen binding domain comprises a heavy chain variable region (V_H FolR1) comprising the sequence of SEQ ID NO:131; the third antigen binding domain comprises a heavy chain variable region (V_H FolR1) comprising the sequence of SEQ ID NO:131; and the common light chains comprise the sequence of SEQ ID NO:132.

In a particular aspect, the anti-FolR1/anti-CD3 bispecific antibody comprises a first
35 heavy chain comprising the amino acid sequence of SEQ ID NO:133, a second heavy chain

comprising the amino acid sequence of SEQ ID NO:134 and three times a common light chain of SEQ ID NO: 135.

Exemplary anti-MCSP/anti-CD3 bispecific antibodies for use in the invention

The present invention further relates to anti-MCSP/anti-CD3 bispecific antibodies and
5 their use in combination with 4-1BB (CD137) agonists, in particular to their use in a method for treating or delaying progression of cancer, more particularly for treating or delaying progression of solid tumors. The anti-MCSP/anti-CD3 bispecific antibodies as used herein are bispecific antibodies comprising a first antigen binding domain that binds to CD3, and a second antigen binding domain that binds to MCSP. In a particular, the anti-MCSP/anti-
10 CD3 bispecific antibodies as used herein comprise a third antigen binding domain that binds to MCSP.

In one aspect, the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_H CD3) and a light chain variable region (V_L CD3), and a second antigen binding domain comprising a heavy chain
15 variable region (V_H MCSP) and a light chain variable region (V_L MCSP). a first antigen binding domain comprising a heavy chain variable region (V_H CD3), a second antigen binding domain comprising a heavy chain variable region (V_H FolR1), a third antigen binding domain comprising a heavy chain variable region (V_H FolR1) and three times a common light chain variable region.

20 In a particular aspect, the anti-MCSP/anti-CD3 bispecific antibody for use in the combination comprises a first antigen binding domain comprising a heavy chain variable region (V_H CD3) comprising CDR-H1 sequence of SEQ ID NO:33, CDR-H2 sequence of SEQ ID NO:34, and CDR-H3 sequence of SEQ ID NO:35; and/or a light chain variable region (V_L CD3) comprising CDR-L1 sequence of SEQ ID NO:36, CDR-L2 sequence of SEQ
25 ID NO:37, and CDR-L3 sequence of SEQ ID NO:38. More particularly, the anti-MCSP/anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_H CD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:39 and/or a light chain variable region (V_L CD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID
30 NO:40. In a further aspect, the anti-MCSP/anti-CD3 bispecific antibody comprises a heavy chain variable region (V_H CD3) comprising the amino acid sequence of SEQ ID NO:39 and/or a light chain variable region (V_L CD3) comprising the amino acid sequence of SEQ ID NO:40.

In another aspect, the anti-MCSP/anti-CD3 bispecific antibody comprises a second antigen binding domain comprising a heavy chain variable region (V_H MCSP) comprising
35 CDR-H1 sequence of SEQ ID NO:151, CDR-H2 sequence of SEQ ID NO:152, and CDR-H3

sequence of SEQ ID NO:153, and/or a light chain variable region (V_LMCSP) comprising CDR-L1 sequence of SEQ ID NO:154, CDR-L2 sequence of SEQ ID NO:155, and CDR-L3 sequence of SEQ ID NO:156.

More particularly, the anti-MCSP/anti-CD3 bispecific comprises a second antigen
5 binding domain comprising a heavy chain variable region (V_HMCSP) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:157 and/or a light chain variable region (V_LMCSP) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:158. In a further aspect, the anti-MCSP/anti-CD3 bispecific comprises a second antigen binding domain comprising a heavy
10 chain variable region (V_HMCSP) comprising the amino acid sequence of SEQ ID NO:157 and/or a light chain variable region (V_LMCSP) comprising the amino acid sequence of SEQ ID NO:158.

In another particular aspect, the anti-MCSP/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to MCSP. In particular, the anti-MCSP/anti-CD3
15 bispecific antibody comprises a third antigen binding domain comprising (a) a heavy chain variable region (V_HMCSP) comprising CDR-H1 sequence of SEQ ID NO:151, CDR-H2 sequence of SEQ ID NO:152, and CDR-H3 sequence of SEQ ID NO:153, and/or a light chain variable region (V_LMCSP) comprising CDR-L1 sequence of SEQ ID NO:154, CDR-L2 sequence of SEQ ID NO:155, and CDR-L3 sequence of SEQ ID NO:156.

20 In a further aspect, the anti-MCSP/anti-CD3 bispecific comprises a third antigen binding domain comprising a heavy chain variable region (V_HMCSP) comprising the amino acid sequence of SEQ ID NO:157 and/or a light chain variable region (V_LMCSP) comprising the amino acid sequence of SEQ ID NO:158.

In a further aspect, the anti-MCSP/anti-CD3 bispecific antibody comprises an Fc
25 domain comprising one or more amino acid substitutions that reduce binding to an Fc receptor and/or effector function. In particular, the anti-MCSP/anti-CD3 bispecific antibody comprises an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

In a particular aspect, the anti-MCSP/anti-CD3 bispecific antibody comprises a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID
30 NO: 147, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 148, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 149, and a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 150. In a further particular embodiment, the bispecific antibody comprises a polypeptide sequence of SEQ ID NO: 147, a polypeptide
35 sequence of SEQ ID NO: 148, a polypeptide sequence of SEQ ID NO: 149 and a polypeptide sequence of SEQ ID NO: 150 (MCSP CD3 TCB).

Particular anti-MCSP/anti-CD3 bispecific antibodies are described in PCT publication no. WO 2014/131712 A1.

Exemplary 4-1BB agonists for use in the invention

5 In particular, the 4-1BB agonists as used in combination with the anti-CEA/anti-CD3 bispecific antibody are molecules comprising 4-1BBL. In particular, the 4-1BB agonist used in the invention comprises three ectodomains of 4-1BBL or fragments thereof.

In a particular aspect, the 4-1BB agonist is a molecule comprising three ectodomains of 4-1BBL or fragments thereof and wherein the ectodomains of 4-1BBL comprise an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8, 10 particularly the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:5.

It has been shown herein, that the 4-1BB agonist is especially useful if it comprises an antigen binding domain that is specific for a tumor-associated antigen, in particular for a target on cancer cells or in the stroma. Thus, in a particular aspect, the 4-1BB agonist is an 15 antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP or CEA.

In a further aspect, the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP, wherein the antigen binding domain capable of specific binding to 20 FAP comprises

(a) 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, 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 25 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, or
(b) a heavy chain variable region (V_H FAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:15, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:16, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:17, and a 30 light chain variable region (V_L FAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:18, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:19, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:20.

In a particular aspect, the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_H FAP) comprising (i) CDR-H1 comprising the 35 amino acid sequence of SEQ ID NO:15, (ii) CDR-H2 comprising the amino acid sequence of

SEQ ID NO:16, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:17, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:18, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:19, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:20.

5 In a further aspect, the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP, wherein 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:21 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:22 or wherein the antigen binding domain capable of specific binding to FAP
10 comprises a heavy chain variable region (V_HFAP) comprising an amino acid sequence of SEQ ID NO:23 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:24. More particularly, the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_HFAP) comprising an amino acid sequence of
15 SEQ ID NO:23 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:24.

In another aspect, the 4-1BB agonist is an antigen binding molecule further comprising a Fc domain composed of a first and a second subunit capable of stable association. In one aspect, the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain,
20 specifically an IgG1 Fc domain or an IgG4 Fc domain. Particularly, the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function. In a particular aspect, the 4-1BB agonist is an antigen binding molecule comprising an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

25 In one aspect, the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one antigen binding domain capable of specific binding to FAP,
(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises
30 one ectodomain of 4-1BBL or a fragment thereof.

In a particular aspect, the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one Fab domain capable of specific binding to FAP, and
(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that
35 (i) the first polypeptide contains a CH1 or CL domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and

wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or

- (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
- (iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

In another aspect, the 4-1BB agonist is an antigen binding molecule comprising

(a) at least one Fab domain capable of specific binding to FAP comprising a heavy chain variable region (V_H FAP) comprising the amino acid sequence of SEQ ID NO:21 and a light chain variable region (V_L FAP) comprising the amino acid sequence of SEQ ID NO:22 or a heavy chain variable region (V_H FAP) comprising the amino acid sequence of SEQ ID NO:23 and a light chain variable region (V_L FAP) comprising the amino acid sequence of SEQ ID NO:24, and

(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

In a particular aspect, the 4-1BB agonist is an antigen binding molecule selected from the group consisting of

a) a molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:67 and a second light chain comprising the amino acid sequence of SEQ ID NO:68; and

- 5 b) a molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:69, a first light chain comprising the amino acid sequence of SEQ ID NO:70, a second heavy chain comprising the amino acid sequence of SEQ ID NO:71 and a second light chain comprising the amino acid sequence of SEQ ID NO:72.

10 Particularly, the 4-1BB agonist is an antigen binding molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:67 and a second light chain comprising the amino acid sequence of SEQ ID NO:68.

15 In another aspect, the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one antigen binding domain capable of specific binding to FAP,
(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers.

20 In one aspect, the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one antigen binding domain capable of specific binding to FAP,
(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers, and
(c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the polypeptide comprising the three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers is fused to the N- or C-terminal amino acid
25 of one of the two subunits of the Fc domain, optionally through a peptide linker.

Particular bispecific antibodies are described in PCT publication No. WO 2016/075278 A1 or in PCT publication No. WO 2016/156291A1.

In a further aspect, the 4-1BB agonist is an anti-FAP/anti-4-1BB bispecific antibody.

30 In another aspect, the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises a heavy chain variable region (V_HCEA) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:49, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:50, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:51,

and a light chain variable region (V_L CEA) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:54.

In a further aspect, the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises a heavy chain variable region (V_H CEA) comprising an amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_L CEA) comprising an amino acid sequence of SEQ ID NO:56.

In another aspect, the 4-1BB agonist is an antigen binding molecule further comprising a Fc domain composed of a first and a second subunit capable of stable association. In one aspect, the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain. Particularly, the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function. In a particular aspect, the 4-1BB agonist is an antigen binding molecule comprising an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

In one aspect, the 4-1BB agonist is an antigen binding molecule comprising (a) at least one antigen binding domain capable of specific binding to CEA, (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

In a particular aspect, the 4-1BB agonist is an antigen binding molecule comprising (a) at least one Fab domain capable of specific binding to CEA, and (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that (i) the first polypeptide contains a CH1 or CL domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two

- ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
- 5 (iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two
- 10 ectodomains of 4-1BBL or fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

- In another aspect, the 4-1BB agonist is an antigen binding molecule comprising
- (a) at least one Fab domain capable of specific binding to CEA comprising a heavy chain
- 15 variable region (V_{HCEA}) comprising the amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_{LCEA}) comprising the amino acid sequence of SEQ ID NO:56, and
- (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID
- 20 NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

- In a particular aspect, the 4-1BB agonist is an antigen binding molecule comprising a
- 25 first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:108 and a second light chain comprising the amino acid sequence of SEQ ID NO:109.

- In another aspect, the 4-1BB agonist is an antigen binding molecule comprising
- 30 (a) at least one antigen binding domain capable of specific binding to CEA,
- (b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers.

- In one aspect, the 4-1BB agonist is an antigen binding molecule comprising
- (a) at least one antigen binding domain capable of specific binding to CEA,
- 35 (b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are

connected to each other by peptide linkers, and

- (c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the polypeptide comprising the three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers is fused to the N- or C-terminal amino acid of one of the two subunits of the Fc domain, optionally through a peptide linker.

In another aspect, the 4-1BB agonist comprises an anti-CEA/anti-4-1BB bispecific antibody.

Agents blocking PD-L1/PD-1 interaction for use in the invention

- In one aspect of the invention, the T-cell activating anti-CD3 bispecific antibodies specific for a tumor-associated antigen, in particular the anti-CEA/anti-CD3 antibodies are for use in a method for treating or delaying progression of cancer, wherein the T-cell activating anti-CD3 bispecific antibodies specific for a tumor-associated antigen are used in combination with a 4-1BB (CD137) agonist and additionally they are combined with an agent blocking PD-L1/PD-1 interaction. In another aspect, the agent blocking PD-L1/PD-1 interaction is only combined with a targeted 4-1BB agonist. In all these aspects, an agent blocking PD-L1/PD-1 interaction is a PD-L1 binding antagonist or a PD-1 binding antagonist. In particular, the agent blocking PD-L1/PD-1 interaction is an anti-PD-L1 antibody or an anti-PD-1 antibody.

- The term “**PD-L1**”, also known as CD274 or B7-H1, refers to any native PD-L1 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), in particular to “human PD-L1”. The amino acid sequence of complete human PD-L1 is shown in UniProt (www.uniprot.org) accession no. Q9NZQ7 (SEQ ID NO:110). The term “**PD-L1 binding antagonist**” refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L1 with either one or more of its binding partners, such as PD-1, B7-1. In some embodiments, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, the PD-L1 binding antagonist inhibits binding of PD-L1 to PD-1 and/or B7-1. In some embodiments, the PD-L1 binding antagonists include anti-PD-L1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L1 with one or more of its binding partners, such as PD-1, B7-1. In one embodiment, a PD-L1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L1 so as to render a dysfunctional T-cell less dysfunctional (e.g., enhancing effector responses to antigen recognition). In particular, a PD-L1 binding antagonist is an anti-PD-L1 antibody. The term “**anti-PD-L1 antibody**” or “antibody binding to human PD-L1” or

"antibody that specifically binds to human PD-L1" or "antagonistic anti-PD-L1" refers to an antibody specifically binding to the human PD-L1 antigen with a binding affinity of KD-value of 1.0×10^{-8} mol/l or lower, in one aspect of a KD-value of 1.0×10^{-9} mol/l or lower. The binding affinity is determined with a standard binding assay, such as surface plasmon resonance technique (BIAcore®, GE-Healthcare Uppsala, Sweden).

In a particular aspect, the agent blocking PD-L1/PD-1 interaction is an anti-PD-L1 antibody. In a specific aspect, the anti-PD-L1 antibody is selected from the group consisting of atezolizumab (MPDL3280A, RG7446), durvalumab (MEDI4736), avelumab (MSB0010718C) and MDX-1105. In a specific aspect, an anti-PD-L1 antibody is YW243.55.S70 described herein. In another specific aspect, an anti-PD-L1 antibody is MDX-1105 described herein. In still another specific aspect, an anti-PD-L1 antibody is MEDI4736 (durvalumab). In yet a further aspect, an anti-PD-L1 antibody is MSB0010718C (avelumab). More particularly, the agent blocking PD-L1/PD-1 interaction is atezolizumab (MPDL3280A). In another aspect, the agent blocking PD-L1/PD-1 interaction is an anti-PD-L1 antibody comprising a heavy chain variable domain VH(PDL-1) of SEQ ID NO:112 and a light chain variable domain VL(PDL-1) of SEQ ID NO:113. In another aspect, the agent blocking PD-L1/PD-1 interaction is an anti-PD-L1 antibody comprising a heavy chain variable domain VH(PDL-1) of SEQ ID NO:114 and a light chain variable domain VL(PDL-1) of SEQ ID NO:115.

The term "**PD-1**", also known as CD279, PD1 or programmed cell death protein 1, refers to any native PD-L1 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), in particular to the human protein PD-1 with the amino acid sequence as shown in UniProt (www.uniprot.org) accession no. Q15116 (SEQ ID NO:111). The term "**PD-1 binding antagonist**" refers to a molecule that inhibits the binding of PD-1 to its ligand binding partners. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2. In particular, a PD-L1 binding antagonist is an anti-PD-L1 antibody. The term "**anti-PD-1 antibody**" or "antibody binding to human PD-1" or "antibody that specifically binds to human PD-1" or "antagonistic anti-PD-1" refers to an antibody specifically binding to the human PD1 antigen with a binding affinity of KD-value of 1.0×10^{-8} mol/l or lower, in one aspect of a KD-value of 1.0×10^{-9} mol/l or lower. The binding affinity is determined with a standard binding assay, such as surface plasmon resonance technique (BIAcore®, GE-Healthcare Uppsala, Sweden).

In one aspect, the agent blocking PD-L1/PD-1 interaction is an anti-PD-1 antibody. In a specific aspect, the anti-PD-1 antibody is selected from the group consisting of MDX 1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, and BGB-108, in particular from pembrolizumab and nivolumab. In
5 another aspect, the agent blocking PD-L1/PD-1 interaction is an anti-PD-1 antibody comprising a heavy chain variable domain VH(PD-1) of SEQ ID NO:116 and a light chain variable domain VL(PD-1) of SEQ ID NO:117. In another aspect, the agent blocking PD-L1/PD-1 interaction is an anti-PD-1 antibody comprising a heavy chain variable domain VH(PD-1) of SEQ ID NO:118 and a light chain variable domain VL(PD-1) of SEQ ID
10 NO:119.

Exemplary 4-1BB agonists for use in combination with agents blocking PD-L1/PD-1 interaction

The present invention also relates to 4-1BB agonists comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method
15 for treating or delaying progression of cancer, wherein the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is used in combination with an agent blocking PD-L1/PD-1 interaction. In one aspect, the 4-1BB agonist is for use in a method for treating or delaying progression of cancer, wherein the agent blocking PD-L1/PD-1 interaction is an anti-PD-L1 antibody or an anti-PD1 antibody, in
20 particular the agent blocking PD-L1/PD-1 interaction is selected from the group consisting of atezolizumab, durvalumab, pembrolizumab and nivolumab. More particularly, the the agent blocking PD-L1/PD-1 interaction is atezolizumab.

The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in in combination with an agent blocking PD-
25 L1/PD-1 interaction is particularly one, wherein the tumor-associated antigen is selected from Fibroblast activation protein (FAP) or CEA. More particularly, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to Fibroblast activation
30 protein (FAP).

In one aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method for treating or delaying progression of cancer in combination with an agent blocking PD-L1/PD-1
interaction is an antigen binding molecule comprising three ectodomains of 4-1BBL or
35 fragments thereof and at least one antigen binding domain capable of specific binding to FAP, 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: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, or
- (b) a heavy chain variable region (V_HFAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:15, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:16, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:17, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:18, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:19, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:20.

In a particular aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method for treating or delaying progression of cancer in combination with an agent blocking PD-L1/PD-1 interaction is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP, wherein 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:21 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:22 or wherein 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:23 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:24.

In another aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in the method is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain. In a further aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in the method is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

In a further aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen a method for treating or delaying progression of cancer in combination with an agent blocking PD-L1/PD-1 interaction is an antigen binding molecule comprising

(a) at least one antigen binding domain capable of specific binding to FAP,

(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that

are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

In another aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method for treating or
5 delaying progression of cancer in combination with an agent blocking PD-L1/PD-1 interaction is an antigen binding molecule comprising

(a) at least one Fab domain capable of specific binding to FAP, and
(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that

- 10 (i) the first polypeptide contains a CH1 or CL domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide
15 linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or
- (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two
20 ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
- (iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second
25 polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to
30 VH or VL by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

In a particular aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in the method an antigen binding molecule comprising

- 35 (a) at least one Fab domain capable of specific binding to FAP comprising a heavy chain variable region ($V_{H\text{FAP}}$) comprising the amino acid sequence of SEQ ID NO:21 and a light chain variable region ($V_{L\text{FAP}}$) comprising the amino acid sequence of SEQ ID NO:22 or a

heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:23 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:24, and

- (b) a first and a second polypeptide that are linked to each other by a disulfide bond,
5 wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID
10 NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

In a specific aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in the method is an antigen binding molecule selected from the group consisting of

- a) a molecule comprising a first heavy chain comprising the amino acid sequence of
15 SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:67 and a second light chain comprising the amino acid sequence of SEQ ID NO:68; and
b) a molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:69, a first light chain comprising the amino acid sequence of SEQ ID NO:70, a
20 second heavy chain comprising the amino acid sequence of SEQ ID NO:71 and a second light chain comprising the amino acid sequence of SEQ ID NO:72.

In another aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method as described herein before is an antigen binding molecule comprising

- 25 (a) at least one antigen binding domain capable of specific binding to FAP,
(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers.

In a further aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in the method is an antigen
30 binding molecule comprising

- (a) at least one antigen binding domain capable of specific binding to FAP,
(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers, and
(c) a Fc domain composed of a first and a second subunit capable of stable association,
35 wherein the polypeptide comprising the three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers is fused to the N- or C-terminal amino acid of one of the two subunits of the Fc domain, optionally through a peptide linker.

In yet another aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method for treating or delaying progression of cancer in combination with an agent blocking PD-L1/PD-1 interaction is an anti-FAP/anti-4-1BB bispecific antibody.

5 In a further aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in the method is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA.

10 In particular the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises (a) a heavy chain variable region (V_H CEA) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:49, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:50, and (iii) CDR-H3 comprising the amino acid sequence of
15 SEQ ID NO:51, and a light chain variable region (V_L CEA) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:54. More particularly, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in the method is an
20 antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises a heavy chain variable region (V_H CEA) comprising an amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_L CEA) comprising an amino acid sequence of SEQ ID NO:56. In one aspect, the 4-
25 1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain. In one aspect, the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

30 In a further aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in the method described herein before is an antigen binding molecule comprising
(a) at least one antigen binding domain capable of specific binding to CEA,
(b) a first and a second polypeptide that are linked to each other by a disulfide bond,
wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that
35 are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

In a particular aspect, the 4-1BB agonist is an antigen binding molecule comprising
 (a) at least one Fab domain capable of specific binding to CEA, and
 (b) a first and a second polypeptide that are linked to each other by a disulfide bond,
 wherein the antigen binding molecule is characterized in that

- 5 (i) the first polypeptide contains a CH1 or CL domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide
 10 linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or
- (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two
 15 ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
- (iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second
 20 polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one
 25 ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

More particularly, the 4-1BB agonist for use in the method is an antigen binding molecule comprising

- (a) at least one Fab domain capable of specific binding to CEA comprising a heavy chain
 30 variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:56, and
- (b) a first and a second polypeptide that are linked to each other by a disulfide bond,
 wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID
 35 NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8. In a specific aspect,

the 4-1BB agonist is an antigen binding molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:108 and a second light chain comprising the amino acid sequence of SEQ ID NO:109.

In another aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in the method is an antigen binding molecule comprising (a) at least one antigen binding domain capable of specific binding to CEA, and (b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers. In a further aspect, the 4-1BB agonist for use in the method is an antigen binding molecule comprising (a) at least one antigen binding domain capable of specific binding to CEA, (b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers, and (c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the polypeptide comprising the three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers is fused to the N- or C-terminal amino acid of one of the two subunits of the Fc domain, optionally through a peptide linker.

In yet another aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in the method is an anti-CEA/anti-4-1BB bispecific antibody.

Preparation of bispecific antibodies for use in the invention

In certain aspects, the therapeutic agents used in the combination comprise multispecific antibodies, e.g. bispecific antibodies. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain aspects, the binding specificities are for different antigens. In certain aspects, the binding specificities are for different epitopes on the same antigen. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, Nature 305: 537 (1983)), WO 93/08829, and Traunecker et al., EMBO J. 10: 3655 (1991)), and “knob-in-hole” engineering (see, e.g., U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking of two or more antibodies or fragments (see, e.g., US Patent

No. 4,676,980, and Brennan et al., Science, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., J. Immunol., 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993)); and using single-chain Fv (scFv) dimers
5 (see, e.g., Gruber et al., J. Immunol., 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. J. Immunol. 147: 60 (1991).

Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

The antibodies or fragmentsa herein also include a "Dual Acting FAb" or "DAF"
10 comprising an antigen binding site that binds to two different antigens (see, US 2008/0069820, for example). "Crossmab" antibodies are also included herein (see e.g. WO 2009/080251, WO 2009/080252, WO2009/080253, or WO2009/080254).

Another technique for making bispecific antibody fragments is the "bispecific T cell engager" or BiTE® approach (see, e.g., WO2004/106381, WO2005/061547,
15 WO2007/042261, and WO2008/119567). This approach utilizes two antibody variable domains arranged on a single polypeptide. For example, a single polypeptide chain includes two single chain Fv (scFv) fragments, each having a variable heavy chain (VH) and a variable light chain (VL) domain separated by a polypeptide linker of a length sufficient to allow intramolecular association between the two domains. This single polypeptide further includes
20 a polypeptide spacer sequence between the two scFv fragments. Each scFv recognizes a different epitope, and these epitopes may be specific for different cell types, such that cells of two different cell types are brought into close proximity or tethered when each scFv is engaged with its cognate epitope. One particular embodiment of this approach includes a scFv recognizing a cell-surface antigen expressed by an immune cell, e.g., a CD3 polypeptide on a
25 T cell, linked to another scFv that recognizes a cell-surface antigen expressed by a target cell, such as a malignant or tumor cell.

As it is a single polypeptide, the bispecific T cell engager may be expressed using any prokaryotic or eukaryotic cell expression system known in the art, e.g., a CHO cell line. However, specific purification techniques (see, e.g., EP1691833) may be necessary to
30 separate monomeric bispecific T cell engagers from other multimeric species, which may have biological activities other than the intended activity of the monomer. In one exemplary purification scheme, a solution containing secreted polypeptides is first subjected to a metal affinity chromatography, and polypeptides are eluted with a gradient of imidazole concentrations. This eluate is further purified using anion exchange chromatography, and
35 polypeptides are eluted using with a gradient of sodium chloride concentrations. Finally, this

eluate is subjected to size exclusion chromatography to separate monomers from multimeric species. In one aspect, the bispecific bispecific antibodies used in the invention are composed of a single polypeptide chain comprising two single chain FV fragments (scFV) fused to each other by a peptide linker.

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

The Fc domain of the antigen binding molecules of the invention consists of a pair of polypeptide chains comprising 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
10 the Fc domain are capable of stable association with each other.

The Fc domain confers favorable pharmacokinetic properties to the antigen binding molecules 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
15 invention to cells expressing Fc receptors rather than to the preferred antigen-bearing cells. Accordingly, in particular aspects, the Fc domain of the antigen binding molecules 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
20 is an Fcγ receptor. In one aspect, the Fc receptor is a human Fc receptor. In a specific aspect, the Fc receptor is an activating human Fcγ receptor, more specifically human FcγRIIIa, FcγRI 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-
25 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
30 cell priming.

In certain aspects, one or more amino acid modifications may be introduced into the Fc region of an antibody 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
35 acid positions.

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

In one aspect, the Fc domain of the antibody 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 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 an antibody according to the invention which comprises an Fc domain with the amino acid substitutions L234A, L235A and P329G ("P329G LALA", EU numbering) 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 Fc γ receptor binding of a human IgG1 Fc domain and is described in International Patent Appl. Publ. No. WO 2012/130831 A1 which also describes methods of preparing such mutant Fc domains and methods for determining its properties such as Fc receptor binding or effector functions.

Fc domains with reduced Fc receptor binding and/or effector function also include those 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 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 substitutions L235E and S228P and P329G (EU numbering). Such IgG4 Fc domain mutants and their Fc γ 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.

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.

Alternatively, binding affinity of Fc domains or cell activating antibodies 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 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 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, ACTITM non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA); and CytoTox 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 aspects, 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)).

Fc domain modifications promoting heterodimerization

The bispecific antigen binding molecules of the invention comprise different antigen-binding sites, fused to one or the other of the two subunits of the Fc domain, thus the two subunits of the Fc domain may be comprised in two non-identical polypeptide chains. Recombinant co-expression of these polypeptides and subsequent dimerization leads to several possible combinations of the two polypeptides. To improve the yield and purity of the bispecific antibodies of the invention in recombinant production, it will thus be advantageous

to introduce in the Fc domain of the bispecific antigen binding molecules of the invention a modification promoting the association of the desired polypeptides.

Accordingly, in particular aspects the invention relates to the bispecific antigen binding molecule comprising (a) at least one antigen binding domain capable of specific binding to a
5 tumor-associated antigen, (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises two ectodomains of 4-1BBL or two fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises only one ectodomain of said 4-1BBL or a fragment thereof, and (c) a Fc domain composed of a
10 first and a second subunit capable of stable association, wherein the Fc domain comprises a modification promoting the association of the first and second subunit of the Fc domain. 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, in one aspect said modification is in the CH3 domain of the Fc domain.

15 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, the invention relates to an antigen binding molecule comprising (a) at least one antigen binding domain capable of specific binding to a tumor-associated antigen, (b) a first and a second polypeptide that are
20 linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises two ectodomains of 4-1BBL or two fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises only one ectodomain of 4-1BBL or a fragment thereof, and (c) a Fc domain composed of a first and a second subunit capable
25 of stable association, wherein the first subunit of the Fc domain comprises knobs and the second subunit of the Fc domain comprises holes according to the knobs into holes method. In a particular aspect, the first subunit of the Fc domain comprises the amino acid substitutions S354C and T366W (EU numbering) and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S and Y407V (numbering according to Kabat EU
30 index).

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
35 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 in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine).

5 Accordingly, in one 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
10 of the Fc domain an 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. 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
15 aspect, in the CH3 domain of the first subunit of the Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and 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). In one aspect, in the second subunit of the Fc domain additionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine
20 residue at position 368 is replaced with an alanine residue (L368A).

In yet a further aspect, in the first subunit of the Fc domain additionally the serine residue 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). Introduction of these two cysteine residues results in formation of a
25 disulfide bridge between the two subunits of the Fc domain, further stabilizing the dimer (Carter (2001), J Immunol Methods 248, 7-15). In a particular aspect, the first subunit of the Fc domain comprises the amino acid substitutions S354C and T366W (EU numbering) and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S and Y407V (numbering according to Kabat EU index).

30 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 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
35 electrostatically unfavorable but heterodimerization electrostatically favorable.

The C-terminus of the heavy chain of the bispecific antibody as reported herein can be a complete C-terminus ending with the amino acid residues PGK. The C-terminus of the heavy chain can be a shortened C-terminus in which one or two of the C terminal amino acid residues have been removed. In one preferred aspect, the C-terminus of the heavy chain is a shortened C-terminus ending PG. In one aspect of all aspects as reported herein, a bispecific antibody comprising a heavy chain including a C-terminal CH3 domain as specified herein, comprises the C-terminal glycine-lysine dipeptide (G446 and K447, numbering according to Kabat EU index). In one embodiment of all aspects as reported herein, a bispecific antibody comprising a heavy chain including a C-terminal CH3 domain, as specified herein, comprises a C-terminal glycine residue (G446, numbering according to Kabat EU index).

Modifications in the Fab domains

In one aspect, the invention relates to a 4-1BBL-containing antigen binding molecule, comprising (a) a Fab fragment capable of specific binding to a tumor-associated antigen, (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises two ectodomains of 4-1BBL or two fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises only one ectodomain of 4-1BBL or a fragment thereof, and (c) a Fc domain composed of a first and a second subunit capable of stable association, wherein in one of 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 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 antigen binding molecule comprising (a) a first Fab fragment capable of specific binding to a tumor associated antigen, (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises two ectodomains of 4-1BBL or two fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises only one ectodomain of 4-1BBL or a fragment thereof, and wherein each of them is linked to a CH1 or CL domain, and (c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the

constant domains CL and CH1 adjacent to 4-1BBL are replaced by each other so that the CH1 domain is part of the light chain and the CL domain is part of the heavy chain.

In another aspect, the invention relates to a bispecific antigen binding molecule, comprising (a) two light chains and two heavy chains of an antibody comprising two Fab
5 fragments capable of specific binding to 4-1BB and the Fc domain, and (b) two additional Fab fragments capable of specific binding to a tumor associated antigen, wherein said additional Fab fragments are each connected via a peptide linker to the C-terminus of the heavy chains of (a). In a particular aspect, the additional Fab fragments are Fab fragments, wherein the variable domains VL and VH are replaced by each other so that the VH domain is part of the
10 light chain and the VL domain is part of the heavy chain.

In another aspect, and to further improve correct pairing, the bispecific antigen binding molecule comprising (a) a first Fab fragment capable of specific binding to a tumor associated antigen, (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises
15 two ectodomains of 4-1BBL or two fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises only one ectodomain of 4-1BBL or a fragment thereof, and wherein each of them is linked to a CH1 or CL domain, and (c) a Fc domain composed of a first and a second subunit capable of stable association, can contain different charged amino acid substitutions (so-called "charged residues"). These
20 modifications are introduced in the crossed or non-crossed CH1 and CL domains. In a particular aspect, the invention relates to a bispecific antigen binding molecule, wherein in one of CL domains 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 one of the CH1 domains the amino acids at position 147 (EU
25 numbering) and at position 213 (EU numbering) have been substituted by glutamic acid (E).

More particularly, the invention relates to a bispecific binding molecule comprising a Fab, wherein in the CL domain adjacent to 4-1BBL 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 adjacent to 4-
30 1BBL the amino acids at position 147 (EU numbering) and at position 213 (EU numbering) have been substituted by glutamic acid (E).

Polynucleotides

The invention further provides isolated polynucleotides encoding an antibody as described herein or a fragment thereof.

The isolated polynucleotides encoding the antibodies of the invention may be expressed 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. For example, the light chain portion of an immunoglobulin may be encoded by a separate polynucleotide from the heavy chain portion of the immunoglobulin. When co-expressed, the heavy chain polypeptides will associate with the light chain polypeptides to form the immunoglobulin.

In some aspects, the isolated polynucleotide encodes the entire antibody according to the invention as described herein. In other embodiments, the isolated polynucleotide encodes a polypeptide comprised in the antibody according to the invention as described herein.

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 (mRNA). RNA of the present invention may be single stranded or double stranded.

Recombinant Methods

Bispecific antibodies as used in 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 antibody or polypeptide fragments thereof, e.g., as described above, is isolated and inserted into one or 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 antibody (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 antibody 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 antibody 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 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 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 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 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 antibody or polypeptide fragments thereof is desired, DNA encoding a signal sequence may be placed upstream of the nucleic acid an antibody 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 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 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 an antibody of the invention or polypeptide fragments thereof.

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) a vector comprising a polynucleotide that encodes (part of) an antibody of the invention 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 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), insect cells, or the like. For example, polypeptides may be produced in bacteria in 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 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 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 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., *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, 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, 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 immunoglobulin, may be engineered so as to also express the other of the immunoglobulin chains such that the expressed product is an immunoglobulin that has both a heavy and a light chain.

In one aspect, a method of producing an antibody of the invention or polypeptide fragments thereof is provided, wherein the method comprises culturing a host cell comprising polynucleotides encoding the antibody of the invention or polypeptide fragments thereof, as provided herein, under conditions suitable for expression of the antibody of the invention or polypeptide fragments thereof, and recovering the antibody of the invention or polypeptide fragments thereof from the host cell (or host cell culture medium).

In certain embodiments the moieties capable of specific binding to a target cell antigen (e.g. Fab fragments) forming part of the antigen binding molecule comprise at least an immunoglobulin variable region capable of binding to an antigen. 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, 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).

Any animal species of immunoglobulin can be used in the invention. Non-limiting immunoglobulins useful in the present invention can be of murine, primate, or human origin. If the fusion protein is intended for human use, a chimeric form of immunoglobulin may be used wherein the constant regions of the immunoglobulin are from a human. A humanized or fully human form of the immunoglobulin can also be prepared in accordance with methods well known in the art (see e. g. U.S. Patent No. 5,565,332 to Winter). Humanization may be achieved by various methods including, but not limited to (a) grafting the non-human (e.g., donor antibody) CDRs onto human (e.g. recipient antibody) framework and constant regions

with or without retention of critical framework residues (e.g. those that are important for retaining good antigen binding affinity or antibody functions), (b) grafting only the non-human specificity-determining regions (SDRs or a-CDRs; the residues critical for the antibody-antigen interaction) onto human framework and constant regions, or (c)

5 transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues. Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front Biosci* 13, 1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332, 323-329 (1988); Queen et al., *Proc Natl Acad Sci USA* 86, 10029-10033 (1989); US Patent Nos. 5,821,337, 7,527,791, 10 6,982,321, and 7,087,409; Jones et al., *Nature* 321, 522-525 (1986); Morrison et al., *Proc Natl Acad Sci* 81, 6851-6855 (1984); Morrison and Oi, *Adv Immunol* 44, 65-92 (1988); Verhoeven et al., *Science* 239, 1534-1536 (1988); Padlan, *Molec Immun* 31(3), 169-217 (1994); Kashmiri et al., *Methods* 36, 25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol Immunol* 28, 489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36, 15 43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36, 61-68 (2005) and Klimka et al., *Br J Cancer* 83, 252-260 (2000) (describing the "guided selection" approach to FR shuffling). Particular immunoglobulins according to the invention are human immunoglobulins. Human antibodies and human variable regions can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr Opin Pharmacol* 5, 368-74 (2001) and Lonberg, *Curr Opin Immunol* 20, 450-459 (2008). Human variable regions can form part of and be derived from human monoclonal antibodies made by the hybridoma method (see e.g. *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)). Human antibodies and human variable regions may also be prepared by administering an 25 immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge (see e.g. Lonberg, *Nat Biotech* 23, 1117-1125 (2005)). Human antibodies and human variable regions may also be generated by isolating Fv clone variable region sequences selected from human-derived phage display libraries (see e.g., Hoogenboom et al. in *Methods in Molecular Biology* 30 178, 1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001); and McCafferty et al., *Nature* 348, 552-554; Clackson et al., *Nature* 352, 624-628 (1991)). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments.

In certain aspects, the antibodies are engineered to have enhanced binding affinity according to, for example, the methods disclosed in PCT publication WO 2012/020006 (see 35 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 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 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 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, 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 Harbor Laboratory, Cold Spring Harbor, NY).

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 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 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 4-1BBL-containing 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.

Assays

The 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.

5 1. Affinity assays

The affinity of the bispecific antigen binding molecules provided herein for the corresponding receptor 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
10 by recombinant expression. The affinity of the bispecific antigen binding molecule for the target cell antigen can also be determined 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. For the FAP-4-1BBL antigen binding molecules the methods have been described in more detail in International
15 Patent Appl. Publ. No. WO 2016/075278 A1. According to one aspect, K_D is measured by surface plasmon resonance using a BIACORE® T100 machine (GE Healthcare) at 25 °C.

2. Binding assays and other assays

In one aspect, the FAP-4-1BBL antigen binding molecules as reported herein are tested for its antigen binding activity as described in more detail in International Patent Appl. Publ.
20 No. WO 2016/075278 A1.

3. Activity assays

In one aspect, assays are provided for identifying the biological activity of FAP-4-1BBL antigen binding molecules.

In certain embodiments, an antibody as reported herein is tested for such biological
25 activity in the *in vitro* co-culture assays with human immune effector cells as described in Example 8, in the *in vitro* co-culture assays with MKN45 cells expressing CEA and PDL-1 as described in Example 9 or in the *in vitro* PBMC activation assay as described in Example 10.

Pharmaceutical Compositions, Formulations and Routes of Administration

In a further aspect, the invention provides pharmaceutical compositions comprising the
30 T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, and 4-1BB agonists provided herein,

e.g., for use in any of the below therapeutic methods. In one embodiment, a pharmaceutical composition comprises an antibody provided herein and at least one pharmaceutically acceptable excipient. In another embodiment, a pharmaceutical composition comprises an antibody provided herein and at least one additional therapeutic agent, e.g., as described
5 below.

In another aspect, provided are pharmaceutical compositions comprising the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, and 4-1BB agonists provided herein

In yet another aspect, the invention provides a T-cell activating anti-CD3 bispecific
10 antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is used in combination with a 4-1BB (CD137) agonist and in combination with an agent blocking PD-L1/PD-1 interaction. In particular, the agent blocking PD-L1/PD-1
15 interaction is an anti-PD-L1 antibody or an anti-PD1 antibody. More particularly, the agent blocking PD-L1/PD-1 interaction is selected from the group consisting of atezolizumab, durvalumab, pembrolizumab and nivolumab. In a specific aspect, the agent blocking PD-L1/PD-1 interaction is atezolizumab.

Pharmaceutical compositions of the present invention comprise a therapeutically
20 effective amount of one or more 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 when administered to an animal, such as, for example, a human, as appropriate. The
25 preparation of a pharmaceutical composition that contains at least one 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 formulations or aqueous solutions. As used herein, "pharmaceutically acceptable
30 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. subcutaneous, intradermal, intralesional, intravenous, intraarterial intramuscular, intrathecal
35 or intraperitoneal injection. For injection, the antigen binding molecules 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 formulatary agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the fusion proteins 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 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 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 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 (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 dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; 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. 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 or gelatin-microcapsules and poly-(methacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles 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 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 antigen binding molecules 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 antigen binding molecules 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 antibodies of the invention 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.

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.

Administration of the T-cell activating anti-CD3 bispecific antibody and the 4-1BB agonist

Both the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody and the 4-1BB agonist (both called substance herein) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. The methods of the present invention are particularly useful, however, in relation to therapeutic agents administered by parenteral, particularly intravenous, infusion.

Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein. In one embodiment, the therapeutic agent is administered parenterally, particularly intravenously. In a particular embodiment, the therapeutic agent is administered by intravenous infusion.

Both the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody, and the 4-1BB agonist would be formulated, 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. Both the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody, and the 4-1BB agonist need not be, but are optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of therapeutic agent present in the formulation, the type of disorder or treatment, and other factors discussed above. These 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.

For the prevention or treatment of disease, the appropriate dosage of the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody and the 4-1BB agonist (when used in their combination or with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of 4-1BB agent, the severity and course of the disease, whether both agents are administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the therapeutic agent, and the discretion of the attending physician. Each substance 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 $\mu\text{g/kg}$ to 15 mg/kg (e.g. 0.1 mg/kg – 10 mg/kg) of the substance can be an initial candidate dosage for administration to the subject, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 $\mu\text{g/kg}$ 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 desired suppression of disease symptoms occurs. One exemplary dosage of each substance would be in the range from about 0.05 mg/kg to about 10 mg/kg . Thus, one or more doses of about 0.5 mg/kg , 2.0 mg/kg , 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the subject. Such doses may be administered intermittently, e.g. every week, every two weeks, or every three weeks (e.g. such that the subject receives from about two to about twenty, or e.g. about six doses of the therapeutic agent). An initial higher loading dose, followed by one or more lower doses, or an initial lower dose, followed by one or more higher doses may be administered. An exemplary dosing regimen comprises administering an initial

dose of about 10 mg, followed by a bi-weekly dose of about 20 mg of the therapeutic agent. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

In one aspect, the administration of both the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody and the 4-1BB agonist is a single administration. In certain aspects, the administration of the therapeutic agent is two or more administrations. In one such aspect, the substances are administered every week, every two weeks, or every three weeks, particularly every two weeks. In one aspect, the substance is administered in a therapeutically effective amount. In one aspect the substance is administered at a dose of about 50 µg/kg, about 100 µg/kg, about 200 µg/kg, about 300 µg/kg, about 400 µg/kg, about 500 µg/kg, about 600 µg/kg, about 700 µg/kg, about 800 µg/kg, about 900 µg/kg or about 1000 µg/kg. In one embodiment, the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody is administered at a dose which is higher than the dose of the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody, in a corresponding treatment regimen without the administration of the 4-1BB agonist. In one aspect the administration of the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody comprises an initial administration of a first dose of the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody, and one or more subsequent administrations of a second dose of the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody, wherein the second dose is higher than the first dose. In one aspect, the administration of the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody comprises an initial administration of a first dose of the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody, and one or more subsequent administrations of a second dose of the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody, wherein the first dose is not lower than the second dose.

In one aspect, the administration of the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody in the treatment regimen according to the invention is the first administration of said T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody to the subject (at least within the same course of treatment). In one aspect, no administration of the 4-1BB agonist is made to the subject prior to the administration of the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody. In another aspect, the 4-1BB agonist is administered prior to the administration of the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody.

In another aspect, the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody is for use in combination with a 4-1BB agonist, wherein a pretreatment with an Type II anti-CD20 antibody, preferably obinutuzumab, is performed prior to the combination treatment, wherein the period of time between the
5 pretreatment and the combination treatment is sufficient for the reduction of B-cells in the individual in response to the Type II anti-CD20 antibody, preferably obinutuzumab.

Activation of T cells can lead to severe cytokine release syndrome (CRS). In a phase 1 study conducted by TeGenero (Suntharalingam et al., N Engl J Med (2006) 355,1018-1028), all 6 healthy volunteers experienced near fatal, severe cytokine release syndrome (CRS)
10 rapidly post-infusion of an inappropriately-dosed, T-cell stimulating super-agonist anti-CD28 monoclonal antibody. The cytokine release associated with administration of a T-cell activating therapeutic agent, such as the anti-CEA/anti-CD3 bispecific antibody, to a subject can be significantly reduced by pre-treatment of said subject with a Type II anti-CD20 antibody, such as obinutuzumab. the use of GAZYVA® pre-treatment (Gpt) should aid in the
15 rapid depletion of B cells, both in the peripheral blood and in secondary lymphoid organs, such that the risk of highly relevant adverse events (AEs) from strong systemic T cell activation by T-cell activating therapeutic agents (e.g. CRS) is reduced, while supporting exposure levels of T-cell activating therapeutic agents that are high enough from the start of dosing to mediate tumour cell elimination. To date, the safety profile of obinutuzumab
20 (including cytokine release) has been assessed and managed in hundreds of patients in ongoing obinutuzumab clinical trials. Finally, in addition to supporting the safety profile of T-cell activating anti-CD3 bispecific antibodies such as the anti-CEA/anti-CD3 bispecific antibody, Gpt should also help prevent the formation of anti-drug antibodies (ADAs) to these unique molecules.

25 In the present invention, the combination of the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody and the 4-1BB agonist can be used in combination with further agents in a therapy. For instance, at least one additional therapeutic agent may be co-administered. In certain aspects, an additional therapeutic agent is an immunotherapeutic agent.

30 In one aspect, the combination of the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody, and the 4-1BB agonist can be used in combination with a PD-1 axis binding antagonist. In one aspect, the PD-1 axis binding antagonist is selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist. In a particular aspect, PD-1 axis binding
35 antagonist is a PD-1 binding antagonist. In one aspect, the PD-1 axis binding antagonist is selected MDX 1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-

0680 (AMP-514), PDR001, REGN2810, and BGB-108. In another particular aspect, the PD-1 axis binding antagonist is a PD-L1 binding antagonist. In one aspect, the PD-1 axis binding antagonist is selected from MPDL3280A (atezolizumab), YW243.55.S70, MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab). More particularly, the
5 combination of the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody, and the 4-1BB agonist can be used in combination with MPDL3280A (atezolizumab).

The period of time between the administration of the PD-1 axis binding antagonist and the administration of the combination therapy comprising T-cell activating anti-CD3
10 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody, and the 4-1BB agonist and the doses are chosen such as to effectively shrink the tumor in the subject prior to administration of the combination therapy.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and
15 separate administration, in which case, administration of the therapeutic agent can occur prior to, simultaneously, and/or following, administration of an additional therapeutic agent or agents. In one embodiment, administration of the therapeutic agent and administration of an additional therapeutic agent occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other.

20 **Administration of the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen and the agent blocking PD-L1/PD-1 interaction**

Both the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen and the agent blocking PD-L1/PD-1 interaction
25 (both called substance herein) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. The methods of the present invention are particularly useful, however, in relation to therapeutic agents administered by parenteral, particularly intravenous, infusion.

Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or
30 subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein. In one embodiment, the therapeutic agent is administered parenterally,

particularly intravenously. In a particular embodiment, the therapeutic agent is administered by intravenous infusion.

Both the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen and the agent blocking PD-L1/PD-1 interaction would be formulated, 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. Both the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen and the agent blocking PD-L1/PD-1 interaction need not be, but are optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of therapeutic agent present in the formulation, the type of disorder or treatment, and other factors discussed above. These 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.

For the prevention or treatment of disease, the appropriate dosage of the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen and the agent blocking PD-L1/PD-1 interaction (when used in their combination or with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of 4-1BB agonist, the severity and course of the disease, whether both agents are administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the therapeutic agent, and the discretion of the attending physician. Each substance 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 $\mu\text{g/kg}$ to 15 mg/kg (e.g. 0.1 mg/kg – 10 mg/kg) of the substance can be an initial candidate dosage for administration to the subject, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 $\mu\text{g/kg}$ 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 desired suppression of disease symptoms occurs. One exemplary dosage of each substance would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the subject. Such doses may be administered intermittently, e.g. every week, every two weeks, or every three weeks (e.g. such that the subject receives from about two to about twenty, or e.g. about six doses of the therapeutic agent). An initial

higher loading dose, followed by one or more lower doses, or an initial lower dose, followed by one or more higher doses may be administered. An exemplary dosing regimen comprises administering an initial dose of about 10 mg, followed by a bi-weekly dose of about 20 mg of the therapeutic agent. However, other dosage regimens may be useful. The progress of this
5 therapy is easily monitored by conventional techniques and assays.

In one aspect, the administration of both the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen and the agent blocking PD-L1/PD-1 interaction is a single administration. In certain aspects, the administration of the therapeutic agent is two or more administrations. In one such aspect, the
10 substances are administered every week, every two weeks, or every three weeks, particularly every two weeks. In one aspect, the substance is administered in a therapeutically effective amount. In one aspect the substance is administered at a dose of about 50 $\mu\text{g/kg}$, about 100 $\mu\text{g/kg}$, about 200 $\mu\text{g/kg}$, about 300 $\mu\text{g/kg}$, about 400 $\mu\text{g/kg}$, about 500 $\mu\text{g/kg}$, about 600 $\mu\text{g/kg}$, about 700 $\mu\text{g/kg}$, about 800 $\mu\text{g/kg}$, about 900 $\mu\text{g/kg}$ or about 1000 $\mu\text{g/kg}$. In one embodiment,
15 the agent blocking PD-L1/PD-1 interaction is administered at a dose which is higher than the dose of the agent blocking PD-L1/PD-1 interaction, in a corresponding treatment regimen without the administration of the 4-1BB agonist. In another embodiment, the agent blocking PD-L1/PD-1 interaction is administered at a dose which is lower than the dose of the agent blocking PD-L1/PD-1 interaction, in a corresponding treatment regimen without the
20 administration of the 4-1BB agonist. In one aspect the administration of the agent blocking PD-L1/PD-1 interaction comprises an initial administration of a first dose of the agent blocking PD-L1/PD-1 interaction and one or more subsequent administrations of a second dose of the agent blocking PD-L1/PD-1 interaction wherein the second dose is higher than the first dose. In one aspect, the administration of the agent blocking PD-L1/PD-1 interaction
25 comprises an initial administration of a first dose of the agent blocking PD-L1/PD-1 interaction and one or more subsequent administrations of a second dose of the the agent blocking PD-L1/PD-1 interaction wherein the first dose is not lower than the second dose.

In one aspect, the administration of the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen in the treatment
30 regimen according to the invention is the first administration of said 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen to the subject (at least within the same course of treatment). In one aspect, no administration of the 4-1BB agonist is made to the subject prior to the administration of the agent blocking PD-L1/PD-1 interaction. In another aspect, the 4-1BB agonist is
35 administered prior to the administration of the agent blocking PD-L1/PD-1 interaction.

In another aspect, the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is for use in combination with the agent blocking PD-L1/PD-1 interaction, wherein a pretreatment with an Type II anti-CD20 antibody, preferably obinutuzumab, is performed prior to the combination treatment, wherein
5 the period of time between the pretreatment and the combination treatment is sufficient for the reduction of B-cells in the individual in response to the Type II anti-CD20 antibody, preferably obinutuzumab.

In the present invention, the combination of the T-cell activating anti-CD3 bispecific antibody, in particular the anti-CEA/anti-CD3 bispecific antibody and the 4-1BB agonist can
10 be used in combination with further agents in a therapy. For instance, at least one additional therapeutic agent may be co-administered. In certain aspects, an additional therapeutic agent is an immunotherapeutic agent.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and
15 separate administration, in which case, administration of the therapeutic agent can occur prior to, simultaneously, and/or following, administration of an additional therapeutic agent or agents. In one embodiment, administration of the therapeutic agent and administration of an additional therapeutic agent occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other.

20 **Therapeutic methods and compositions**

Bispecific antibodies recognizing two cell surface proteins on different cell populations hold the promise to redirect cytotoxic immune cells for destruction of pathogenic target cells.

In one aspect, provided is a method for treating or delaying progression of cancer in a subject comprising administering to the subject an effective amount of a T-cell activating anti-
25 CD3 bispecific antibody, in particular a anti-CEA/anti-CD3 bispecific antibody, and a 4-1BB agonist.

In one such aspect, the method further comprises administering to the subject an effective amount of at least one additional therapeutic agent. In further embodiments, herein is provided a method for tumor shrinkage comprising administering to the subject an effective
30 amount of a T-cell activating anti-CD3 bispecific antibody, in particular an anti-CEA/anti-CD3 bispecific antibody, and a 4-1BB agonist. An “individual” or a “subject” according to any of the above aspects is preferably a human.

In further aspects, a composition for use in cancer immunotherapy is provided comprising a T-cell activating anti-CD3 bispecific antibody, in particular a anti-CEA/anti-CD3 bispecific antibody, and a 4-1BB agonist. In certain embodiments, a composition comprising a T-cell activating anti-CD3 bispecific antibody, in particular a anti-CEA/anti-CD3 bispecific antibody, and a 4-1BB agonist for use in a method of cancer immunotherapy is provided.

In a further aspect, herein is provided for the use of a composition comprising a T-cell activating anti-CD3 bispecific antibody, in particular a anti-CEA/anti-CD3 bispecific antibody, and a 4-1BB agonist in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of solid tumors. In a further embodiment, the medicament is for use in a method of tumor shrinkage comprising administering to an individual having a solid tumor an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent. In a further embodiment, the medicament is for treating solid tumors. In some aspects, the individual has CEA positive cancer. In some aspects, CEA positive cancer is colon cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, breast cancer, kidney cancer, esophageal cancer, or prostate cancer. In some aspects, the breast cancer is a breast carcinoma or a breast adenocarcinoma. In some aspects, the breast carcinoma is an invasive ductal carcinoma. In some aspects, the lung cancer is a lung adenocarcinoma. In some embodiments, the colon cancer is a colorectal adenocarcinoma. An "individual" according to any of the above embodiments may be a human.

The combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody as reported herein can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent or agents. In one embodiment, administration of a T-cell activating anti-CD3 bispecific antibody, in particular a anti-CEA/anti-CD3 bispecific antibody, and a 4-1BB agonist and optionally the administration of an additional therapeutic agent occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other.

Both the T-cell activating anti-CD3 bispecific antibody, in particular a anti-CEA/anti-CD3 bispecific antibody, and the 4-1BB agonist as reported herein (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or

subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are
5 contemplated herein.

Both the T-cell activating anti-CD3 bispecific antibody, in particular a anti-CEA/anti-CD3 bispecific antibody, and the 4-1BB agonist as reported herein would be formulated, 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
10 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. The antibodies need not be, but are optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of
15 antibodies present in the formulation, the type of disorder or treatment, and other factors discussed above. These 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.

Articles of Manufacture (Kits)

20 In another aspect of the invention, a kit containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The kit comprises at least one 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 container holds a
25 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 two active agents in the kit are a T-cell activating anti-CD3 bispecific antibody, in particular a anti-CEA/anti-CD3 bispecific antibody, and an
30 4-1BB agonist of the invention.

In a particular aspect, provided is a kit for treating or delaying progression of cancer in a subject, comprising a package comprising (A) a first composition comprising as active ingredient a T-cell activating anti-CD3 bispecific antibody, in particular a anti-CEA/anti-CD3 bispecific antibody, and a pharmaceutically acceptable carrier; (B) a second composition

comprising as active ingredient a 4-1BB agonist and a pharmaceutically acceptable carrier, and (C) instructions for using the compositions in a combination therapy.

The label or package insert indicates how the composition is used for treating the condition of choice and provides the instructions for using the compositions in a combination therapy. Moreover, the kit may comprise (a) a first container with a composition contained therein, wherein the composition comprises a T-cell activating anti-CD3 bispecific antibody, in particular a anti-CEA/anti-CD3 bispecific antibody, of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises an 4-1BB agonist of the invention. In addition, the kit may comprise one or more further containers comprising further active ingredients that can be used in combination. The article of 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 kit 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 further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Table C (Sequences):

SEQ ID NO:	Name	Sequence
1	Human (hu) 4-1BBL (71-254)	REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLG VHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSRSE
2	hu 4-1BBL (85-254)	DLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLG VHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSRSE
3	hu 4-1BBL (80-254)	DPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLG VHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSRSE
4	hu 4-1BBL (52-254)	PWAVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLG

SEQ ID NO:	Name	Sequence
		VHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSRSE
5	Human (hu) 4-1BBL (71-248)	REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGL
6	hu 4-1BBL (85-248)	DLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGL
7	hu 4-1BBL (80-248)	DPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGL
8	hu 4-1BBL (52-248)	PWAVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGL
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(4B9) CDR-H1	SYAMS
16	FAP(4B9) CDR-H2	AIIGSGASTYYADSVKG
17	FAP(4B9) CDR-H3	GWFGGFNY
18	FAP(4B9) CDR-L1	RASQSVTSSYLA
19	FAP(4B9) CDR-L2	VGSRRAT
20	FAP(4B9) CDR-L3	QQGIMLPPT
21	FAP(28H1) VH	EVQLLESGLVQPGGSLRLSCAASGFTFSSHAMSWVRQAPGKGLEWVSAIWASGEQYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKGWLGNFYWGQGTLLTVSS
22	FAP(28H1) VL	EIVLTQSPGTLSPGERATLSCRASQSVRSYLAWEYQQKPGQAPRLLIIGASTRATGIPDRFSGSGTDFITLTISRLEPEDFAVYYCQQGQVIPPTFGQGTKVEIK
23	FAP(4B9) VH	EVQLLESGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIIGSGASTYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCA

SEQ ID NO:	Name	Sequence
		KGWFGGFNYWGQGLTVTVSS
24	FAP(4B9) VL	EIVLTQSPGTLSPGERATLSCRASQSVTSSYL AWYQQKPGQAPRLINVGSRRTATGIPDRFSGSG SGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQ GTKVEIK
25	dimeric hu 4-1BBL (71-254) connected by (G4S) ₂ linker	REGPELSPDDPAGLLDLRQGMFAQLVAQNVLL IDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALH LQPLRSAAGAAALALTVDLPPASSEARNSAFGF QGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGLPSRSEGGGGSGGGG SREGPELSPDDPAGLLDLRQGMFAQLVAQNVLL IDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALH LQPLRSAAGAAALALTVDLPPASSEARNSAFGF QGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGLPSRSE
26	dimeric hu 4-1BBL (85-254) connected by (G4S) ₂ linker	DLRQGMFAQLVAQNVLLIDGPLSWYSDPGLA GVSLTGGLSYKEDTKELVAKAGVYYVFFQLE LRRVVAGEGSGSVSLALHLQPLRSAAGAAALA LTVDLPPASSEARNSAFGFQGRLLHLSAGQRLG VHLHTEARARHAWQLTQGATVLGLFRVTPEIP AGLPSRSEGGGGSGGGGSLDLRQGMFAQLVA QNVLLIDGPLSWYSDPGLAGVSLTGGLSYKED TKELVAKAGVYYVFFQLELRRVVAGEGSGSV SLALHLQPLRSAAGAAALALTVDLPPASSEARN SAFGFQGRLLHLSAGQRLGVHLHTEARARHA WQLTQGATVLGLFRVTPEIPAGLPSRSE
27	dimeric hu 4-1BBL (80-254) connected by (G4S) ₂ linker	DPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYS DPGLAGVSLTGGLSYKEDTKELVAKAGVYY VFFQLELRRVVAGEGSGSVSLALHLQPLRSAA GAAALALTVDLPPASSEARNSAFGFQGRLLHLS AGQRLGVHLHTEARARHAWQLTQGATVLGLF RVTPEIPAGLPSRSEGGGGSGGGGSDPAGLLD LRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGV SLTGGLSYKEDTKELVAKAGVYYVFFQLELR RVVAGEGSGSVSLALHLQPLRSAAGAAALALT VDLPPASSEARNSAFGFQGRLLHLSAGQRLGV HLHTEARARHAWQLTQGATVLGLFRVTPEIPA GLPSRSE
28	dimeric hu 4-1BBL (52-254) connected by (G4S) ₂ linker	PWAVSGARASPGSAAAPRLREGPELSPDDPAGL DLRQGMFAQLVAQNVLLIDGPLSWYSDPGLA GVSLTGGLSYKEDTKELVAKAGVYYVFFQLE LRRVVAGEGSGSVSLALHLQPLRSAAGAAALA LTVDLPPASSEARNSAFGFQGRLLHLSAGQRLG VHLHTEARARHAWQLTQGATVLGLFRVTPEIP AGLPSRSEGGGGSGGGGSPWAVSGARASPGS AASPRLREGPELSPDDPAGLLDLRQGMFAQLV AQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKE DTKELVAKAGVYYVFFQLELRRVVAGEGSGS VSLALHLQPLRSAAGAAALALTVDLPPASSEAR NSAFGFQGRLLHLSAGQRLGVHLHTEARARHA

SEQ ID NO:	Name	Sequence
		WQLTQGATVLGLFRVTPEIPAGLPSRSE
29	dimeric hu 4-1BBL (71-248) connected by (G4S) ₂ linker	REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGLGGGGSGGGGSREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGL
30	dimeric hu 4-1BBL (85-248) connected by (G4S) ₂ linker	LDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGLGGGGSGGGGSLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGL
31	dimeric hu 4-1BBL (80-248) connected by (G4S) ₂ linker	DPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGLGGGGSGGGGSDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGL
32	dimeric hu 4-1BBL (52-248) connected by (G4S) ₂ linker	PWAVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGLGGGGSGGGGSPWAVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGL
33	CD3-HCDR1	TYAMN
34	CD3-HCDR2	RIRSKYNNYATYYADSVKG
35	CD3-HCDR3	HGNFGNSYVSWFAY

SEQ ID NO:	Name	Sequence
36	CD3-LCDR1	GSSTGAVTTSNYAN
37	CD3-LCDR2	GTNKRAP
38	CD3-LCDR3	ALWYSNLWV
39	CD3 VH	EVQLLES G GGLVQP G GSLRLSCAASGFTFSTYA MNWVRQAPGKGLEWVSRIISKYNNYATYYA DSVKGRFTISRDDSKNTLYLQMNSLR AEDTAV YYCVRHGNFGNSYVSWFAYWGQGLVTVSS
40	CD3 VL	QAVVTQEP SLTVSPGGTVTLTCSSTGAVTTSN YANWVQEKPGQA FRGLIGGTNKRAPGTPARFS GSL LGKAALTLSGAQPEDEAEYYCALWYSNL WVFGG GTKLTVL
41	CEA-HCDR1	EFGMN
42	CEA-HCDR2	WINTKTGEATYVEEFKG
43	CEA-HCDR3	WDFAYYVEAMDY
44	CEA-LCDR1	KASAAVGTYYVA
45	CEA-LCDR2	SASYRKR
46	CEA-LCDR3	HQYYTYPLFT
47	CEA VH	<u>QVQLVQSGAEVKKPGASVKV SCKASGYTFTEF</u> <u>GMNWVRQAPGQGLEWMGWINTKTGEATYVE</u> <u>EFGKGRVTITDTSTSTAYMELRSLRSDDTAVY</u> <u>YCARWDFAYYVEAMDYWGQGT TTVTVSS</u>
48	CEA VL	<u>DIQMTQSPSSLSASVGDRVTITCKASAAVGTYY</u> <u>AWYQQKPGKAPKLLIYSASYRKRGVPSRFSGS</u> <u>GSGTDFLTITISLQPEDFATYYCHQYYTYPLFTF</u> <u>GQGTKLEIK</u>
49	CEA-HCDR1 (CEACAM5)	DTYMH
50	CEA-HCDR2 (CEACAM5)	RIDPANGNSKYVPKFQG
51	CEA-HCDR3 (CEACAM5)	FGYYVSDYAMAY
52	CEA-LCDR1 (CEACAM5)	RAGESVDIFGVGFLH
53	CEA-LCDR2 (CEACAM5)	RASNRAT
54	CEA-LCDR3 (CEACAM5)	QQTNEDPYT
55	CEA VH (CEACAM5)	QVQLVQSGAEVKKPGSSVKV SCKASGFNIKDT YMHWVRQAPGQGLEWMGRIDPANGNSKYVP KFQGRVTITADTSTSTAYMELSSLRSEDTAVYY CAPFGYYVSDYAMAYWGQGLVTVSS
56	CEA VL (CEACAM5)	EIVLTQSPATLSLSPGERATLSCRAGESVDIFGV GFLHWYQQKPGQAPRLLIYRASNRATGIPARFS GSGSGTDFLTITISLQPEDFAVYYCQQTNEDPY TFGQGTKLEIK
57	CD3 VH-CL (CEACAM5 TCB)	EVQLLES G GGLVQP G GSLRLSCAASGFTFSTYA MNWVRQAPGKGLEWVSRIISKYNNYATYYA DSVKGRFTISRDDSKNTLYLQMNSLR AEDTAV YYCVRHGNFGNSYVSWFAYWGQGLVTVSSA SVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC
58	humanized CEA VH-CH1(EE)-Fc (hole, P329G LALA)	QVQLVQSGAEVKKPGSSVKV SCKASGFNIKDT YMHWVRQAPGQGLEWMGRIDPANGNSKYVP KFQGRVTITADTSTSTAYMELSSLRSEDTAVYY

SEQ ID NO:	Name	Sequence
	(CEACAM5 TCB)	CAPFGYYVSDYAMAYWGQGLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSC DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS RTPEVTCVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALGAPIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKS RWQQGNVFCFSVMHEALHNHYTQKSLSLSP
59	humanized CEA VH- CH1(EE)-CD3 VL-CH1-Fc (knob, P329G LALA) (CEACAM5 TCB)	QVQLVQSGAEVKKPGSSVKVSCKASGFNIKDT YMHVVRQAPGQGLEWMGRIDPANGNSKYVP KFQGRVTITADTSTSTAYMELSSLRSEDYAVYY CAPFGYYVSDYAMAYWGQGLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSC DGGGGSGGGGSQAVVTQEPSTVSPGGTVTLT CGSSTGAVTTSNYANWVQEKPGQAFRGLIGGT NKRAPGTPARFSGSLLGGKAALTLGAQPEDE AEYYCALWYSNLWVFGGGTKLTVLSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS RTPEVTCVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALGAPIEKTISKAKGQPREPVY TLPPCRDELTKNQVSLWCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDK SRWQQGNVFCFSVMHEALHNHYTQKSLSLSP
60	humanized CEA VL-CL(RK) (CEACAM5 TCB)	EIVLTQSPATLSLSPGERATLSCRAGESVDIFGV GFLHWYQQKPGQAPRLLIYRASNRATGIPARFS GSGSGTDFTLTISSLEPEDFAVYYCQQTNEPDY TFGQGTKLEIKRTVAAPSVFIFPPSDRKLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC
61	Light chain „CEA _{2F1} ” (CEA TCB)	DIQMTQSPSSLSASVGDRTITCKASAAVGTYY <u>AWYQQKPGKAPKLLIYSAS</u> YRKRGVPSRFSGS GSGTDFTLTISSLQPEDFATYYCHQYYTYPLFTF GQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC
62	Light Chain humanized CD3 _{CH2527} (Crossfab, VL- CH1) (CEA TCB)	QAVVTQEPSTVSPGGTVTLT <u>CGSSTGAVTTSN</u> <u>YANWVQEKPGQAFRGLIGGT</u> NKRAPGTPARFS GSLLGGKAALTLGAQPEDEAEYYCALWYSNL <u>WVFGGGTKLTVLSSASTKG</u> PSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV

SEQ ID NO:	Name	Sequence
		NHKPSNTKVDKKVEPKSC
63	CEA _{CH1A1A 98/99} — humanized CD3 _{CH2527} (Crossfab VH-Ck)—Fc(knob) P329GLALA (CEA TCB)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEF <u>GMNWVRQAPGQGLEWMGWINTKTGEATYVE</u> <u>EFKGRVTFTTDTSTSTAYMELRSLRSDDTAVY</u> <u>YCARWDFAYYVEAMDYWGQGT</u><u>TVTVSSAST</u> KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTPSSSLGTQTYICNVNHKPSNTKVDKKVEPK SCDGGGGSGGGGSEVQLLESGGGLVQPGGSLR LSCAASGFTFSTYAMNWVRQAPGKGLEWVSRI <u>RSKYNNYATYYADSVKGRFTISRDDSKNTLYL</u> <u>QMNSLRAEDTAVYYCVRHGNFGNSYVSWFAY</u> <u>WGQGT</u><u>LVTVSSASVAAPSVFIFPPSDEQLKSGT</u> ASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKSTYSLSSITLTLTKADYEKHKVYA CEVTHQGLSSPVTKSFNRGECDKTHTCPPCPAP EAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LGAPIEKTISKAKGQPREPQVYTLPPCRDELTK NQVSLWCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
64	CEA _{CH1A1A 98/99} (VH-CH1)—Fc(hole) P329GLALA (CEA TCB)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEF GMNWVRQAPGQGLEWMGWINTKTGEATYVE EFKGRVTFTTDTSTSTAYMELRSLRSDDTAVY YCARWDFAYYVEAMDYWGQGTTVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTPSSSLGTQTYICNVNHKPSNTKVDKKVEPK SCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALGAPIEKTISKAKGQPREPQ VCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK
65	Dimeric hu 4-1BBL (71-248) – CL* Fc knob chain (Construct 2.4 and 5.4)	REGPELSPDDPAGLLDLRQGMFAQLVAQNVLL IDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRV VAGEGSGSVSLALH LQPLRSAAGAAALALTVDLPPASSEARNSAFGF QGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGLGGGGSGGGGSRGPE LSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPL SWYSDPGLAGVSLTGGLSYKEDTKELV VAKA GVYYVFFQLELRRV VAGEGSGSVSLALHLQPL RSAAGAAALALTVDLPPASSEARNSAFGFQGR LLHLSAGQRLGVHLHTEARARHAWQLTQ GAT VLGLFRVTPEIPAGLGGGGSGGGGSRVTAAPSV FIFPPSDRKLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKSTYSLSSITLTL

SEQ ID NO:	Name	Sequence
		SKADYEKHKVYACEVTHQGLSSPVTKSFNRGE CDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALGAPIEKTISKAKGQPREPQV YTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK
66	Monomeric hu 4-1BBL (71-248) – CH1* (Construct 2.4 and 5.4)	REGPELSPDDPAGLLDLRQGMFAQLVAQNVLL IDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALH LQPLRSAAGAAALALTVDLPPASSEARNSAFGF QGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGLGGGSGGGGSASTK GPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHHKPSNTKVDKVEPKSC
67	anti-FAP (4B9) Fc hole chain (Construct 2.4)	EVQLLESGGGLVQPGGSLRLSCAASGFTSSYA MSWVRQAPGKGLEWVSAIIGSGASTYYYADSVK GRFTISRDNKNTLYLQMNSLRAEDTAVYYCA KGWFGGFNYWGQGTLLTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHHKPSNTKVDKVEPKSCDKTHTCP PCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALGAPIEKTISKAKGQPREPQVCTLPPSRDE LTKNQVSLSCAVKGFYPSDIAVEWESNGQPN NYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
68	anti-FAP (4B9) light chain (Construct 2.4)	EIVLTQSPGTLISLSPGERATLSCRASQSVTSSYL AWYQQKPGQAPRLINVGSRRTGIPDRFSGSG SGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQ GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKSTYLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC
69	Dimeric hu 4-1BBL (71-254) – CL* Fc knob chain (Construct 1.2)	REGPELSPDDPAGLLDLRQGMFAQLVAQNVLL IDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALH LQPLRSAAGAAALALTVDLPPASSEARNSAFGF QGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGLPSRSEGGGGSGGGG SREGPELSPDDPAGLLDLRQGMFAQLVAQNVLL IDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALH LQPLRSAAGAAALALTVDLPPASSEARNSAFGF QGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGLPSRSEGGGGSGGGG SRTVAAPSVFIFPPSDRKLKSGTASVVCLLNNF

SEQ ID NO:	Name	Sequence
		YPREAKVQWKVDNALQSGNSQESVTEQDSKD STYLSSTLTLSKADYEEKHKVYACEVTHQGLSS PVTKSFNRGECDKTHTCPPCPAPEAAGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVYTLPPCRDELTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFCFSVMHEALHN HYTQKSLSLSPGK
70	Monomeric hu 4-1BBL (71-254) –CH1* (Construct 1.2)	REGPELSPDDPAGLLDLRQGMFAQLVAQNVL IDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV VAKAGVYYVFFQLELRRVVAGEGSGSVSLALH LQPLRSAAGAAALALTVDLPPASSEARNSAFGF QGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGLPSPRSEGGGGSGGGG SASTKGPSVFPLAPSSKSTSGGTAALGCLVEDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKVE EPKSC
71	anti-FAP(28H1) Fc hole chain	EVQLLESQGGGLVQPGGSLRLSCAASGFTFSSHA MSWVRQAPGKGLEWVSAIWASGEQYYADSV KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYC AKGWLGNFDYWGQGT LVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNAKALGAPIEKTISKAKGQPREPQVCTLP PSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFCFSVMHEALHNHYTQKSLSLSPGK
72	anti-FAP (28H1) light chain	EIVLTQSPGTLSPGERATLSCRASQSVSRSYL AWYQQKPGQAPRLLIIGASTRATGIPDRFSGSG SGTDFTLTISRLEPEDFAVYYCQQGQVIPPTFGQ GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKSTYLSSTLTLSKADYEEKHKVYACEVT HQGLSSPVTKSFNRGEC
73	DP47 Fc hole chain	EVQLLESQGGGLVQPGGSLRLSCAASGFTFSSYA MSWVRQAPGKGLEWVSAISGSGGSTYYADSV KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYC AKGSGFDYWGQGT LVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALGAPIEKTISKAKGQPREPQVCTLPSPRDEL

SEQ ID NO:	Name	Sequence
		TKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSGDSFFLVSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK
74	anti-FAP (4B9) Fc hole chain fused to dimeric hu 4-1BBL (71-254)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYA MSWVRQAPGKGLEWVSAIIGSGASTYYADSVK GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA KGWFGGFNYWGQGT LTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVT VSWNSG ALTSGVHTFPAVLQSSGLYSLSSVTV PSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAAGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALGAPIEKTISKAKGQPREPQVCTLPSPRDE LTKNQVSLSCAVKGFYPSDIAVEWESNGQPE NYKTTTPVLDSGDSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGGGGGGSGG GGSGREGPELSPDDPAGLLDLRQGMFAQLVAQN VLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKE LVVAKAGVYYYVFFQLELRRV VAGEGSGSVSLA LHLQPLRSAAGAAALALTVDLPPASSEARNSAF GFQGRLLHLSAGQRLGVHLHTEARARHAWQL TQGATVLGLFRVTPEIPAGLPSPRSEGGGGSGG GGSGREGPELSPDDPAGLLDLRQGMFAQLVAQN VLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKE LVVAKAGVYYYVFFQLELRRV VAGEGSGSVSLA LHLQPLRSAAGAAALALTVDLPPASSEARNSAF GFQGRLLHLSAGQRLGVHLHTEARARHAWQL TQGATVLGLFRVTPEIPAGLPSPRSE
75	anti-FAP (4B9) Fc knob chain fused to monomeric hu 4- 1BBL (71-254)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYA MSWVRQAPGKGLEWVSAIIGSGASTYYADSVK GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA KGWFGGFNYWGQGT LTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVT VSWNSG ALTSGVHTFPAVLQSSGLYSLSSVTV PSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAAGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALGAPIEKTISKAKGQPREPQVYTLPPCRD ELTKNQVSLWCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGGGGGGSG GGSGREGPELSPDDPAGLLDLRQGMFAQLVAQ NVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDT KELVVAKAGVYYYVFFQLELRRV VAGEGSGSVS LALHLQPLRSAAGAAALALTVDLPPASSEARN SAFGFQGRLLHLSAGQRLGVHLHTEARARHAW QLTQGATVLGLFRVTPEIPAGLPSPRSE
76	anti-FAP (28H1) Fc hole chain fused to dimeric hu 4-1BBL (71-254)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSHA MSWVRQAPGKGLEWVSAIWASGEQYYADSV KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYC

SEQ ID NO:	Name	Sequence
		AKGWLGNFDYWGQGTTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNAKALGAPIEKTISKAKGQPREPQVCTLPSS RDELTKNQVSLSCAVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMEALHNHYTQKSLSLSPGGGGG SGGGSREGPELSPDDPAGLLDLRQGMFAQLV AQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKE DTKELVVAAGVYYVFFQLELRRVVAGEGSGS VSLALHLQPLRSAAGAAALALTVDLPPASSEAR NSAFGFQGRLLHLSAGQRLGVHLHTEARARHA WQLTQGATVLGLFRVTPEIPAGLPSRSEGGG SGGGSREGPELSPDDPAGLLDLRQGMFAQLV AQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKE DTKELVVAAGVYYVFFQLELRRVVAGEGSGS VSLALHLQPLRSAAGAAALALTVDLPPASSEAR NSAFGFQGRLLHLSAGQRLGVHLHTEARARHA WQLTQGATVLGLFRVTPEIPAGLPSRSE
77	anti-FAP (28H1) Fc knob chain fused to monomeric hu 4-1BBL (71-254)	EVQLLESAGGGLVQPGGSLRLSCAASGFTFSSHA MSWVRQAPGKGLEWVSAIWASGEQYYADSV KGRFTISRDNKNTLYLQMNSLRAEDTAVYYC AKGWLGNFDYWGQGTTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNAKALGAPIEKTISKAKGQPREPQVYTLPPC RDELTKNQVSLWCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMEALHNHYTQKSLSLSPGGGGG SGGGSREGPELSPDDPAGLLDLRQGMFAQLV AQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKE DTKELVVAAGVYYVFFQLELRRVVAGEGSGS VSLALHLQPLRSAAGAAALALTVDLPPASSEAR NSAFGFQGRLLHLSAGQRLGVHLHTEARARHA WQLTQGATVLGLFRVTPEIPAGLPSRSE
78	DP47 Fc hole chain fused to dimeric hu 4-1BBL (71-254)	EVQLLESAGGGLVQPGGSLRLSCAASGFTFSSYA MSWVRQAPGKGLEWVSAIIGSGASTYYADSVK GRFTISRDNKNTLYLQMNSLRAEDTAVYYCA KGWFGGFNYWGQGTTLVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR

SEQ ID NO:	Name	Sequence
		EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALGAPIEKTISKAKGQPREPQVCTLPSPRDE LTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSSFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGGGGGSGG GGSREGPELSPDDPAGLLDLRQGMFAQLVAQN VLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKE LVVAKAGVYYVFFQLELRRV VAGEGSGSVSLA LHLQPLRSAAGAAALALTVDLPPASSEARNSAF GFQGRLLHLSAGQRLGVHLHTEARARHAWQL TQGATVLGLFRVTPEIPAGLPSRSEGGGSGG GGSREGPELSPDDPAGLLDLRQGMFAQLVAQN VLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKE LVVAKAGVYYVFFQLELRRV VAGEGSGSVSLA LHLQPLRSAAGAAALALTVDLPPASSEARNSAF GFQGRLLHLSAGQRLGVHLHTEARARHAWQL TQGATVLGLFRVTPEIPAGLPSRSE
79	DP47 Fc knob chain fused to monomeric hu 4-1BBL (71-254)	EVQLLESGLLVQPGGSLRLSCAASGFTFSSYA MSWVRQAPGKGLEWVSAIIGSGASTYYADSVK GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA KGWFGGFNYWGQGT LTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVT VSWNSG ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAAGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALGAPIEKTISKAKGQPREPQVYTLPPCRD ELTKNQVSLWCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSDGSSFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGGGGGSG GGSREGPELSPDDPAGLLDLRQGMFAQLVAQ NVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDT KELVVAKAGVYYVFFQLELRRV VAGEGSGSVS LALHLQPLRSAAGAAALALTVDLPPASSEARN AFGFQGRLLHLSAGQRLGVHLHTEARARHAW QLTQGATVLGLFRVTPEIPAGLPSRSE
80	Human (hu) FAP	UniProt no. Q12884
81	hu FAP ectodomain+poly-lys-tag+his ₆ -tag	RPSRVHNSEENTMRALTLKDILNGTFSYKTFEP NWISGQEYLHQ SADNNIVLYNIETGQSYTILSN RTMKSVNASNYGLSPDRQFVYLESDYSKLWR YSYTATYYIYDL SNGEFVRGNELPRPIQYLCWS PVGSKLAYVYQNNIYLKQRP GDPPFQITFN GRE NKIFNGIPDWVYEEEMLATKYALWWSPNGKFL AYAEFNDTDIPVIAYSYYGDEQYPTINIPYKA GAKNPVVRIFIIDTTYPAYVGPQEV PVPAMIASS DYYFSWLTWVTD ERVCLQWLKRVQNVSVLSI CDFREDWQ TWDCPKTQEHIEESRTGWAGGFFV STPVFSYDAISYYKIFSDKDG YKHIHYIKDTVEN AIQITSGKWEAINIFRVTQDSL FYSSNEFEEY PG RRNIYRISIGSYPPSKKCVTCHLRKERCQYYTAS FSDYAKYYALVCYGP GIPISTLHDGR TDQEIKIL

SEQ ID NO:	Name	Sequence
		EENKELENALKNIQLPKEEIKKLEVDEITLWYK MILPPQFDRSKKYPLLIQVYGGPCSQSRSVF VNWISYLASKEGMVIALVDGRGTAQGDKLLY AVYRKLGVEVEDQITAVRKFIEMGFIDEKRIA IWGWSYGGYVSSLALASGTGLFKCGIAVAPVS SWEYYASVYTERFMGLPTKDDNLEHYKNSTV MARAHEYFRNVDYLLIHGTADDNVHFQNSAQIA KALVNAQVDFQAMWYSDQNHGLSGLSTNHLY THMTHFLKQCFSLSDGKKKKKKGGHHHHHH
82	mouse FAP	UniProt no. P97321
83	Murine FAP ectodomain+poly-lys- tag+his ₆ -tag	RPSRVYKPEGNTKRALTLKDILNGTFSYKTYFP NWISEQEYLHQSEDDNIVFYNIETRESYILSNST MKS VNATDYGLSPDRQFVYLESDYSKLWRY YTATYYIYDLQNGEFVRGYELPRPIQYLCWSPV GSKLAYVYQNNIYLKQRPDPPFQITYTGREN IFNGIPDWVYEEEMLATKYALWWSPDGKFLAY VEFNDSIPIIAYSYYGDGQYPRITINIPYKAGA KNPVVRVFIVDTTYPHHVGPMEVPVPEMIASSD YYFSWLTWVSSERVCLQWLKRVQNVSVLSICD FREDWHAWECPKNQEHEESRTGWAGGFFVS TPAFSQDATSYKIFSDKDGKHHIHYIKDTVEN AIQITSGKWEAIYIFRVTQDSLFISSNEFEGYPG RRNIYRISIGNSPPSKKCVTCHLRKERCQYYTAS FSYKAKYYALVCYGPGLPISTLHDGRDQEIQV LEENKELENSLRNIQLPKEEIKKLDGGLTFWY KMILPPQFDRSKKYPLLIQVYGGPCSQSRSVF AVNWITYLASKEGIVIALVDGRGTAQGDKFL HAVYRKLGVEVEDQLTAVRKFIEMGFIDEERI AIWGWSYGGYVSSLALASGTGLFKCGIAVAPV SSWEYYASISYERFMGLPTKDDNLEHYKNSTV MARAHEYFRNVDYLLIHGTADDNVHFQNSAQIA KALVNAQVDFQAMWYSDQNHGILSGRSQNH YTHMTHFLKQCFSLSDGKKKKKKGGHHHHHH
84	Cynomolgus FAP ectodomain+poly-lys- tag+his ₆ -tag	RPPRVHNSEENTMRALTLKDILNGTFSYKTFFP NWISEQEYLHQSEDDNIVLYNIETGQSYTILSN RTMKS VNASNYGLSPDRQFVYLESDYSKLWR YSYTATYYIYDLNNGEFVRGNELPRPIQYLCWS PVGSKLAYVYQNNIYLKQRPDPPFQITFNGRE NKIFNGIPDWVYEEEMLATKYALWWSPNGKFL AYAEFNDTDIPVIAYSYYGDEQYPRITINIPYKA GAKNPVVRIFIIDTTPAYVGPQEVVPVAMIASS DYYFSWLTWVTDERVCLQWLKRVQNVSVLSI CDFREDWQTWDCPKTQEHEESRTGWAGGFFV STPVFSYDAISYYKIFSDKDGKHHIHYIKDTVEN AIQITSGKWEAINIFRVTQDSLFISSNEFEDYPG RRNIYRISIGSYPPSKKCVTCHLRKERCQYYTAS FSDYAKYYALVCYGPPISTLHDGRDQEI EENKELENALKNIQLPKEEIKKLEVDEITLWYK MILPPQFDRSKKYPLLIQVYGGPCSQSRSVF VNWISYLASKEGMVIALVDGRGTAQGDKLLY AVYRKLGVEVEDQITAVRKFIEMGFIDEKRIA

SEQ ID NO:	Name	Sequence
		IWGWSYGGYVSSLALASGTGLFKCGIAVAPVS SWEYYASVYTERFMGLPTKDDNLEHYKNSTV MARAEYFRNVDYLLIHGTADDNVHFQNSAQIA KALVNAQVDFQAMWYSDQNHGLSGLSTNHL YTHMTHFLKQCFSLSGKGGGGGGHHHHHH
85	human CEA	UniProt no. P06731
86	full length 4-1BBL	UniProt No. P41273
87	4-1BBL (50-254)	ACPWAVSGARASPGSAASPRLREGPELSPDDPA GLDLRQGMFAQLVAQNVLIDGPLSWYSDPG LAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQ LELRRV VAGEGSGSVSLALHLQPLRSAAGAAA LALTVDLPPASSEARNSAFGFQGRLLHLSAGQR LGVHLHTEARARHAWQLTQGATVLGLFRVTP EIPAGLPSRSE
88	human 4-1BB	UniProt accession No. Q07011
89	murine 4-1BB	UniProt accession No. P20334
90	cynomolgus 4-1BB	Uniprot accession No. F6W5G6
91	G4S peptide linker	GGGGS
92	(G4S) ₂	GGGSGGGGS
93	(SG4) ₂	SGGGSGGGG
94	peptide linker	GGGSGGGSGGGG
95	peptide linker	GSPGSSSSGS
96	(G4S) ₃ peptide linker	GGGSGGGSGGGGS ₃
97	(G4S) ₄ peptide linker	GGGSGGGSGGGSGGGGS
98	peptide linker	GSGSGSGS
99	peptide linker	GSGSGNGS
100	peptide linker	GGSGSGSG
101	peptide linker	GGSGSG
102	peptide linker	GGSG
103	peptide linker	GGSGNGSG
104	peptide linker	GGNGSGSG
105	peptide linker	GGNGSG
106	human CD3ε	UniProt No. P07766
107	cynomolgus CD3ε	NCBI GenBank no. BAB71849.1 Uniprot Q05LI5
108	anti-CEACAM5 Fc hole chain (Construct 5.4)	QVQLVQSGAEVKKPGSSVKVSKASGFNIKDT YMHWRVQAPGQGLEWMGRIDPANGNSKYVP KFQGRVTITADTSTSTAYMELSSLRSED TAVYYCAPFGYYVSDYAMAYWGQGLVTVSSASTKG PSVFLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALGAPIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKS RWQQGNVFCFSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:	Name	Sequence
		K
109	anti-CEACAM5 light chain (Construct 5.4)	EIVLTQSPATLSLSPGERATLSCRAGESVDIFGV GFLHWYQQKPGQAPRLLIYRASNRATGIPARFS GSGSGTDFTLTISSLEPEDFAVYYCQQTNEPDY TFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDYSLSSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC
110	human PD-L1 (Uniprot Q9NZQ7)	MRIFAVFIFMTYWHLNNAFTVTPKDLVYVVEY GSNMTIECKFPVEKQLDLAALIVYWEMEDKNII QFVHGEEDLVQVHSSYRQARLLKDQLSLGNA ALQITDVKLQDAGVYRCMISYGGADYKRITVK VNAPYNKINQRILVVDPTSEHELTCQAEGYPK AEVIWTSSDHQVLSGKTTTTNSKREEKLFNVT TLRINTTTNEIFYCTFRRLDPEENHTAELVPELP LAHPPNERTHLVILGAILLCLGVALTFIFRLRKG RMMDVKKCGIQDTNSKKQSDTHLEET
111	human PD-1 (Uniprot Q15116)	MQIPQAPWPVWVAVLQLGWRPGWFLDSPDRP WNPPTFSPALLVTEGDNATFTCSFSNTSESV LNWYRMSPSNQTDKLAAPEDRSQPGQDCRFR VTQLPNGRDFHMSVVRARRNDSGTLYLCGAISL APKAQIKESLRAELRVTERRAEVPTAHPSPSPRP AGQFQTLVVGTVGGLLGSLLVWVLAVICSR AARGTIGARRTGQPLKEDPSAVPVFSDYGE DFQWREKTPEPPVPCVPEQTEYATIVFSPGMGT SSPARRGSADGPRSAQPLRPEDGHCSWPL
112	VH (PD-L1)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDS WIHWVRQAPGKGLEWVAWISPYGGSTYYADS VKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CARRHWPGGFDYWGQGTLVTVSS
113	VL (PD-L1)	DIQMTQSPSSLSASVGRVTITCRASQDVSTAV AWYQQKPGKAPKLLIYSASFYSGVPSRFSGSG SGTDFTLTISSLQPEDFATYYCQQYLYHPATFG QGTKVEIK
114	VH (PD-L1)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRY WMSWVRQAPGKGLEWVANIKQDGSEKYYVD SVKGRFTISRDNKNSLYLQMNSLRAEDTAVY YCAREGGWFGELAFDYWGQGTLVTVSS
115	VL (PD-L1)	EIVLTQSPGTLSPGERATLSCRASQRVSSSYL AWYQQKPGQAPRLLIYDASSRATGIPDRFSGSG SGTDFTLTISRLEPEDFAVYYCQQYGLPWTFG QGTKVEIK
116	VH (PD-1)	QVQLVQSGVEVKKPGASVKVSCASGYTFTN YYMYWVRQAPGQGLEWMGINPSNGGTNFN EKFKNRVTLTDSSTTTAYMELKSLQFDDTAV YYCARRDYRFDMGFYWGQGTTVTVSS
117	VL (PD-1)	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGY SYLHWYQQKPGQAPRLLIYLAISYLESGVPARFS GSGSGTDFTLTISSLEPEDFAVYYCQHSRDLPLT FGGGTKVEIK
118	VH (PD-1)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSG MHWVRQAPGKGLEWVAWIWYDGSKRYYADS

SEQ ID NO:	Name	Sequence
		VKGRFTISRDN SKNTLFLQMNSLRAEDTAVYY CATND DYWGQGLTVTVSS
119	VL (PD-1)	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLA WYQQKPGQAPRLLIYDASN RATGIPARFSGSGS GTDFLTITSSLEPEDFAVYYCQSSNWPRTFGQ GTKVEIK
120	DP47 light chain	EIVLTQSPGTLSPGERATLSCRASQSVSSSYL AWYQQKPGQAPRLLIYGASSRATGIPDRFSGSG SGTDFLTISRLEPEDFAVYYCQQYGSSPLTFGQ GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNFPYPREAKVQWKVDNALQSGNSQESVTE QDSKDYSTYSLSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC
121	(CH2527) CD3-HCDR1	TYAMN
122	(CH2527) CD3-HCDR2	RIRSKYNNYATYYADSVKG
123	(CH2527) CD3-HCDR3	HGNFGNSYVSWFAY
124	(16D5) FoIR1-HCDR1	NAWMS
125	(16D5) FoIR1-HCDR2	RIKSKTDGGTTDYAAPVKG
126	(16D5) FoIR1-HCDR3	PWEWSWYDY
127	(CH2527-VL7-46-13)-LCDR1	GSSTGAVTTSNYAN
128	(CH2527-VL7-46-13)-LCDR2	GTNKRAP
129	(CH2527-VL7-46-13)-LCDR3	ALWYSNLWV
130	(CH2527) CD3 VH	EVQLLES GGGGLVQPGGSLRLSCAASGFTFSTYA MNWVRQAPGKGLEWVSRI RSKYNNYATYYA DSVKGRFTISRDDSKNTLYLQMNSLRAEDTAV YYCVRHGNFGNSYVSWFAYWGQGLTVTVSS
131	(16D5) FoIR1 VH	EVQLVES GGGGLVKPGGSLRLSCAASGFTFSNA WMSWVRQAPGKGLEWVGRIKSKTDGGTTDY AAPVKGRFTISRDDSKNTLYLQMNSLKTEDTA VYYCTTPWEWSWYDYWGQGLTVTVSS
132	(CH2527-VL7-46-13)VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSN YANWVQEKPGQAFRGLIGGTNKRAPGTPARFS GSLLGGKAALTLGAQPEDEAEYYCALWYSNL WVFGGGTKLTVL
133	(16D5)VH-CH1- (CH2527)VH-CH1 Fc knob PGLALA	EVQLVES GGGGLVKPGGSLRLSCAASGFTFSNA WMSWVRQAPGKGLEWVGRIKSKTDGGTTDY AAPVKGRFTISRDDSKNTLYLQMNSLKTEDTA VYYCTTPWEWSWYDYWGQGLTVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DGGGGSGGGGSEVQLLES GGGGLVQPGGSLRLS CAASGFTFSTYAMNWVRQAPGKGLEWVSRI RSKYNNYATYYADSVKGRFTISRDDSKNTLYLQM NSLRAEDTAVYYCVRHGNFGNSYVSWFAYWG QGLTVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPS VFLFPPKPKDTLMISRTPEVTCVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKT

SEQ ID NO:	Name	Sequence
		ISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
134	(16D5)VH-CH1-Fc hole PGLALA H435R-Y436F	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEWVGRIKSKTDGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCTTPWEWSWYDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNRYTQKSLSLSPGK
135	(CH2527-VL7-46-13)VL-CL (common light chain)	QAVVTQEPSTLVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNKRAPGTPARFSGSLGGKAALTLGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTPSKQSNNKYAASSYLSLTPEQWKSHRYSYCQVTHEGSTVEKTVAPTECS
136	di-mu4-1BBL-CL Fc knob chain	see Table 21
137	mono-mu4-1BBL-CH1 chain	see Table 21
138	VHCH1 (4B9) Fc hole chain	see Table 21
139	VLCL(4B9) Light chain	see Table 21
140	VHCH1 (MU137-1)-Heavy chain Fc-DD-VL (28H1)	see Table 23
141	VHCH1 (20H4.9)-Heavy chain Fc-KK-VH (28H1)	see Table 23
142	VLCL-Light chain (MU137-1)	see Table 23
143	VHCH1 (MU137-1)-Heavy chain Fc-DD-VH (28H1)	see Table 24
144	VHCH1 (MU137-1)-Heavy chain Fc-KK-VL (28H1)	see Table 24
145	murine PD-L1 antibody heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLLTVSAAKTTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTVNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLITITLTPKVTCTVV

SEQ ID NO:	Name	Sequence
		VDISKDAPEVQFSWFVDDDEVHTAQTQPREEQ FNSTFRSVSELPIMHQDWLNGKEFKCRVNSAA FGAPIEKTISKTKGRPKAPQVYTIPPPKEQMAK DKVSLTCMITDFFPEDITVEWQWNGQPAENYK NTQPIMDTDGSIYFVYSKLVQKSNWEAGNTFT CSVLHEGLHNHHTTEKSLSHSPGK
146	murine PD-L1 antibody light chain	DIQMTQSPSSLSASVGDRTITCRASQDVSTAV AWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSG SGTDFLTITSLQPEDFATYYCQQYLYHPATFG QGTKVEIKRADAAPTVSIFPPSSEQLTSGGASVV CFLNMFYPKDINVKWKIDGSRQNGVLNSWTD QDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEAT HKTSTSPIVKSFNRNEC
147	MCSP CD3 TCB (MCSP) light chain	DIQMTQSPSSLSASVGDRTITCRASQGIRNYL NWKYQQKPGKAPKLLIYTTSSLHSGVPSRFSGSG SGTDYTLTITSLQPEDFATYYCQQYSALPWTFG QGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDYSTYSLSTLTLSKADYEEKHKVYACEV THQGLSSPVTKSFNRGEC
148	MCSP CD3 TCB (CD3) light chain	QAVVTQEPSTLVSPGGTVTLTCGSSTGAVTTSN YANWVQEKPGQAFRGLIGGTNKRAPGTPARFS GSLGGAALTLGAQPEDEAEYYCALWYSNL WVFGGGTKLTVLSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSC
149	MCSP CD3 TCB heavy chain 1	QVQLQESGPGLVKPSQTLSTCTVSGGSITSGY YWNWIRQHPGKGLEWIGYITFDGSNNYNPSLK SRVTISRDTSKNQFSLKLSSVTAADTAVYYCAD FDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDGGGGSGGGGSEV QLLESGGGLVQPGGSLRLSCAASGFTSTYAM NWVRQAPGKGLEWVSRIRSKYNNYATYYADS VKGRFTISRDDSKNTLYLQMNSLRAEDTAVYY CVRHGNFGNSYVSWFAYWGQGTLLTVSSASV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDYSTYSL STLTLSKADYEEKHKVYACEVTHQGLSSPVTKS FNRGECDKTHTCPPCPAPEAAGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVYTLPPCRDELTKNQVSLWCLVKGFYP SDIAVEWESNGQPENNYKTTTPVLDSDGSFFLY SKLTVDKSRWQQGNVFCFSVMHEALHNHYTQ KSLSLSPGK
150	MCSP CD3 TCB heavy chain 2	QVQLQESGPGLVKPSQTLSTCTVSGGSITSGY YWNWIRQHPGKGLEWIGYITFDGSNNYNPSLK SRVTISRDTSKNQFSLKLSSVTAADTAVYYCAD

SEQ ID NO:	Name	Sequence
		FDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS HEDPEVKFNWYVDGVEVHAKTKPREEQYNST YRVVSVLTVQLHQLNGLSKYSKCKVSNKALGA PIEKTISKAKGQPREPQVCTLPSPRDELTKNQVS LSCAVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSGDGSFFLVSKLTVDKS RWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
151	MCSP-HCDR1	SGYYWN
152	MCSP-HCDR2	YITFDGSNNY NPSLKS
153	MCSP-HCDR3	FDY
154	MCSP-LCDR1	RASQGIRNYLN
155	MCSP-LCDR2	YTSSLHS
156	MCSP-LCDR3	QQYSALPWT
157	MCSP VH	QVQLQESGPGLVKPSQTLSTCTVSGGSITSGY YWNWIRQHPGKGLEWIGYITFDGSNNYNPSLK SRVTISRDTSKNQFSLKLSSVTAADTAVYYCAD FDYWGQGTLVTVSS
158	MCSP VL	DIQMTQSPSSLSASVGDRTITCRASQGIRNYL NWKYQQKPGKAPKLLIYTSSLHSGVPSRFSGSG SGTDYTLTISSLQPEDFATYYCQQYSALPWTFG QGTEKVEIK
159	CD20	UniProt accession No. P11836

- 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). Amino acids of antibody chains are numbered and referred to according to the 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.

Aspects of the Invention

- 10 In the following, some of the aspects of the invention are listed.

1. A T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method for treating or delaying progression of cancer, wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is used in combination with a 4-1BB (CD137) agonist.

2. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of aspect 1, wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is an anti-CEA/anti-CD3 bispecific antibody.

3. The anti-CEA/anti-CD3 bispecific antibody for use in a method of aspects 1 or 2,
5 wherein the T-cell activating anti-CD3 bispecific antibody and the 4-1BB agonist are administered together in a single composition or administered separately in two or more different compositions.

4. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist comprises three ectodomains of 4-1BBL or fragments
10 thereof.

5. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is a molecule comprising three ectodomains of 4-1BBL or fragments thereof and wherein the ectodomains of 4-1BBL comprise an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID
15 NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8, particularly the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:5.

6. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable
20 of specific binding to a tumor-associated antigen.

7. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to Fibroblast activation protein (FAP).

8. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP, wherein the antigen binding domain capable of specific binding to FAP comprises
25 (a) a heavy chain variable region ($V_{\text{H}}\text{FAP}$) 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, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:11, and a light chain variable region ($V_{\text{L}}\text{FAP}$) 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
30 (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:14, or
35 (b) a heavy chain variable region ($V_{\text{H}}\text{FAP}$) comprising (i) CDR-H1 comprising the amino

acid sequence of SEQ ID NO:15, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:16, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:17, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:18, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:19, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:20.

9. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP, wherein 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:21 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:22 or wherein 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:23 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:24.

10. The anti-CEA/anti-CD3 bispecific antibody for use in a method of of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain.

11. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

12. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising (a) at least one antigen binding domain capable of specific binding to FAP, (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

13. The anti-CEA/anti-CD3 bispecific antibody for use in a method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising (a) at least one Fab domain capable of specific binding to CD19, and (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that (i) the first polypeptide contains a CH1 or CL domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked

to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or

- (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
- (iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

14. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

(a) at least one Fab domain capable of specific binding to FAP comprising a heavy chain variable region (V_H FAP) comprising the amino acid sequence of SEQ ID NO:21 and a light chain variable region (V_L FAP) comprising the amino acid sequence of SEQ ID NO:22 or a heavy chain variable region (V_H FAP) comprising the amino acid sequence of SEQ ID NO:23 and a light chain variable region (V_L FAP) comprising the amino acid sequence of SEQ ID NO:24, and

(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

15. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule selected from the group consisting of

a) a molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:67 and a second light chain comprising the amino acid sequence of SEQ ID NO:68; and

b) a molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:69, a first light chain comprising the amino acid sequence of SEQ ID NO:70, a second heavy chain comprising the amino acid sequence of SEQ ID NO:71 and a second light chain comprising the amino acid sequence of SEQ ID NO:72.

16. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

(a) at least one antigen binding domain capable of specific binding to FAP,
(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers.

17. The anti-CEA/anti-CD3 bispecific antibody for use in a method of of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

(a) at least one antigen binding domain capable of specific binding to FAP,
(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers, and
(c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the polypeptide comprising the three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers is fused to the N- or C-terminal amino acid of one of the two subunits of the Fc domain, optionally through a peptide linker.

18. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an anti-FAP/anti-4-1BB bispecific antibody.

19. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA.

20. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises (a) a heavy chain variable region (V_H CEA) comprising (i) CDR-H1

comprising the amino acid sequence of SEQ ID NO:49, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:50, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:51, and a light chain variable region (V_LCEA) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:54.

21. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises a heavy chain variable region (V_HCEA) comprising an amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_LCEA) comprising an amino acid sequence of SEQ ID NO:56.

22. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain.

23. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

24. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising (a) at least one antigen binding domain capable of specific binding to CEA, (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

25. The anti-CEA/anti-CD3 bispecific antibody for use in a method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising (a) at least one Fab domain capable of specific binding to CEA, and (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that (i) the first polypeptide contains a CH1 or CL domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments

thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or

- 5 (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected
10 via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
- (iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two
15 ectodomains of 4-1BBL or fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

26. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding
20 aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
- (a) at least one Fab domain capable of specific binding to CEA comprising a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:56, and
- (b) a first and a second polypeptide that are linked to each other by a disulfide bond,
25 wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID
30 NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

27. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid
35 sequence of SEQ ID NO:108 and a second light chain comprising the amino acid sequence of SEQ ID NO:109.

28. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

- (a) at least one antigen binding domain capable of specific binding to CEA,
 - (b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are
- 5 connected to each other by peptide linkers.

29. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

- (a) at least one antigen binding domain capable of specific binding to CEA,
 - (b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are
- 10 connected to each other by peptide linkers, and
- (c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the polypeptide comprising the three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers is fused to the N- or C-terminal amino acid of one of the two subunits of the Fc domain, optionally through a peptide linker.

- 15 30. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the 4-1BB agonist is an anti-CEA/anti-4-1BB bispecific antibody.

31. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_H CD3) and a light chain variable region

20 (V_L CD3), and a second antigen binding domain comprising a heavy chain variable region (V_H CEA) and a light chain variable region (V_L CEA).

32. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the first antigen binding domain comprises a heavy chain variable region (V_H CD3) comprising CDR-H1 sequence of SEQ ID NO:33, CDR-H2 sequence of SEQ ID
- 25 NO:34, and CDR-H3 sequence of SEQ ID NO:35; and/or a light chain variable region (V_L CD3) comprising CDR-L1 sequence of SEQ ID NO:36, CDR-L2 sequence of SEQ ID NO:37, and CDR-L3 sequence of SEQ ID NO:38.

33. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the first antigen binding domain comprises a heavy chain variable region
- 30 (V_H CD3) comprising the amino acid sequence of SEQ ID NO:39 and/or a light chain variable region (V_L CD3) comprising the amino acid sequence of SEQ ID NO:40.

34. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the second antigen binding domain comprises

- (a) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43, and/or a
- 35 light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:44, CDR-

L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46, or

- (b) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:49, CDR-H2 sequence of SEQ ID NO:50, and CDR-H3 sequence of SEQ ID NO:51, and/or a light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54.

35. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the second antigen binding domain comprises a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:48 or wherein the second antigen binding domain comprises a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:56.

36. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to CEA.

37. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the third antigen binding domain comprises a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:48 or wherein the second antigen binding domain comprises a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:56.

38. The anti-CEA/anti-CD3 bispecific antibody for use in a method of any one of the preceding aspects, wherein the first antigen binding domain is a cross-Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged, and the second and third, if present, antigen binding domain is a conventional Fab molecule.

39. The anti-CEA/anti-CD3 bispecific antibody for use in a method of any one of the preceding aspects, wherein (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at

the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

40. The anti-CEA/anti-CD3 bispecific antibody for use in a method of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody comprises an Fc domain
5 comprising one or more amino acid substitutions that reduce binding to an Fc receptor and/or effector function.

41. The anti-CEA/anti-CD3 bispecific antibody for use in a method of any one of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody comprises an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

10 42. An anti-CEA/anti-CD3 bispecific antibody for use in a method of any one of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody is used in combination with a 4-1BB (CD137) agonist and wherein the combination is administered at intervals from about about one week to three weeks.

43. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated
15 antigen for use in a method of any one of the preceding aspects, wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is used in combination with a 4-1BB (CD137) agonist and in combination with an agent blocking PD-L1/PD-1 interaction.

44. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated
20 antigen for use in a method of aspect 43, wherein the agent blocking PD-L1/PD-1 interaction is a anti-PD-L1 antibody or an anti-PD1 antibody.

45. A pharmaceutical product comprising (A) a first composition comprising as active ingredient an anti-CEA/anti-CD3 bispecific antibody and a pharmaceutically acceptable carrier; and (B) a second composition comprising as active ingredient a 4-1BB agonist and a
25 pharmaceutically acceptable carrier, for use in the combined, sequential or simultaneous, treatment of a disease, in particular cancer.

46. A pharmaceutical composition comprising anti-CEA/anti-CD3 bispecific antibody and a 4-1BB agonist.

47. The pharmaceutical composition of aspect 46 for use in the treatment of solid
30 tumors.

48. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist comprises three ectodomains of 4-1BBL or fragments thereof.

49. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is a molecule comprising three ectodomains of 4-1BBL or fragments thereof and
35 wherein the ectodomains of 4-1BBL comprise an amino acid sequence selected from the

group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8, particularly the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:5.

50. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to a tumor associated antigen.

51. The pharmaceutical composition of any one of the preceding aspects, wherein the tumor associated antigen is FAP.

52. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP, wherein 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: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_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, or

(b) a heavy chain variable region (V_H FAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:15, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:16, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:17, and a light chain variable region (V_L FAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:18, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:19, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:20.

53. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP, wherein the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_H FAP) comprising an amino acid sequence of SEQ ID NO:21 and a light chain variable region (V_L FAP) comprising an amino acid sequence of SEQ ID NO:22 or wherein the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_H FAP) comprising an amino acid sequence of SEQ ID NO:23 and a light chain variable region (V_L FAP) comprising an amino acid sequence of SEQ ID NO:24.

54. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule further comprising a Fc domain composed of a first and a second subunit capable of stable association.

55. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain.

56. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

57. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

58. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one antigen binding domain capable of specific binding to FAP,
(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

59. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one Fab domain capable of specific binding to FAP, and
(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that

(i) the first polypeptide contains a CH1 or CL domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or
(ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second

polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
(iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein
5 the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one
10 ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

60. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

- (a) at least one Fab domain capable of specific binding to FAP comprising a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:21 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:22 or a
15 heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:23 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:24, and
(b) a first and a second polypeptide that are linked to each other by a disulfide bond,
20 wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID
25 NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

61. The pharmaceutical composition of any one of the preceding aspects, wherein wherein the 4-1BB agonist is an antigen binding molecule selected from the group consisting of

- a) a molecule comprising a first heavy chain comprising the amino acid sequence of
30 SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:67 and a second light chain comprising the amino acid sequence of SEQ ID NO:68; and
b) a molecule comprising a first heavy chain comprising the amino acid sequence of
SEQ ID NO:69, a first light chain comprising the amino acid sequence of SEQ ID NO:70, a
35 second heavy chain comprising the amino acid sequence of SEQ ID NO:71 and a second light chain comprising the amino acid sequence of SEQ ID NO:72.

62. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
- (a) at least one antigen binding domain capable of specific binding to FAP,
 - (b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are
- 5 connected to each other by peptide linkers.
63. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
- (a) at least one antigen binding domain capable of specific binding to FAP,
 - (b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are
- 10 connected to each other by peptide linkers, and
- (c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the polypeptide comprising the three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers is fused to the N- or C-terminal amino acid of one of the two subunits of the Fc domain, optionally through a peptide linker.
- 15 64. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an anti-FAP/anti-4-1BB bispecific antibody.
65. The pharmaceutical composition of any one of the preceding aspects, wherein the tumor associated antigen is CEA.
66. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-
- 20 1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises a heavy chain variable region (V_H CEA) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:49, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:50, and
- 25 (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:51, and a light chain variable region (V_L CEA) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:54.
67. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-
- 30 1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises a heavy chain variable region (V_H CEA) comprising an amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_L CEA) comprising an amino acid sequence of SEQ ID NO:56.

68. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule further comprising a Fc domain composed of a first and a second subunit capable of stable association.

69. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain.

70. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

71. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

72. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one antigen binding domain capable of specific binding to CEA,
(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

73. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one Fab domain capable of specific binding to CEA, and
(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that

(i) the first polypeptide contains a CH1 or CL domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or
(ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second

polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
(iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein
5 the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one
10 ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

74. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

- (a) (a) at least one Fab domain capable of specific binding to CEA comprising a heavy chain variable region (V_{HCEA}) comprising the amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_{LCEA}) comprising the amino acid sequence of SEQ ID NO:56, and
15 (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and
20 SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

75. The pharmaceutical composition of any one of the preceding aspects, wherein wherein the 4-1BB agonist is an antigen binding molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:108 and a second light chain comprising the amino acid sequence of
25 SEQ ID NO:109.

76. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
30

- (a) at least one antigen binding domain capable of specific binding to CEA,
(b) a polypeptide comprising ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers.

77. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
35

- (a) at least one antigen binding domain capable of specific binding to CEA,

(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers, and

(c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the polypeptide comprising the three ectodomains of 4-1BBL or fragments thereof
5 that are connected to each other by peptide linkers is fused to the N- or C-terminal amino acid of one of the two subunits of the Fc domain, optionally through a peptide linker.

78. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an anti-CEA/anti-4-1BB bispecific antibody.

79. The pharmaceutical composition of any one of the preceding aspects, wherein the
10 anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds to CD3, and a second antigen binding domain that binds to CEA.

80. The pharmaceutical composition of any one of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_H CD3) and a light chain variable region (V_L CD3), and a
15 second antigen binding domain comprising a heavy chain variable region (V_H CEA) and a light chain variable region (V_L CEA).

81. The pharmaceutical composition of any one of the preceding aspects, wherein the first antigen binding domain comprises a heavy chain variable region (V_H CD3) comprising CDR-H1 sequence of SEQ ID NO:33, CDR-H2 sequence of SEQ ID NO:34, and CDR-H3
20 sequence of SEQ ID NO:35; and/or a light chain variable region (V_L CD3) comprising CDR-L1 sequence of SEQ ID NO:36, CDR-L2 sequence of SEQ ID NO:37, and CDR-L3 sequence of SEQ ID NO:38.

82. The pharmaceutical composition of any one of the preceding aspects, wherein the first antigen binding domain comprises a heavy chain variable region (V_H CD3) comprising
25 the amino acid sequence of SEQ ID NO:39 and/or a light chain variable region (V_L CD3) comprising the amino acid sequence of SEQ ID NO:40.

83. The pharmaceutical composition of any one of the preceding aspects, wherein the second antigen binding domain comprises

(a) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43, and/or a
30 light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:44, CDR-L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46, or

(b) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:49, CDR-H2 sequence of SEQ ID NO:50, and CDR-H3 sequence of SEQ ID NO:51, and/or a
35 light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54.

84. The pharmaceutical composition of any one of the preceding aspects, wherein the second antigen binding domain comprises a heavy chain variable region (V_HCEA) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_LCEA) comprising the amino acid sequence of SEQ ID NO:48 or wherein the second antigen binding domain comprises a heavy chain variable region (V_HCEA) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_LCEA) comprising the amino acid sequence of SEQ ID NO:56.

85. The pharmaceutical composition of any one of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to CEA.

86. The pharmaceutical composition of any one of the preceding aspects, wherein the third antigen binding domain comprises

(a) a heavy chain variable region (V_HCEA) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43, and/or a light chain variable region (V_LCEA) comprising CDR-L1 sequence of SEQ ID NO:44, CDR-L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46, or

(b) a heavy chain variable region (V_HCEA) comprising CDR-H1 sequence of SEQ ID NO:49, CDR-H2 sequence of SEQ ID NO:50, and CDR-H3 sequence of SEQ ID NO:51, and/or a light chain variable region (V_LCEA) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54.

87. The pharmaceutical composition of any one of the preceding aspects, wherein the third antigen binding domain comprises a heavy chain variable region (V_HCEA) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_LCEA) comprising the amino acid sequence of SEQ ID NO:48 or wherein the second antigen binding domain comprises a heavy chain variable region (V_HCEA) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_LCEA) comprising the amino acid sequence of SEQ ID NO:56.

88. The pharmaceutical composition of any one of the preceding aspects, wherein the first antigen binding domain is a cross-Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged, and the second and third, if present, antigen binding domain is a conventional Fab molecule.

89. The pharmaceutical composition of any one of the preceding aspects, wherein (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab

heavy chain to the N-terminus of the second subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

90. The pharmaceutical composition of any one of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody comprises an Fc domain comprising one or more amino acid substitutions that reduce binding to an Fc receptor and/or effector function.

10 91. The pharmaceutical composition of any one of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody comprises an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

92. The pharmaceutical composition of any one of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody is used in combination with a 4-1BB (CD137) agonist and wherein the combination is administered at intervals from about one week to three weeks.

93. The pharmaceutical composition of any one of the preceding aspects for use in treating or delaying progression of a proliferative disease, in particular cancer.

20 94. The pharmaceutical composition of any one of the preceding aspects for use in the treatment of colon cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, breast cancer, kidney cancer, esophageal cancer, or prostate cancer.

25 95. A kit for treating or delaying progression of cancer in a subject, comprising a package comprising (A) a first composition comprising as active ingredient an anti-CEA/anti-CD3 bispecific antibody and a pharmaceutically acceptable carrier; (B) a second composition comprising as active ingredient a 4-1BB agonist and a pharmaceutically acceptable carrier, and (C) instructions for using the compositions in a combination therapy.

30 96. Use of a combination of an anti-CEA/anti-CD3 bispecific antibody and a 4-1BB agonist in the manufacture of a medicament for treating or delaying progression of a proliferative disease, in particular cancer.

97. Use of a combination of an anti-CEA/anti-CD3 bispecific antibody and a 4-1BB agonist in the manufacture of a medicament, wherein the medicament is for the treatment of solid tumors.

98. A method for treating or delaying progression of cancer in a subject comprising administering to the subject an effective amount of an anti-CEA/anti-CD3 antibody and a 4-1BB agonist.

99. A method for treating or delaying progression of cancer in a subject comprising
5 administering to the subject an effective amount of an anti-CEA/anti-CD3 antibody and a 4-1BB agonist, wherein the 4-1BB agonist is an antigen binding molecule.

100. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a Fc domain.

101. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an
10 antigen binding molecule comprising a Fc domain with modifications reducing Fc γ receptor binding and/or effector function.

102. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof.

103. The method of any one of the preceding aspects, wherein the -1BB agonist is an
15 antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and an antigen binding domain capable of specific binding to a tumor associated antigen.

104. The method of any one of the preceding aspects, wherein the 4-1BB agonist is a molecule comprising three ectodomains of 4-1BBL or fragments thereof and wherein the
20 ectodomains of 4-1BBL comprise an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8, particularly the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:5.

105. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and
25 at least one moiety capable of specific binding to FAP, 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: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
30 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, or
(b) a heavy chain variable region (V_HFAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:15, (ii) CDR-H2 comprising the amino acid sequence of SEQ
35 ID NO:16, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:17, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid

sequence of SEQ ID NO:18, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:19, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:20.

106. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and
5 at least one antigen binding domain capable of specific binding to FAP, wherein 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:21 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:22 or wherein the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region
10 (V_HFAP) comprising an amino acid sequence of SEQ ID NO:23 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:24.

107. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule further comprising a Fc domain composed of a first and a second subunit capable of stable association.

15 108. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain.

109. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid
20 substitution that reduces binding to an Fc receptor and/or effector function.

110. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one antigen binding domain capable of specific binding to FAP,
(b) a first and a second polypeptide that are linked to each other by a disulfide bond,
25 wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

111. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
30 (a) at least one Fab domain capable of specific binding to FAP, and
(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that
(i) the first polypeptide contains a CH1 or CL domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked
35 to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments

thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or

- 5 (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected
10 via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
- (iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two
15 ectodomains of 4-1BBL or fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

112. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an
20 antigen binding molecule comprising
- (a) at least one Fab domain capable of specific binding to FAP comprising a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:21 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:22 or a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:23
25 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:24, and
- (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID
30 NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

113. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an
35 antigen binding molecule selected from the group consisting of

a) a molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:67 and a second light chain comprising the amino acid sequence of SEQ ID NO:68; and

- 5 b) a molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:69, a first light chain comprising the amino acid sequence of SEQ ID NO:70, a second heavy chain comprising the amino acid sequence of SEQ ID NO:71 and a second light chain comprising the amino acid sequence of SEQ ID NO:72.

114. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an
10 antigen binding molecule comprising

- (a) at least one antigen binding domain capable of specific binding to FAP,
(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers.

115. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an
15 anti-FAP/anti-4-1BB bispecific antibody.

116. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one moiety capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises
20 a heavy chain variable region (V_H CEA) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:49, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:50, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:51, and a light chain variable region (V_L CEA) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and
25 (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:54.

117. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises a heavy chain variable region
30 (V_H CEA) comprising an amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_L CEA) comprising an amino acid sequence of SEQ ID NO:56.

118. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule further comprising a Fc domain composed of a first and a second subunit capable of stable association.

119. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain.

120. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

121. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

- (a) at least one antigen binding domain capable of specific binding to CEA,
- 10 (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

122. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

- (a) at least one Fab domain capable of specific binding to CEA, and
- (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that
 - (i) the first polypeptide contains a CH1 or CL domain and the second polypeptide
 - 20 contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or
 - (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
 - (iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein
 - 30 the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to
 - 35

VH or VL by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

123. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an
5 antigen binding molecule comprising
(a) at least one Fab domain capable of specific binding to CEA comprising a heavy chain variable region (V_{HCEA}) comprising an amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_{LCEA}) comprising an amino acid sequence of SEQ ID NO:56, and
(b) a first and a second polypeptide that are linked to each other by a disulfide bond,
10 wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID
15 NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

124. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:108 and a second
20 light chain comprising the amino acid sequence of SEQ ID NO:109.

125. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one antigen binding domain capable of specific binding to FAP,
(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are
25 connected to each other by peptide linkers.

126. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an anti-CEA/anti-4-1BB bispecific antibody.

127. The method of any one of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds to CD3, and a second
30 antigen binding domain that binds to CEA.

128. The method of any one of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_{HCD3}) and a light chain variable region (V_{LCD3}), and a second antigen binding domain comprising a heavy chain variable region (V_{HCEA}) and a light chain variable
35 region (V_{LCEA}).

129. The method of any one of the preceding aspects, wherein the first antigen binding domain comprises a heavy chain variable region (V_H CD3) comprising CDR-H1 sequence of SEQ ID NO:33, CDR-H2 sequence of SEQ ID NO:34, and CDR-H3 sequence of SEQ ID NO:35; and/or a light chain variable region (V_L CD3) comprising CDR-L1 sequence of SEQ ID NO:36, CDR-L2 sequence of SEQ ID NO:37, and CDR-L3 sequence of SEQ ID NO:38.

130. The method of any one of the preceding aspects, wherein the first antigen binding domain comprises a heavy chain variable region (V_H CD3) comprising the amino acid sequence of SEQ ID NO:39 and/or a light chain variable region (V_L CD3) comprising the amino acid sequence of SEQ ID NO:40.

131. The method of any one of the preceding aspects, wherein the second antigen binding domain comprises
(a) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43, and/or a light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:44, CDR-L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46, or
(b) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:49, CDR-H2 sequence of SEQ ID NO:50, and CDR-H3 sequence of SEQ ID NO:51, and/or a light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54.

132. The method of any one of the preceding aspects, wherein the second antigen binding domain comprises a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:48 or wherein the second antigen binding domain comprises a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:56.

133. The method of any one of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to CEA.

134. The method of any one of the preceding aspects, wherein the third antigen binding domain comprises
(a) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43, and/or a light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:44, CDR-L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46, or
(b) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:49, CDR-H2 sequence of SEQ ID NO:50, and CDR-H3 sequence of SEQ ID NO:51, and/or a

light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54.

135. The method of any one of the preceding aspects, wherein the third antigen binding domain comprises a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:48 or wherein the third antigen binding domain comprises a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:56.

10 136. The method of any one of the preceding aspects, wherein the first antigen binding domain is a cross-Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged, and the second and third, if present, antigen binding domain is a conventional Fab molecule.

137. The method of any one of the preceding aspects, wherein (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

25 138. The method of any one of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody comprises an Fc domain comprising one or more amino acid substitutions that reduce binding to an Fc receptor and/or effector function.

139. The method of any one of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody comprises an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

140. The method of any one of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody is used in combination with a 4-1BB (CD137) agonist and wherein the combination is administered at intervals from about one week to three weeks.

141. The method of any one of the preceding aspects, wherein the anti-CEA/anti-CD3 bispecific antibody and the 4-1BB agonist are administered together in a single composition or administered separately in two or more different compositions.

142. The method of any one of the preceding aspects, wherein the the anti-CEA/anti-CD3 bispecific antibody and the 4-1BB (CD137) agonist are administered intravenously or subcutaneously.

143. The method of any one of the preceding aspects, wherein the anti-CEA/anti-CD3
5 bispecific antibody is administered concurrently with, prior to, or subsequently to the 4-1BB agonist.

144. The method of any one of the preceding aspects, wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is used in combination with a 4-1BB (CD137) agonist and in combination with an agent blocking PD-L1/PD-1 interaction.

10 145. The method of any one of the preceding aspects, wherein the agent blocking PD-L1/PD-1 interaction is an anti-PD-L1 antibody or an anti-PD1 antibody.

146. The method of any one of the preceding aspects, wherein the agent blocking PD-L1/PD-1 interaction is selected from the the group consisting of atezolizumab, durvalumab, pembrolizumab and nivolumab.

15 147. The method of any one of the preceding aspects, wherein the agent blocking PD-L1/PD-1 interaction is atezolizumab.

148. A T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method for treating or delaying progression of cancer of any of the preceding aspects, wherein the T-cell activating anti-CD3 bispecific antibody specific for a
20 tumor-associated antigen is used in combination with a 4-1BB (CD137) agonist and wherein the 4-1BB agonist acts synergistically with the T-cell activating anti-CD3 bispecific antibody.

149. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method for treating or delaying progression of cancer of the preceding aspects, wherein the T-cell activating anti-CD3 bispecific antibody is an anti-FolR1/anti-CD3
25 bispecific antibody.

150. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method for treating or delaying progression of cancer of the preceding aspects, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_H CD3), a second antigen binding
30 domain comprising a heavy chain variable region (V_H FolR1) and a common light chain variable region.

151. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method for treating or delaying progression of cancer of the preceding aspects, wherein the first antigen binding domain comprises a heavy chain variable region
35 (V_H CD3) comprising CDR-H1 sequence of SEQ ID NO:121, CDR-H2 sequence of SEQ ID

NO:122, and CDR-H3 sequence of SEQ ID NO:123; the second antigen binding domain comprises a heavy chain variable region (V_HFolR1) comprising CDR-H1 sequence of SEQ ID NO:124, CDR-H2 sequence of SEQ ID NO:125, and CDR-H3 sequence of SEQ ID NO:126; and wherein the common light chain comprises a CDR-L1 sequence of SEQ ID NO:127,
5 CDR-L2 sequence of SEQ ID NO:128, and CDR-L3 sequence of SEQ ID NO:129.

152. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method for treating or delaying progression of cancer of the preceding aspects, wherein the first antigen binding domain comprises a heavy chain variable region (V_HCD3) comprising the sequence of SEQ ID NO:130; the second antigen binding domain
10 comprises a heavy chain variable region (V_HFolR1) comprising the sequence of SEQ ID NO:131; and wherein the common light chain comprises the sequence of SEQ ID NO:132.

153. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method for treating or delaying progression of cancer of the preceding aspects, wherein the anti-FolR1/anti-CD3 bispecific antibody comprises a third antigen
15 binding domain that binds to FolR1.

154. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method for treating or delaying progression of cancer of the preceding aspects, wherein the anti-FolR1/anti-CD3 bispecific antibody comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO:133, a second heavy chain comprising the
20 amino acid sequence of SEQ ID NO:134 and a common light chain of SEQ ID NO: 135.

155. A pharmaceutical product comprising (A) a first composition comprising as active ingredient an anti-FolR1/anti-CD3 bispecific antibody and a pharmaceutically acceptable carrier; and (B) a second composition comprising as active ingredient a 4-1BB agonist and a pharmaceutically acceptable carrier, for use in the combined, sequential or simultaneous,
25 treatment of a disease, in particular cancer.

156. A pharmaceutical composition comprising anti-FolR1/anti-CD3 bispecific antibody and a 4-1BB agonist.

157. A method for treating or delaying progression of cancer in a subject comprising administering to the subject an effective amount of an anti-FolR1/anti-CD3 antibody and a 4-
30 1BB agonist, wherein the 4-1BB agonist is an antigen binding molecule.

158. A 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is used in combination with
35 an agent blocking PD-L1/PD-1 interaction.

159. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of aspect 158, wherein the agent blocking PD-L1/PD-1 interaction is an anti-PD-L1 antibody or an anti-PD1 antibody.

160. The 4-1BB agonist comprising at least one antigen binding domain capable of
5 specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the agent blocking PD-L1/PD-1 interaction is selected from the group consisting of atezolizumab, durvalumab, pembrolizumab and nivolumab.

161. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of the preceding
10 aspects, wherein the agent blocking PD-L1/PD-1 interaction is atezolizumab.

162. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the tumor-associated antigen is selected from Fibroblast activation protein (FAP) or CEA.

15 163. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to Fibroblast activation protein (FAP).

20 164. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP, wherein the antigen binding domain capable of specific binding to FAP
25 comprises

(a) 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, 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
30 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, or
(b) a heavy chain variable region (V_H FAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:15, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:16, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:17, and a
35 light chain variable region (V_L FAP) comprising (iv) CDR-L1 comprising the amino acid

sequence of SEQ ID NO:18, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:19, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:20.

165. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP, wherein the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_H FAP) comprising an amino acid sequence of SEQ ID NO:21 and a light chain variable region (V_L FAP) comprising an amino acid sequence of SEQ ID NO:22 or wherein the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_H FAP) comprising an amino acid sequence of SEQ ID NO:23 and a light chain variable region (V_L FAP) comprising an amino acid sequence of SEQ ID NO:24.

166. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain.

167. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

168. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one antigen binding domain capable of specific binding to FAP,
(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

169. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one Fab domain capable of specific binding to FAP, and
(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that

- (i) the first polypeptide contains a CH1 or CL domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or
- (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
- (iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

170. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

(a) at least one Fab domain capable of specific binding to FAP comprising a heavy chain variable region ($V_{\text{H}}\text{FAP}$) comprising the amino acid sequence of SEQ ID NO:21 and a light chain variable region ($V_{\text{L}}\text{FAP}$) comprising the amino acid sequence of SEQ ID NO:22 or a heavy chain variable region ($V_{\text{H}}\text{FAP}$) comprising the amino acid sequence of SEQ ID NO:23 and a light chain variable region ($V_{\text{L}}\text{FAP}$) comprising the amino acid sequence of SEQ ID NO:24, and

(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

171. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule selected from the group consisting of

5 a) a molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:67 and a second light chain comprising the amino acid sequence of SEQ ID NO:68; and

10 b) a molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:69, a first light chain comprising the amino acid sequence of SEQ ID NO:70, a second heavy chain comprising the amino acid sequence of SEQ ID NO:71 and a second light chain comprising the amino acid sequence of SEQ ID NO:72.

172. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, 15 wherein the 4-1BB agonist is an antigen binding molecule comprising

(a) at least one antigen binding domain capable of specific binding to FAP,

(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers.

173. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, 20 wherein the 4-1BB agonist is an antigen binding molecule comprising

(a) at least one antigen binding domain capable of specific binding to FAP,

(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers, and

25 (c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the polypeptide comprising the three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers is fused to the N- or C-terminal amino acid of one of the two subunits of the Fc domain, optionally through a peptide linker.

174. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, 30 wherein the 4-1BB agonist is an anti-FAP/anti-4-1BB bispecific antibody.

175. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4- 35 1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA.

176. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises (a) a heavy chain variable region (V_H CEA) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:49, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:50, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:51, and a light chain variable region (V_L CEA) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:54.

177. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises a heavy chain variable region (V_H CEA) comprising an amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_L CEA) comprising an amino acid sequence of SEQ ID NO:56.

178. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain.

179. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

180. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising (a) at least one antigen binding domain capable of specific binding to CEA, (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

181. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

(a) at least one Fab domain capable of specific binding to CEA, and

5 (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that

(i) the first polypeptide contains a CH1 or CL domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and
10 wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or

15 (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected
20 via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or

(iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two
25 ectodomains of 4-1BBL or fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

182. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

(a) at least one Fab domain capable of specific binding to CEA comprising a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:56, and

35 (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and

SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

183. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:108 and a second light chain comprising the amino acid sequence of SEQ ID NO:109.

184. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one antigen binding domain capable of specific binding to CEA,
(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers.

185. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one antigen binding domain capable of specific binding to CEA,
(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers, and
(c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the polypeptide comprising the three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers is fused to the N- or C-terminal amino acid of one of the two subunits of the Fc domain, optionally through a peptide linker.

186. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of the preceding aspects, wherein the 4-1BB agonist is an anti-CEA/anti-4-1BB bispecific antibody.

187. A pharmaceutical product comprising (A) a first composition comprising as active ingredient an agent blocking PD-L1/PD-1 interaction and a pharmaceutically acceptable carrier; and (B) a second composition comprising as active ingredient a 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen and a pharmaceutically acceptable carrier, for use in the combined, sequential or simultaneous, treatment of a disease, in particular cancer.

188. A pharmaceutical composition comprising an agent blocking PD-L1/PD-1 interaction and a 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen.

189. The pharmaceutical composition of aspect 188 for use in the treatment of solid
5 tumors.

190. The pharmaceutical composition of any one of the preceding aspects, wherein the agent blocking PD-L1/PD-1 interaction is an anti-PD-L1 antibody or an anti-PD1 antibody.

191. The pharmaceutical composition of any one of the preceding aspects, wherein the agent blocking PD-L1/PD-1 interaction is selected from the group consisting of atezolizumab,
10 durvalumab, pembrolizumab and nivolumab.

192. The pharmaceutical composition of any one of the preceding aspects, wherein the agent blocking PD-L1/PD-1 interaction is atezolizumab.

193. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to
15 a tumor-associated antigen is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof.

194. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is a molecule comprising three ectodomains of 4-1BBL or
20 fragments thereof and wherein the ectodomains of 4-1BBL comprise an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8, particularly the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:5.

195. The pharmaceutical composition of any one of the preceding aspects, wherein the
25 4-1BB agonist is an antigen binding molecule an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP.

196. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or
30 fragments thereof and at least one antigen binding domain capable of specific binding to FAP, wherein 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: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
35 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, or
(b) a heavy chain variable region (V_HFAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:15, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:16, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:17, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:18, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:19, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:20.

197. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP, wherein 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:21 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:22 or wherein 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:23 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:24.

198. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain.

199. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

200. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

201. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one antigen binding domain capable of specific binding to FAP,
(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

202. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one Fab domain capable of specific binding to FAP, and

(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that

- (i) the first polypeptide contains a CH1 or CL domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or
- (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
- (iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

203. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

(a) at least one Fab domain capable of specific binding to FAP comprising a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:21 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:22 or a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:23 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:24, and

(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence

selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

204. The pharmaceutical composition of any one of the preceding aspects, wherein
5 of wherein the 4-1BB agonist is an antigen binding molecule selected from the group consisting of

a) a molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:67 and a second light chain comprising the amino acid sequence of SEQ ID NO:68; and

10 b) a molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:69, a first light chain comprising the amino acid sequence of SEQ ID NO:70, a second heavy chain comprising the amino acid sequence of SEQ ID NO:71 and a second light chain comprising the amino acid sequence of SEQ ID NO:72.

205. The pharmaceutical composition of any one of the preceding aspects, wherein the
15 4-1BB agonist is an antigen binding molecule comprising

(a) at least one antigen binding domain capable of specific binding to FAP,
(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers.

206. The pharmaceutical composition of any one of the preceding aspects, wherein the
20 4-1BB agonist is an antigen binding molecule comprising

(a) at least one antigen binding domain capable of specific binding to FAP,
(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers, and

(c) a Fc domain composed of a first and a second subunit capable of stable association,
25 wherein the polypeptide comprising the three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers is fused to the N- or C-terminal amino acid of one of the two subunits of the Fc domain, optionally through a peptide linker.

207. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an anti-FAP/anti-4-1BB bispecific antibody.

30 208. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA.

209. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or
35 fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises a heavy

chain variable region (V_H CEA) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:49, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:50, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:51, and a light chain variable region (V_L CEA) comprising (iv) CDR-L1 comprising the amino acid sequence of
5 SEQ ID NO:52, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:54.

210. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA,
10 wherein the antigen binding domain capable of specific binding to CEA comprises a heavy chain variable region (V_H CEA) comprising an amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_L CEA) comprising an amino acid sequence of SEQ ID NO:56.

211. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an
15 IgG1 Fc domain or an IgG4 Fc domain.

212. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

213. The pharmaceutical composition of any one of the preceding aspects, wherein the
20 4-1BB agonist is an antigen binding molecule comprising an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

214. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one antigen binding domain capable of specific binding to CEA,
25 (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

215. The pharmaceutical composition of any one of the preceding aspects, wherein the
30 4-1BB agonist is an antigen binding molecule comprising
(a) at least one Fab domain capable of specific binding to CEA, and
(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that
(i) the first polypeptide contains a CH1 or CL domain and the second polypeptide
35 contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and

wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or

- (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
- (iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

216. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

- (a) at least one Fab domain capable of specific binding to CEA comprising a heavy chain variable region (V_{HCEA}) comprising the amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_{LCEA}) comprising the amino acid sequence of SEQ ID NO:56, and
- (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

217. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:108 and a second light chain comprising the amino acid sequence of SEQ ID NO:109.

218. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

(a) at least one antigen binding domain capable of specific binding to CEA,

5 (b) a polypeptide comprising ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers.

219. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

(a) at least one antigen binding domain capable of specific binding to CEA,

10 (b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers, and

(c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the polypeptide comprising the three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers is fused to the N- or C-terminal amino acid of one of the two subunits of the Fc domain, optionally through a peptide linker.

15 220. The pharmaceutical composition of any one of the preceding aspects, wherein the 4-1BB agonist is an anti-CEA/anti-4-1BB bispecific antibody.

221. Use of a combination of an agent blocking PD-L1/PD-1 interaction and a 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen in the manufacture of a medicament for treating or delaying
20 progression of a proliferative disease, in particular cancer.

222. Use of a combination of an agent blocking PD-L1/PD-1 interaction and a 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen in the manufacture of a medicament, wherein the medicament is for the treatment of solid tumors.

25 223. A method for treating or delaying progression of cancer in a subject comprising administering to the subject an effective amount of an agent blocking PD-L1/PD-1 interaction and a 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen.

30 224. A method for treating or delaying progression of cancer in a subject comprising administering to the subject an effective amount of an agent blocking PD-L1/PD-1 interaction and a 4-1BB agonist, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and an antigen binding domain capable of specific binding to a tumor associated antigen.

35 225. The method of any one of the preceding aspects, wherein the 4-1BB agonist is a molecule comprising three ectodomains of 4-1BBL or fragments thereof and wherein the ectodomains of 4-1BBL comprise an amino acid sequence selected from the group consisting

of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8, particularly the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:5.

226. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one moiety capable of specific binding to FAP, wherein 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: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_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, or

(b) a heavy chain variable region (V_H FAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:15, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:16, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:17, and a light chain variable region (V_L FAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:18, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:19, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:20.

227. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP, wherein the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_H FAP) comprising an amino acid sequence of SEQ ID NO:21 and a light chain variable region (V_L FAP) comprising an amino acid sequence of SEQ ID NO:22 or wherein the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V_H FAP) comprising an amino acid sequence of SEQ ID NO:23 and a light chain variable region (V_L FAP) comprising an amino acid sequence of SEQ ID NO:24.

228. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain.

229. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

230. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

- (a) at least one antigen binding domain capable of specific binding to FAP,
(b) a first and a second polypeptide that are linked to each other by a disulfide bond,
wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that
are connected to each other by a peptide linker and in that the second polypeptide comprises
5 one ectodomain of 4-1BBL or a fragment thereof.

231. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an
antigen binding molecule comprising

- (a) at least one Fab domain capable of specific binding to FAP, and
(b) a first and a second polypeptide that are linked to each other by a disulfide bond,
10 wherein the antigen binding molecule is characterized in that
- (i) the first polypeptide contains a CH1 or CL domain and the second polypeptide
contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked
to the first polypeptide by a disulfide bond between the CH1 and CL domain, and
wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments
15 thereof that are connected to each other and to the CH1 or CL domain by a peptide
linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or
a fragment thereof connected via a peptide linker to the CL or CH1 domain of said
polypeptide, or
- (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a
20 CH3 domain, respectively, and wherein the first polypeptide comprises two
ectodomains of 4-1BBL or fragments thereof that are connected to each other and to
the C-terminus of the CH3 domain by a peptide linker and wherein the second
polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected
via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
- (iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second
25 polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein
the second polypeptide is linked to the first polypeptide by a disulfide bond between
the CH1 and CL domain, and wherein the first polypeptide comprises two
ectodomains of 4-1BBL or fragments thereof that are connected to each other and to
30 VH or VL by a peptide linker and wherein the second polypeptide comprises one
ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or
VH of said polypeptide.

232. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an
antigen binding molecule comprising

- 35 (a) at least one Fab domain capable of specific binding to FAP comprising a heavy chain
variable region ($V_{H\text{FAP}}$) comprising the amino acid sequence of SEQ ID NO:21 and a light
chain variable region ($V_{L\text{FAP}}$) comprising the amino acid sequence of SEQ ID NO:22 or a

heavy chain variable region (V_H FAP) comprising the amino acid sequence of SEQ ID NO:23 and a light chain variable region (V_L FAP) comprising the amino acid sequence of SEQ ID NO:24, and

- (b) a first and a second polypeptide that are linked to each other by a disulfide bond,
5 wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID
10 NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

233. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule selected from the group consisting of

- a) a molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a
15 second heavy chain comprising the amino acid sequence of SEQ ID NO:67 and a second light chain comprising the amino acid sequence of SEQ ID NO:68; and
b) a molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:69, a first light chain comprising the amino acid sequence of SEQ ID NO:70, a second heavy chain comprising the amino acid sequence of SEQ ID NO:71 and a second light
20 chain comprising the amino acid sequence of SEQ ID NO:72.

234. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

- (a) at least one antigen binding domain capable of specific binding to FAP,
(b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are
25 connected to each other by peptide linkers.

235. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an anti-FAP/anti-4-1BB bispecific antibody.

236. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and
30 at least one moiety capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises
a heavy chain variable region (V_H CEA) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:49, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:50, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:51, and a light
35 chain variable region (V_L CEA) comprising (iv) CDR-L1 comprising the amino acid sequence

of SEQ ID NO:52, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:54.

237. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and
5 at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises a heavy chain variable region (V_HCEA) comprising an amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_LCEA) comprising an amino acid sequence of SEQ ID NO:56.

238. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an
10 antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain.

239. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

15 240. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one antigen binding domain capable of specific binding to CEA,
(b) a first and a second polypeptide that are linked to each other by a disulfide bond,
wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that
20 are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

241. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
(a) at least one Fab domain capable of specific binding to CEA, and
25 (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that
(i) the first polypeptide contains a CH1 or CL domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and
30 wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or
35 (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two

- ectodomains of 4-1BBL or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to the C-terminus of the CH3 domain of said polypeptide, or
- 5 (iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two
- 10 ectodomains of 4-1BBL or fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

242. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising

- 15 (a) at least one Fab domain capable of specific binding to CEA comprising a heavy chain variable region (V_HCEA) comprising an amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_LCEA) comprising an amino acid sequence of SEQ ID NO:56, and
- (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises
- 20 the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

- 25 243. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:108 and a second light chain comprising the amino acid sequence of SEQ ID NO:109.

- 30 244. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an antigen binding molecule comprising
- (a) at least one antigen binding domain capable of specific binding to FAP,
- (b) a polypeptide comprising three ectodomains of 4-1BBL or fragments thereof that are connected to each other by peptide linkers.

- 35 245. The method of any one of the preceding aspects, wherein the 4-1BB agonist is an anti-CEA/anti-4-1BB bispecific antibody.

246. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method for treating or delaying progression of cancer, wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is used in combination with a 4-1BB agonist and wherein a pretreatment with an Type II anti-CD20 antibody is performed prior to the combination treatment, wherein the period of time between the pretreatment and the combination treatment is sufficient for the reduction of B-cells in the individual in response to the Type II anti-CD20 antibody.

247. The T-cell activating anti-CD3 bispecific antibody for use in a method of aspect 246, wherein the Type II anti-CD20 antibody is obinutuzumab.

247. A method for treating or delaying progression of cancer in a subject comprising administering to the subject a combination of an effective amount of a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen and of an effective amount of a 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, wherein a pretreatment with an Type II anti-CD20 antibody is performed prior to the treatment with the combination, wherein the period of time between the pretreatment and the combination treatment is sufficient for the reduction of B-cells in the individual in response to the Type II anti-CD20 antibody.

248. A method for treating or delaying progression of cancer in a subject comprising administering to the subject a combination of an effective amount of an agent blocking PD-L1/PD-1 interaction and of an effective amount of a 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, wherein a pretreatment with an Type II anti-CD20 antibody is performed prior to the treatment with the combination, wherein the period of time between the pretreatment and the combination treatment is sufficient for the reduction of B-cells in the individual in response to the Type II anti-CD20 antibody.

249. The method of aspects 247 or 248, wherein the Type II anti-CD20 antibody is obinutuzumab.

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.

5 **Recombinant DNA techniques**

Standard methods were used to manipulate DNA as described in Sambrook et al., 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
10 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.

DNA sequencing

DNA sequences were determined by double strand sequencing.

Gene synthesis

15 Desired gene segments were either generated by PCR using appropriate templates or were synthesized by Geneart AG (Regensburg, Germany) from synthetic oligonucleotides and PCR products by automated gene synthesis. In cases where no exact gene sequence was available, oligonucleotide primers were designed based on sequences from closest homologues and the genes were isolated by RT-PCR from RNA originating from the
20 appropriate tissue. The gene segments flanked by singular restriction endonuclease cleavage sites were cloned into standard cloning / sequencing vectors. The plasmid DNA was purified from transformed bacteria and concentration determined by UV spectroscopy. The DNA sequence of the subcloned gene fragments was confirmed by DNA sequencing. Gene segments were designed with suitable restriction sites to allow sub-cloning into the respective
25 expression vectors. All constructs were designed with a 5'-end DNA sequence coding for a leader peptide which targets proteins for secretion in eukaryotic cells.

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
30 Yamada, K.M. (eds.), John Wiley & Sons, Inc.

Protein purification

Proteins were purified from filtered cell culture supernatants referring to standard protocols. In brief, antibodies were applied to a Protein A Sepharose column (GE healthcare) and washed with PBS. Elution of antibodies was achieved at pH 2.8 followed by immediate
5 neutralization of the sample. Aggregated protein was separated from monomeric antibodies by size exclusion chromatography (Superdex 200, GE Healthcare) in PBS or in 20 mM Histidine, 150 mM NaCl pH 6.0. Monomeric antibody fractions were pooled, concentrated (if required) using e.g., a MILLIPORE Amicon Ultra (30 MWCO) centrifugal concentrator, frozen and stored at -20°C or -80°C. Part of the samples were provided for subsequent protein
10 analytics and analytical characterization e.g. by SDS-PAGE, size exclusion chromatography (SEC) or mass spectrometry.

SDS-PAGE

The NuPAGE® Pre-Cast gel system (Invitrogen) was used according to the manufacturer's instruction. In particular, 10% or 4-12% NuPAGE® Novex® Bis-TRIS Pre-
15 Cast gels (pH 6.4) and a NuPAGE® MES (reduced gels, with NuPAGE® Antioxidant running buffer additive) or MOPS (non-reduced gels) running buffer was used.

Analytical size exclusion chromatography

Size exclusion chromatography (SEC) for the determination of the aggregation and oligomeric state of antibodies was performed by HPLC chromatography. Briefly, Protein A
20 purified antibodies were applied to a Tosoh TSKgel G3000SW column in 300 mM NaCl, 50 mM KH₂PO₄/K₂HPO₄, pH 7.5 on an Agilent HPLC 1100 system or to a Superdex 200 column (GE Healthcare) in 2 x PBS on a Dionex HPLC-System. The eluted protein was quantified by UV absorbance and integration of peak areas. BioRad Gel Filtration Standard 151–1901 served as a standard.

25 Mass spectrometry

This section describes the characterization of the multispecific antibodies with VH/VL exchange (VH/VL CrossMabs) with emphasis on their correct assembly. The expected primary structures were analyzed by electrospray ionization mass spectrometry (ESI-MS) of the deglycosylated intact CrossMabs and deglycosylated/plasmin digested or alternatively
30 deglycosylated/limited LysC digested CrossMabs.

The VH/VL CrossMabs were deglycosylated with N-Glycosidase F in a phosphate or Tris buffer at 37°C for up to 17 h at a protein concentration of 1 mg/ml. The plasmin or limited LysC (Roche) digestions were performed with 100 µg deglycosylated VH/VL

CrossMabs in a Tris buffer pH 8 at room temperature for 120 hours and at 37°C for 40 min, respectively. Prior to mass spectrometry the samples were desalted via HPLC on a Sephadex G25 column (GE Healthcare). The total mass was determined via ESI-MS on a maXis 4G UHR-QTOF MS system (Bruker Daltonik) equipped with a TriVersa NanoMate source
5 (Advion).

Determination of binding and binding affinity of multispecific antibodies to the respective antigens using surface plasmon resonance (SPR) (BIACORE)

Binding of the generated antibodies to the respective antigens is investigated by surface plasmon resonance using a BIACORE instrument (GE Healthcare Biosciences AB, Uppsala,
10 Sweden). Briefly, for affinity measurements Goat-Anti-Human IgG, JIR 109-005-098 antibodies are immobilized on a CM5 chip via amine coupling for presentation of the antibodies against the respective antigen. Binding is measured in HBS buffer (HBS-P (10 mM HEPES, 150 mM NaCl, 0.005% Tween 20, pH 7.4), 25°C (or alternatively at 37°C). Antigen (R&D Systems or in house purified) was added in various concentrations in solution.
15 Association was measured by an antigen injection of 80 seconds to 3 minutes; dissociation was measured by washing the chip surface with HBS buffer for 3 - 10 minutes and a KD value was estimated using a 1:1 Langmuir binding model. Negative control data (e.g. buffer curves) are subtracted from sample curves for correction of system intrinsic baseline drift and for noise signal reduction. The respective Biacore Evaluation Software is used for analysis of
20 sensorgrams and for calculation of affinity data.

Example 1

Preparation, purification and characterization of FAP-4-1BBL antigen binding molecules

FAP-targeted 4-1BB ligand trimer-containing Fc fusion antigen binding molecules were
25 prepared as described in International Patent Appl. Publ. No. WO 2016/075278 A1.

In particular, the following molecules were made:

a) Monovalent FAP-targeted and untargeted 4-1BB ligand trimer-containing Fc fusion antigen binding molecules

A polypeptide encoding a dimeric 4-1BB ligand fused to human CL domain was
30 subcloned in frame with the human IgG1 heavy chain CH2 and CH3 domains on the knob (Merchant, Zhu et al. 1998, Nature Biotechnol. 16, 677-681). A polypeptide containing one ectodomain of the 4-1BB ligand was fused to the human IgG1-CH1 domain. In Construct 2.4, in order to improve correct pairing the following mutations were additionally introduced in

the crossed CH-CL (charged variant). In the dimeric 4-1BB ligand fused to human CL, E123R and Q124K, in the monomeric 4-1BB ligand fused to human CH1, K147E and K213E.

The variable region of heavy and light chain DNA sequences encoding a binder specific for FAP, clone 28H1 or clone 4B9, were subcloned in frame with either the constant heavy chain of the hole or the constant light chain of human IgG1. 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 WO 2012/130831. Combination of the dimeric ligand-Fc knob chain containing the S354C/T366W mutations, the monomeric CH1 fusion, the targeted anti-FAP-Fc hole chain containing the Y349C/T366S/L368A/Y407V mutations and the anti-FAP light chain allows generation of a heterodimer, which includes an assembled trimeric 4-1BB ligand and a FAP binding Fab (**Figure 1B**). An untargeted version has been prepared accordingly by replacing the FAP binder by germline DP47 (**Figure 1D**).

Table 1: Monovalent Constructs used in the experiments

	Example in WO 2016/075278	composed of
mono FAP(4B9)-4-1BBL (Charged variant)	Example 2.1.4 (Construct 2.4)	SEQ ID NO:65, SEQ ID NO:66 SEQ ID NO:67 and SEQ ID NO:68
mono FAP(28H1)-4-1BBL	Example 1.1 (Construct 1.2)	SEQ ID NO:69, SEQ ID NO:70 SEQ ID NO:71 and SEQ ID NO:72
mono untargeted DP47-4-1BBL	Example 1.4 (Control B)	SEQ ID NO:69, SEQ ID NO:70 SEQ ID NO:73 and SEQ ID NO:120

a) Bivalent FAP-targeted and untargeted 4-1BB ligand trimer-containing Fc fusion antigen binding molecules

The DNA sequences encoding the heavy and light chain variable regions of heavy and light chain specific a binder specific for FAP, clone 28H1 or clone 4B9, were subcloned in frame with either the constant heavy chain of the hole, the knob or the constant light chain of human IgG1. The Pro329Gly, Leu234Ala and Leu235Ala mutations were 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 WO 2012/130831. Furthermore, a polypeptide

comprising two ectodomains of 4-1BB ligand was fused to the C-terminus of human IgG1 Fc hole chain and a polypeptide comprising one ectodomain of 4-1BB ligand was fused to the C-terminus of human IgG1 Fc knob chain. Combination of the anti-FAP huIgG1 hole dimeric ligand heavy chain containing the Y349C/T366S/L368A/Y407V mutations, the anti-FAP huIgG1 knob monomeric ligand heavy chain containing the S354C/T366W mutations and the anti-FAP light chains allowed generation of a heterodimer, which included an assembled trimeric 4-1BB ligand and two FAP binding Fabs (**Figure 1C**). An untargeted version has been prepared accordingly by replacing the FAP binder by germline DP47 (**Figure 1E**).

Table 2: Bivalent Constructs used in the experiments

	Example in WO 2016/075278	composed of
bi FAP(4B9)-4-1BBL	Example 2.1.3 (Construct 2.3)	2 x SEQ ID NO:68, SEQ ID NO:74 and SEQ ID NO:75
bi FAP(28H1)-4-1BBL	Example 1.1 (Construct 1.5)	2 x SEQ ID NO:72, SEQ ID NO:76 and SEQ ID NO:77
bi untargeted DP47-4-1BBL	Example 2.2 (Control C)	2 x SEQ ID NO:73, SEQ ID NO:78 and SEQ ID NO:79

The production and characterization of the FAP-targeted and untargeted 4-1BB ligand trimer-containing Fc fusion antigen binding molecules is described in detail in WO 2016/075278, Examples 1 to 6, respectively.

Example 2

Preparation, purification and characterization of T-cell bispecific (TCB) antibodies

TCB molecules have been prepared according to the methods described in WO 2014/131712 A1 or WO 2016/079076 A1.

The preparation of the anti-CEA/anti-CD3 bispecific antibody (CEA CD3 TCB or CEA TCB) used in the experiments is described in Example 3 of WO 2014/131712 A1. CEA CD3 TCB is a “2+1 IgG CrossFab” antibody and is comprised of two different heavy chains and two different light chains. Point mutations in the CH3 domain (“knobs into holes”) were introduced to promote the assembly of the two different heavy chains. Exchange of the VH

and VL domains in the CD3 binding Fab were made in order to promote the correct assembly of the two different light chains. 2 +1 means that the molecule has two antigen binding domains specific for CEA and one antigen binding domain specific for CD3. CEACAM5 CD3 TCB has the same format, but comprises another CEA binder and comprises point mutations in the CH and CL domains of the CD3 binder in order to support correct pairing of the light chains.

CEA CD3 TCB comprises the amino acid sequences of SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63 and SEQ ID NO:64. CEACAM5 CD TCB comprises the amino acid sequences of SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59 and SEQ ID NO:60. A schematic scheme of the bispecific antibody in 2+1 format is shown in **Figure 1A**.

The preparation of the anti-FolR1/anti-CD3 bispecific antibody (FolR1 CD3 TCB or FolR1 TCB) used in the experiments is described in WO 2016/079076 A1. FolR1 CD3 TCB is shown as “FolR1 TCB 2+1 classical (common light chain)” in Figure 1D of WO 2016/079076 and is comprised of two different heavy chains and three times the same VLCL light chain (common light chain). Point mutations in the CH3 domain (“knobs into holes”) were introduced to promote the assembly of the two different heavy chains. 2 +1 means that the molecule has two antigen binding domains specific for FolR1 and one antigen binding domain specific for CD3. The CD3 binder is fused at the C-terminus of the Fab heavy chain to the N-terminus of of the first subunit of the Fc domain comprising the knob mutation.

FolR1 CD3 TCB comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO:133, a second heavy chain comprising the amino acid sequence of SEQ ID NO:134 and three times a common light chain of SEQ ID NO: 135.

The preparation of the anti-MCSP/anti-CD3 bispecific antibody (MCSP CD3 TCB) used in the experiments is also described in WO 2014/131712 A1. As CEA CD3 TCB, it is a “2+1 IgG CrossFab” antibody and is comprised of two different heavy chains and two different light chains. MCSP CD3 TCB comprises the amino acid sequences of SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149 and SEQ ID NO:150.

Example 3

Potent anti-tumor effect by combination therapy of FAP-4-1BBL and CEA TCB *in vivo*

30 a) Experiments with mono- and bivalent FAP(4B9)-4-1BBL

The human monovalent FAP-targeted 4-1BBL (mono FAP-4-1BBL, FAP binder 4B9) was tested as single agent and in combination with the human CEA CD3 TCB against the

bivalent FAP-targeted 4-1BBL (bi FAP-4-1BBL, FAP binder 4B9) and the monovalent and bivalent untargeted-4-1BBL as single agent and in combination. Human gastric MKN45 cancer cells were corafted subcutaneously with a mouse fibroblast cell line (3T3) in NOG humanized mice from the Jackson Laboratory.

- 5 Human MKN45 cells (human gastric carcinoma) were originally obtained from ATCC and after expansion deposited in the Glycart internal cell bank. Cells were cultured in DMEM containing 10% FCS at 37 °C in a water-saturated atmosphere at 5 % CO₂. In vitro passage 9 was used for subcutaneous injection at a viability of 97%. Human fibroblasts NIH-3T3 were originally obtained from ATCC, engineered at Roche Nutley to express human FAP and
- 10 cultured in DMEM containing 10% Calf serum, 1 x Sodium Pyruvate and 1.5 µg/ml Puromycin. Clone 39 was used at an in vitro passage number 16 and at a viability of 98%.

50 microliters cell suspension (1x10⁶ MKN45 cells + 1x10⁶ 3T3-huFAP) mixed with 50 microliters Matrigel were injected subcutaneously in the flank of anaesthetized mice with a 22G to 30G needle.

- 15 NSG female mice transferred with human stem cells were delivered by Jackson laboratories; Mice were maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). Experimental study protocol was reviewed and approved by local government (P ZH193/2014). After arrival animals were maintained for one week to get accustomed to new
- 20 environment and for observation. Continuous health monitoring was carried out on regular basis.

- 7 days before cell injection mice were bled and screened for the amount of human t-cells in the blood. Mice were injected sub cutaneously on study day 0 with 1x10⁶ MKN45 cells mixed with 1x10⁶ 3T3 fibroblasts. Tumors were measured 2 to 3 times per week during
- 25 the whole experiment by Caliper. On day 6 mice were randomized for tumor size and human T-cell count with an average T-cell count/µl blood of 165 and an average tumor size of 190-200 mm³. On the day of randomization mice were injected i.v. with Vehicle, CEA CD3 TCB, monovalent FAP-4-1BBL, bivalent FAP-4-1BBL, monovalent untargeted (DP47) 4-1BBL, bivalent untargeted 4-1BBL or the combinations of the 4-1BBL constructs with CEA CD3
- 30 TCB for 3 weeks.

All mice were injected i.v. with 200 µl of the appropriate solution. The mice in the vehicle group were injected with Histidine Buffer and the treatment groups with the 4-1BBL containing constructs, the CEA CD3 TCB or the combinations. To obtain the proper amount of compound per 200 µl, the stock solutions were diluted with Histidine Buffer when

necessary. The dose and schedule used for CEA TCB was 2.5 mg/kg, twice/week whereas the 4-1BBL constructs were given at a dose of 10 mg/kg, once/week.

The experiment was terminated at study day 23. Tumors, blood and spleen were harvested in PBS, single cell suspensions were generated and stained for different immune
5 cell markers and analysed by FACS.

Parts of tumors at termination were formalin fixed and afterwards embedded in Paraffin. Samples were cut and stained for CD3 and CD8.

Table 3: Compositions used in the *in vivo* experiments

Compound	Dose (mg/kg)	Formulation buffer	Concentration (mg/mL)
monovalent untargeted 4-1BBL (DP47)-4-1BBL	10	20 mM Histidine, 140 mM NaCl, 0.01% Tween-20, pH 6.0	3.64 (=stock concentration)
bivalent untargeted 4-1BBL (DP47)-4-1BBL	10	20 mM Histidine, 140 mM NaCl, pH 6.0, 0.01% Tween20	3.17 (=stock concentration)
monovalent FAP (4B9) 4-1BBL	10	20 mM Histidine, 140 mM NaCl, pH 6.0, 0.01% Tween20	3.96 (=stock concentration)
bivalent FAP (4B9) 4-1BBL	10	20 mM Histidine, 140 mM NaCl, pH 6.0, 0.01% Tween20	1.14 (=stock concentration)
CEA CD3 TCB	2.5	20 mM Histidine, 140 mM NaCl, pH 6.0	4.77 (=stock concentration)

10 **Figure 2** shows that the combination CEA CD3 TCB + mono FAP (4B9)-4-1BBL mediated superior efficacy in terms of Tumor growth inhibition compared to all other groups.

Table 4: Tumor growth inhibition (TGI) at study day 21 and 23

Group	TGI day 21 [%]	TGI day 23 [%]
CEA CD3 TCB	43.5	39
monovalent untarg.4-1BBL	16.9	13.6
bivalent untarg.4-1BBL	23.1	9.5
monovalent FAP 4-1BBL	2.1	-5.5
bivalent FAP 4-1BBL	53.3	34.8
CEA CD3 TCB monovalent untarg. 4-1BBL	54.6	68

CEA CD3 TCB bivalent untarg. 4-1BBL	45.2	39
CEA CD3 TCB monovalent FAP 4-1BBL	93.1	91.2
CEA CD3 TCB bivalent FAP 4-1BBL	65.4	57.3

The T cell infiltration in tumor (Figures 3A and 3B), blood (Figures 3C and 3D) and spleen (Figures 3E and 3F) at study termination has been analyzed. The combination of FAP-targeted 4-1BBL compounds with CEA CD3 TCB increases intra-tumoral T-cell frequencies compared to mono-therapies (appr. 300-fold increase compared to vehicle). This increase is mainly due to CD8⁺ T-cell increase. The FAP-targeted 4-1BBL monovalent compound, when combined with CEA CD3 TCB, is superior in mediating the increase in intra-tumoral CD8⁺ T-cell frequency. Intra-tumoral CD8⁺/CD4⁺ ratio strongly increases in the group receiving combination treatment of FAP-targeted 4-1BBL (especially in the monovalent format) with CEA CD3 TCB. FAP-targeted 4-1BBL compounds (especially in the monovalent format) also mediated an increase in T-cell frequency in spleen and blood of the treated mice. A strong increase in intra-tumoral T-cell frequency paralleled by a strong increase in intra-tumoral CD8⁺/CD4⁺ T-cell ratio can be identified as an immuno-PD biomarker of anti-tumor activity of FAP-targeted 4-1BBL compounds, with the monovalent format showing superior activity than the bivalent format. The results of a histological analysis at the end of the study are shown in **Figure 4**.

b) Experiments with FAP(28H1)-4-1BBL

The human monovalent FAP (28H1) 4-1BBL (mono FAP-4-1BBL) was tested as single agent and in combination with the human CEA CD3 TCB against the bivalent FAP (28H1) 4-1BBL (bi FAP-4-1BBL) and the monovalent untargated-4-1BBL as single agent and in combination. Human gastric MKN45 cells were injected subcutaneously in NOG mice from Taconic. 2 Days before the first therapy mice were injected with human PBMCs isolated from buffy coat.

Human MKN45 cells (human gastric carcinoma) were originally obtained from ATCC and after expansion deposited in the Glycart internal cell bank. Cells were cultured in DMEM containing 10% FCS, cells were cultured at 37 °C in a water-saturated atmosphere at 5 % CO₂. In vitro passage 8 was used for subcutaneous injection, at a viability of 97%. 50 microliters cell suspension (1x10⁶ MKN45 cells) mixed with 50 microliters Matrigel were injected subcutaneously in the flank of anaesthetized mice with a 22G to 30G needle on day 0.

Buffy coats were obtained from Züricher Blutspende and PBMCs purified freshly before injection on day 9. 10 Mio PBMCs were injected i.p. in 200ul volume of RPMI w/o at a viability of 94,3%. Buffy coat number H0140143019428 was used. .

NOG female mice were delivered by Taconic. Mice were maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). Experimental study protocol was reviewed and approved by local government (P ZH193/2014). After arrival animals were maintained for one week to get accustomed to new environment and for observation. Continuous health monitoring was carried out on regular basis.

Mice were injected sub cutaneously on study day 0 with 1x10⁶ MKN45 cells. Tumors were measured 2 to 3 times per week during the whole experiment by Caliper. Freshly purified PBMCs were injected on day9, 2 days before the first therapy. One day after randomization, on day 11, mice were injected i.v. with Vehicle, CEA tcb, mono FAP-4-1BBL, bi-FAP-4-1BBL, mono untarg.4-1BBL or the combinations of the 4-1BBL constructs with CEA tcb for up to 3 weeks.

All mice were injected i.v. with 200 µl of the appropriate solution. The mice in the vehicle group were injected with Histidine Buffer and the treatment groups with the 4-1BBL containing constructs, the CEA tcb or the combinations. To obtain the proper amount of compound per 200 µl, the stock solutions were diluted with Histidine Buffer when necessary. The dose and schedule used for CEA tcb was 2.5mg/kg, twice/week whereas the 4-1BBL constructs were given at a dose of 10mg/kg, once/week.

Table 5: Compositions used in the *in vivo* experiments

Compound	Dose (mg/kg)	Formulation buffer	Concentration (mg/mL)
monovalent untargeted 4-1BBL (DP47)-4-1BBL	10	20 mM Histidine, 140 mM NaCl, 0.01% Tween-20, pH 6.0	3.07 (=stock concentration)
monovalent FAP (28H1) 4-1BBL	10	20 mM Histidine, 140 mM NaCl, pH 6.0, 0.01% Tween20	2.3 (=stock concentration)
bivalent FAP (28H1) 4-1BBL	10	20 mM Histidine, 140 mM NaCl, pH 6.0, 0.01% Tween20	3.07 (=stock concentration)
CEA CD3 TCB	2.5	20 mM Histidine, 140 mM NaCl, pH 6.0	4.87 (=stock concentration)

The experiment was terminated at study day 25. Tumors, blood and spleen were harvested in PBS, single cell suspensions were stained for different immune cell markers and analysed by FACS.

Parts of tumors at termination were formalin fixed and afterwards embedded in Paraffin.

5 Samples were cut and stained for CD3 and CD8.

Figure 5 shows that the combination CEA CD3 TCB + mono FAP (28H1)-4-1BBL mediated superior efficacy in terms of Tumor growth inhibition compared to all other groups.

Table 6: Tumor growth inhibition (TGI) at study day 21 and 23

Group	TGI day 21 [%]	TGI day 23 [%]
CEA CD3 TCB	26.7	36.4
monovalent untarg.4-1BBL	24.1	32.2
monovalent FAP 4-1BBL	28.1	27.8
bivalent FAP 4-1BBL	48.4	51.9
CEA CD3 TCB monovalent untarg. 4-1BBL	13.2	72.1
CEA CD3 TCB monovalent FAP 4-1BBL	28.6	44.7
CEA CD3 TCB bivalent FAP 4-1BBL	40.7	53.5

10 To test for significant differences in group means for multiple comparisons, the standard analysis of variance (ANOVA) is automatically produced, using the Dunnett's method. Dunnett's method tests whether means are different from the mean of a control group.

Table 7: p-values: Comparison with a control using Dunnett's method

Group	p-value day 23 vs group A
A: Vehicle	1
B: CEA CD3 TCB	0.255
C: monovalent FAP 4-1BBL	0.247
D: monovalent untarg.4-1BBL	0.087
E: Bivalent FAP-4-1BBL	0.010*
F: CEA CD3 TCB + monovalent FAP-4-1BBL	0.015*
G: CEA CD3 TCB + monovalent untarg 4-BBL	0.033*
H: CEA CD3 TCB + bivalent FAP-4-1BBL	0.022*

The T cell infiltration (CD8/CD4 ratio) in tumor and blood after termination of the study (at day 25) is shown in Figures 6A and 6B.

Example 4

5 **Combination therapy of different concentrations of FAP-4-1BBL and CEA TCB *in vivo***

a) Experimental Procedure

The human monovalent FAP-targeted 4-1BBL (FAP-4-1BBL, FAP binder 4B9) was tested in 2 different concentrations in combination with the human CEA CD3 TCB. Human gastric MKN45 cancer cells were co-grafted subcutaneously with a mouse fibroblast cell line
10 (3T3) in NOG mice humanized mice with human stem cells.

Human MKN45 cells (human gastric carcinoma) were originally obtained from ATCC and after expansion deposited in the Glycart internal cell bank. Cells were cultured in DMEM containing 10% FCS at 37 °C in a water-saturated atmosphere at 5 % CO₂. In vitro passage 12 was used for subcutaneous injection at a viability of 97%. Human fibroblasts NIH-3T3 were
15 originally obtained from ATCC, engineered at Roche Nutley to express human FAP and cultured in DMEM containing 10% Calf serum, 1x Sodium Pyruvate and 1.5ug/ml Puromycin. Clone 39 was used at an in vitro passage number 18 and at a viability of 98.2%.

50 microliters cell suspension (1x10⁶ MKN45 cells + 1x10⁶ 3T3-huFAP) mixed with 50 microliters Matrigel were injected subcutaneously in the flank of anaesthetized mice with a
20 22G to 30G needle.

NOG female mice (purchased from Taconic), aged 5 weeks at start of the experiments, were maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). Experimental study protocol was reviewed and approved by local government authorities (2011-128). After
25 arrival, animals were maintained for one week to get accustomed to the new environment and for observation. Continuous health monitoring was carried out on a daily basis.

For humanization, mice were injected with Busulfan (20mg/kg) followed 24 hours later by injection of 100,000 human HSC (purchased from StemCell Technologies). 15 weeks after stem cell injection, humanized mice were screened for human T-cell frequencies in blood by
30 flow cytometry, and were randomized in the different study groups (see 5.4). Only humanized mice that revealed a humanization rate greater than 20% (i.e. 20% circulating human immune cells within all leucocytes) were used for the experiment.

7-14 days before cell injection mice were bled and screened for the amount of human t-cells in the blood. Mice were randomized for human T cells with an average T cell count/ul blood of 75-81. Mice were injected sub cutaneously on study day 0 with 1×10^6 MKN45 cells mixed with 1×10^6 3T3 fibroblasts. Tumors were measured 2 to 3 times per week during the whole experiment by Caliper. On day16 mice were randomized for tumor size with an average tumor size of 150 mm^3 . One day after randomization, day17, mice were injected i.v. with Vehicle, CEA CD3 TCB and the combinations of the 3 mg/kg or 1mg/kg FAP-4-1BBL with CEA CD3 TCB for 5 weeks.

All mice were injected i.v. with 200 μl of the appropriate solution. The mice in the vehicle group were injected with Histidine Buffer and the treatment groups with the CEA CD3 TCB or the combination of CEA CD3 TCB and FAP-4-1BBL in 2 different doses. To obtain the proper amount of compound per 200 μl , the stock solutions were diluted with Histidine Buffer when necessary. The dose and schedule used for CEA CD3 TCB was 2.5mg/kg, twice/week whereas the 4-1BBL construct was given at a dose of 3mg/kg or 1mg/kg once/week.

The experiment was terminated at study day 52. From vehicle group 6/9 mice were alive, from CEA CD3 treated group 6/9 mice were alive, from CEA CD3 TCB + FAP-4-1BBL 3mg/kg treated group 3/9 were alive and from the combination group with 1mg/kg 2/9 mice were alive. Some mice had to be sacrificed due to bad health status during the experiment.

Spleen and tumor from all remaining mice per group were analysed by flow cytometry at termination. Single cell suspensions were stained for CD45, CD3, CD4 and CD8 and the amount of cells was analysed. Parts of tumors at termination and from animals during the experiment were formalin fixed and afterwards embedded in Paraffin. Samples were cut and stained for CD3 and CD8.

Immunohistochemical images of human MKN45 gastric subcutaneous tumors cogenerated with 3T3 murine fibroblasts derived from the indicated treatment groups in humanized NOG mice were generated. Tissue samples were prepared for immunohistochemical staining. Subcutaneous tumors were harvested from animals at day 52 and during the experiment, fixed in formalin 10% (Sigma, Germany) and later processed for FFPE (Leica 1020, Germany). 4 μm paraffin sections were subsequently cut in a microtome (Leica RM2235, Germany). HuCD8 and HuCD3 immunohistochemistry was performed with anti-human CD8 (Cell Marque Corporation, California) and anti-human CD3 (ThermoFischer Scientific, USA) in the Leica autostainer (Leica ST5010, Germany) following the manufacture's protocols. Quantification of huCD3 and huCD8 positive T cells was performed with Definiens software

(Definiens, Germany). Statistics were analyzed by one way ANOVA with multiple comparison tests. Results showed very low number of T cells in the MNK45/3T3 sc tumors from untreated mice. There is a significant increase of positive CD3 (A) and CD8 (B) T cell number in the CEA CD3 TCB + FAP-4-1BBL 3mg/kg group compared to vehicle and CEA CD3 TCB monotherapy. (statistics One Way ANOVA, Tukey's multiple comparison test, $p < 0.05$). Animals analysed in histology are from different experiment days to increase the number of samples. (Vehicle: 5x day52; CEA CD3 TCB: 5x day52; CEA CD3 TCB + FAP-4-1BBL 3mg/kg: 2xday52, 1x day32, 2xday29; CEA CD3 TCB + FAP-4-1BBL 1mg/kg: 1xday52, 2xday50, 1xday37, 1xday35)

10 b) Results

Already in previous experiments it was shown that CEA CD3 TCB can mediate efficacy in xenograft mouse models via T cell dependent killing of tumor cells. For testing our human constructs human immune cells and specially T cells have to be present in the mouse system. For this reason we use humanized mice meaning mice transferred with human stemcells. These mice develop over time a partially human immune system consisting mainly of T and B cells.

We coinjected MKN45, a CEA expressing human gastric cancer cell line, and 3T3 fibroblasts which improve the stroma component and FAP expression in the tumor. CEA is targeted by the CEA CD3 TCB, crosslinking T cells with tumor cells and inducing T cell mediated killing of tumor cells and T cell activation. Upon T cell activation 4-1BB is upregulated mainly on CD8 positive T cells. FAP-4-1BBL crosslinks FAP expressing fibroblasts and 4-1BB expressing T cells and therefor is inducing 4-1BB signaling. This leads to improved killing capacity, survival and proliferation of the T cells.

We could prove in this study that combination therapy of FAP-4-1BBL in a dose of 3 mg/kg or 1 mg/kg and CEA CD3 TCB leads to superior efficacy compared to the monotherapy of CEA CD3 TCB. As is shown in **Figures 10A and 10B**, T cell infiltration in the tumor at the end of the study is significantly increased in the combination groups compared to all other groups shown by flow cytometry. In histology, the significant increase could only be observed in the combination of CEA CD3 TCB and FAP-4-1BBL 3 mg/kg.

30 **Table 8: Compositions used in the *in vivo* experiments**

Compound	Dose	Formulation buffer	Concentration (mg/mL)
FAP (4B9)-4-1BBL	3 mg/kg and 1 mg/kg	20 mM Histidine, 140 mM NaCl, pH6.0	1.77 (= stock solution)

-185-

		0.01% Tween20	
CEA CD3 TCB	2.5 mg/kg	20 mM Histidine, 240 mM Sucrose, 10 mM Methionine, 0.05% Tween20 , pH 5.5	4.92 (= stock solution)

Tumor growth inhibition based on medians was calculated at study day 35, 39 and 46. In the CEA CD3 TCB monotherapy group 9/9 mice were alive on study day 35 and 39 and 8/9 mice at study day 46. In the combination group with 3mg/kg FAP-4-1BBL at day 35 4/9 and at day 39 and 46 3 /9 mice were alive. In the combination group with 1mg/kg at day 35 6/9 mice were alive and at day39 and 46 5/9 mice were alive. The Group treated with CEA CD3 TCB + FAP-4-1BBL 1mg/kg shows the strongest inhibition of tumor growth.

Table 9: Tumor growth inhibition (TGI) at study day 35, 39 and 46

Group	CEA CD3 TCB	CEA CD3 TCB + FAP-1-1BBL 3mg/kg	CEA CD3 TCB + FAP-4-1BBL 1mg/kg
TGI day35 [%]	33.6	69.8	85.2
TGI day39 [%]	44.6	65.2	94.6
TGI day46 [%]	27.7	69.5	98.5

To test for significant differences in group means for multiple comparisons, the standard analysis of variance (ANOVA) is automatically produced, using the Dunnett's method. Dunnett's method tests whether means are different from the mean of a control group.

Table 10: p-values: Comparison with a control using Dunnett's method (AUC=area under the curve)

Comparison with CEA CD3 TCB using Dunnett's method (AUC)	Day 35		Day 46	
	Mice alive	p-value	Mice alive	p-value
CEA CD3 TCB	9/9	1	8/9	1
Vehicle	8/9	0.3936	8/9	0.0769
CEA CD3 TCB + FAP-41BBL (LEAD) 3mg/kg	4/9	0.4896	3/9	0.0510
CEA CD3 TCB + FAP-41BBL (LEAD) 1mg/kg	6/9	0.0280*	5/9	0.0006*

In **Figures 8A and 8B** the pharmacokinetic profile of the injected compounds during the first week is shown. The T cell infiltration in tumor and spleen at study termination is illustrated in **Figure 9**.

Example 5

5 **Combination therapy of different concentrations of FAP-4-1BBL and CEACAM5 TCB *in vivo***

a) Experimental Procedure

The human FAP-targeted 4-1BBL (FAP-4-1BBL, FAP binder 4B9) was tested in a concentration of 10mg/kg and 1mg/kg in combination with the human CEACAM5 CD3 TCB
10 in a human gastric MKN45 cancer model. MKN45 cells were cocultured sub cutaneously with a mouse fibroblast cell line (3T3) in NSG humanized mice.

Human MKN45 cells (human gastric carcinoma) were originally obtained from DSMZ and after expansion deposited in the Glycart internal cell bank. Cells were cultured in DMEM containing 10% FCS at 37 °C in a water-saturated atmosphere at 5 % CO₂. In vitro passage
15 14 was used for subcutaneous injection at a viability of 99.2%. Human fibroblasts NIH-3T3 were originally obtained from ATCC, engineered at Roche Nutley to express human FAP and cultured in DMEM containing 10% Calf serum, 1x Sodium Pyruvate and 1.5ug/ml Puromycin. Clone 39 was used at an in vitro passage number 9 and at a viability of 98.2%.

50 microliters cell suspension (1x10⁶ MKN45 cells + 1x10⁶ 3T3-huFAP) mixed with
20 50 microliters Matrigel were injected subcutaneously in the flank of anaesthetized mice with a 22G to 30G needle.

NSG female mice (purchased from Charles River), age 5 weeks at start of the experiment, were maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG).
25 Experimental study protocol was reviewed and approved by local government authorities . After arrival, animals were maintained for one week to get accustomed to the new environment and for observation. Continuous health monitoring was carried out on a daily basis.

For humanization, mice were injected with Busulfan (20mg/kg) followed 24 hours later
30 by injection of 100,000 human HSC (purchased from StemCell Technologies).

7-14 days before cell injection mice were bled and screened for the amount of human t-cells in the blood. Mice were randomized for human T cells with an average T cell count/ul

- blood of 40-42. Mice were injected sub cutaneously on study day 0 with 1×10^6 MKN45 cells mixed with 1×10^6 3T3 fibroblasts. Tumors were measured 2 to 3 times per week during the whole experiment by Caliper. On day23 mice were randomized for tumor size with an average tumor size of 350mm³. On day of randomization mice were injected weekly i.v. with
- 5 Vehicle, CEACAM5 CD3 TCB, FAP-4-1BBL 10mg/kg, FAP-4-1BBL 1mg/kg and the combinations of FAP-4-1BBL 10mg/kg or 1mg/kg with CEACAM5 CD3 TCB for 4 weeks.

Table 11: Compositions used in the *in vivo* experiments

Compound	Dose	Formulation buffer	Concentration (mg/mL)
FAP (4B9)-4-1BBL	10 mg/kg and 1 mg/kg	20 mM Histidine, 140 mM NaCl, pH6.0 0.01% Tween20	1.6 (= stock solution)
CEACAM5 CD3 TCB	0.5 mg/kg	20 mM Histidine, 140 mM NaCl, pH6.0 0.01% Tween20	3.1 (= stock solution)

- All mice were injected i.v. with 200 μ l of the appropriate solution. The mice in the
- 10 vehicle group were injected with Histidine Buffer and the treatment groups with the CEACAM5 CD3 TCB, FAP-4-1BBL or the combinations. To obtain the proper amount of compound per 200 μ l, the stock solutions were diluted with Histidine Buffer when necessary. The dose and schedule used for CEACAM5 CD3 TCB was 0.5mg/kg once/week whereas FAP-4-1BBL was given at a dose of 10mg/kg or 1mg/kg once/week.

- 15 Four additional mice were treated in the vehicle group, CEACAM5 CD3 TCB group and the combination groups. The additional mice were taken as scouts on day 33, 72h after the second treatment. The tumors of scouts were analysed for cytokines by Multiplex and T cell infiltration by histology and FACS.

The experiment was terminated at study day 50.

20 **Table 12: Mice alive on day 50**

Group	Vehicle	CEACAM5 CD3 TCB	FAP-4-1BBL 10mg/kg	FAP-4-1BBL 1mg/kg	CEACAM5 CD3 TCB + FAP-4-1BBL 10mg/kg	CEACAM5 CD3 TCB + FAP-4-1BBL 1mg/kg
mice alive day 50	1/10	8/9	3/8	3/8	5/10	3/9

Some mice had to be sacrificed due to bad health status during the experiment.

Spleen and tumor from all remaining mice per group were analysed by flow cytometry and histology at termination day 50. Single cell suspensions were stained for CD45, CD3, CD4 and CD8 and the amount of cells was analysed. Parts of tumors at termination and from animals during the experiment were formalin fixed and afterwards embedded in Paraffin. Samples were cut and stained for CD3 and CD8.

b) Results

In previous experiments it was shown that FAP-4-1BBL can improve the efficacy of CEA CD3 TCB in xenograft mouse models. In this study we wanted to confirm that FAP-4-1BBL can also enhance efficacy of CEACAM5 CD3 TCB mainly mediated by T cells. We used a suboptimal dosis of CEACAM5 CD3 TCB as this construct is already very potent on its own.

To test our human constructs human immune cells and specifically T cells have to be present in the mouse system. For this reason we use humanized mice meaning mice transferred with human stemcells. These mice develop over time a partially human immune system consisting mainly of T and B cells.

We coinjected MKN45, a CEA expressing human gastric cancer cell line, and 3T3 fibroblasts which improve the stroma component and FAP expression in the tumor. CEA is targeted by the CEA CD3 TCB, crosslinking T cells with tumor cells and inducing T cell mediated killing of tumor cells and T cell activation. Upon T cell activation 4-1BB is upregulated mainly on CD8 positive T cells. FAP-4-1BBL crosslinks FAP expressing fibroblasts and 4-1BB expressing T cells and therefor is inducing 4-1BB signaling. This leads to improved killing capacity, survival and proliferation of the T cells.

We could prove in this study that combination therapy of FAP-4-1BBL in a dose of 10mg/kg and CEACAM5 CD3 TCB leads to slightly improved efficacy compared to the monotherapy of CEACAM5 CD3 TCB (see **Figure 11**). T cell infiltration in the tumor in scouts on day 33 as well as at the end of the study is significantly increased in the combination groups (one or both, depending on time point and analysis) compared to all other groups shown by flow cytometry and histology. A strong increase of intratumoral cytokines and chemokines could be observed in scouts at day 33 in the combination of CEACAM5 CD3 TCB and FAP-4-1BBL 10mg/kg.

Table 13: Tumor growth inhibition (TGI) at study day 44 and 47

Group	CEACAM5 CD3 TCB	FAP-1- 1BBL 10mg/kg	FAP-4- 1BBL 1mg/kg	CEACAM5 CD3 TCB + FAP-4- 1BBL 10mg/kg	CEACAM5 CD3 TCB + FAP-4- 1BBL 1mg/kg
Mice alive day 44	8/9	4/8	4/8	6/10	6/9
TGI day44 [%]	47.8	-5.9	36.7	62.1	12.4
Mice alive day47	8/9	3/8	3/8	5/10	4/9
TGI day47[%]	49.0	5.2	33.0	75.9	15.4

To test for significant differences in group means for multiple comparisons, the standard analysis of variance (ANOVA) is automatically produced, using the Dunnett's method.

- 5 Dunnett's method tests whether means are different from the mean of a control group.

Table 14: p-values: Comparison with a control using Dunnett's method (AUC=area under the curve)

Comparison with Vehicle using Dunnett's method (AUC)	Day 47	
	Mice alive	p-value
CEACAM5 CD3 TCB	8/9	0.1683
FAP-1-1BBL 10mg/kg	3/8	0.9508
FAP-4-1BBL 1mg/kg	3/8	0.7808
CEACAM5 CD3 TCB + FAP-4-1BBL 1mg/kg	4/10	0.5380
CEACAM5 CD3 TCB + FAP-4-1BBL 10mg/kg	5/9	0.0500

- 10 In order to study the pharmacokinetic profile of injected compounds during the first week, 2 mice per Group were bled 1h and 72h after 1st and 2nd therapy. Injected compounds were analysed by ELISA (see Figures 12A and 12B).

- 15 (A) Biotinylated human 4-1BB, test sample, Digoxigenin labelled anti-huCH1 antibody and anti-Digoxigenin detection antibody (POD) are added stepwise to a 96-well streptavidin-coated microtiter plate and incubated after every step for 1h at room temperature. The plate is washed three times after each step to remove unbound substances. Finally, the peroxidase-bound complex is visualized by adding ABTS substrate solution to form a colored reaction product. The reaction product intensity, which is photometrically determined at 405nm (with

reference wavelength at 490nm), is proportional to the analyte concentration in the serum sample.

(B) Biotinylated anti-huCD3 -CDR antibody, test sample, Digoxigenin labelled anti-huFc antibody and anti-Digoxigenin detection antibody (POD) are added stepwise to a 96-well streptavidin-coated microtiter plate and incubated after every step for 1h at room temperature. The plate is washed three times after each step to remove unbound substances. Finally, the peroxidase-bound complex is visualized by adding ABTS substrate solution to form a colored reaction product. The reaction product intensity, which is photometrically determined at 405nm (with reference wavelength at 490nm), is proportional to the analyte concentration in the serum sample.

4-1BBL was detected via 4-1BB binding (A), whereas CEA CD3 TCB was detected via binding to anti-CD3 CDR antibody (B).

All groups injected with compounds show comparable exposure of the molecules between the different groups (dose dependent), either FAP-4-1BBL or CEA CD3 TCB.

The T cell infiltration in tumor at study termination was analysed by FACS. Tumors from 3-4 mice/group were analysed by flow cytometry at day 33 and at termination on day 50. Single cell suspensions were stained for CD45, CD3, CD4 and CD8 and the amount of cells was analysed. One Way ANOVA and Tukey's multiple comparison test (with $p < 0.05$) was used as statistical methods (see Figures 13A and 13B).

Combining FAP-4-1BBL 10mg/kg with CEACAM CD3 TCB leads to a statistically increased infiltration of CD8 and CD4 positive T-cells in the tumor at day 33 and/or day 50 compared to vehicle or the TCB (dependent on timepoint).

Immunohistochemical images of human MKN45 gastric subcutaneous tumors co-grafted with 3T3 murine fibroblasts derived from the indicated treatment groups in humanized NOG mice were generated. Tissue samples were prepared for immunohistochemical staining. Subcutaneous tumors were harvested from animals at day 33 (vehicle, CEACAM5 CD3 TCB and the combinations) and at the end of the experiment (except vehicle, only one tumor still available at that timepoint). Tumors were fixed in formalin 10% (Sigma, Germany) and later processed for FFPE (Leica 1020, Germany). 4 μ m paraffin sections were subsequently cut in a microtome (Leica RM2235, Germany). HuCD8 immunohistochemistry was performed with anti-human CD8 (Cell Marque Corporation, California) in the Leica autostainer (Leica ST5010, Germany) following the manufacturer's protocols. Quantification of huCD8 positive T cells was performed with Definiens software (Definiens, Germany). Statistics were analyzed by one way ANOVA with multiple comparison tests. Results showed very low

number of T cells in the MNK45/3T3 sc tumors from untreated mice at day 33 and at termination day 50. Also monotherapies of FAP-4-1BBL don't show an increase of T-cells in the tumor at the end of the experiment. There is a significant increase of positive CD8 T cells in the CEACAM5 CD3 TCB + FAP-4-1BBL 1mg/kg group compared to CEACAM5 CD3 TCB monotherapy on day33 (see **Figure 14A**). At study termination on day50 both combination groups show a significant increase of CD8 T cells compared to CEACAM5 CD3 TCB monotherapy (see **Figure 14B**). (statistics One Way ANOVA, Tukey's multiple comparison test, $p < 0.05$).

No differences could be observed in CD4 T cell infiltration (data not shown).

For the Cytokine Analysis in tumor and serum at the end of the study, serum was collected and 20-30 mg of snap-frozen tumor tissues were processed for whole protein isolation at day 33. Briefly, tissue samples were meshed by using the Tissue Lyser system and stainless steel beads in a total volume of 150 μ l of lysis buffer. Meshed samples were cleared by centrifugation and whole protein content was analysis by BCA protein assay kit in the supernatant. At total of 200 μ g of whole protein of tumor and spleen lysates as well as a 1:10 dilution of serum samples were used for the analysis of different cytokines/chemokines by the Bio-Plex system following instructions of manufacturer (Bio-Plex Pro™ Human Cytokine 40-plex Assay, BioRad).

An increase of several cytokines was observed in the serum and in tumor upon combination of CEACAM5 CD3 TCB and FAP-4-1BBL compared to the monotherapy of CEACAM5 CD3 TCB.

Statistical significant increased cytokines in the tumor CEACAM5 CD3 TCB+ FAP-4-1BBL 10mg/kg vs. CEACAM5 CD3 TCB alone were: CXCL10, 13, 5, CCL1, 11, 21, 22, IL12, MIP-3a, MIP-1a, TECK, MCP3 and MCP4 (statistics analysed with One-way ANOVA, values not shown).

In Figures 15A, 15B and 15C examples of increases of intratumoral cytokines are shown: CCL1, TECK, CCL2, CXCL13, CXCL12, IL16, MIP-3a.

Example 6

Preparation, purification and characterization of CEA-4-1BBL antigen binding molecules

CEA-targeted 4-1BB ligand trimer-containing Fc fusion antigen binding molecules were prepared as described in International Patent Appl. Publ. No. WO 2016/075278 A1.

In particular, monovalent CEA-targeted and untargeted 4-1BB ligand trimer-containing Fc fusion antigen binding molecules were made.

A polypeptide encoding a dimeric 4-1BB ligand fused to the human CL domain was subcloned in frame with the human IgG1 heavy chain CH2 and CH3 domains on the knob
 5 (Merchant, Zhu et al. 1998, Nature Biotechnol. 16, 677-681). A polypeptide containing one ectodomain of the 4-1BB ligand was fused to the human IgG1-CH1 domain. In order to improve correct pairing the following mutations were additionally introduced in the crossed CH-CL (charged variant): In the dimeric 4-1BB ligand fused to human CL, E123R and Q124K, in the monomeric 4-1BB ligand fused to human CH1, K147E and K213E.

10 The variable region of heavy and light chain DNA sequences encoding a binder specific for CEA, clone T84.66-LCHA (CEACAM5), were subcloned in frame with either the constant heavy chain of the hole or the constant light chain of human IgG1. The Pro329Gly, Leu234Ala and Leu235Ala mutations were introduced in the constant region of the knob and
 15 described in WO 2012/130831. Combination of the dimeric ligand-Fc knob chain containing the S354C/T366W mutations, the monomeric CH1 fusion, the targeted anti-CEA-Fc hole chain containing the Y349C/T366S/L368A/Y407V mutations and the anti-CEA light chain allows generation of a heterodimer, which includes an assembled trimeric 4-1BB ligand and a CEA binding Fab (in analogy to **Figure 1B**, anti-FAP replaced by anti-CEA).

20 **Table 15: Monovalent Construct used in the experiments**

	Example in WO 2016/075278	composed of
mono CEA (T84.66-LCHA)-4-1BBL (Charged variant)	Example 11.2.4 (Construct 5.4)	SEQ ID NO:65, SEQ ID NO:66 SEQ ID NO:108 and SEQ ID NO:109

The production and characterization of the CEA-targeted and untargeted 4-1BB ligand trimer-containing Fc fusion antigen binding molecules is described in detail in WO 2016/075278, Examples 11 to 13, respectively.

Example 7**Combination therapy of different concentrations of CEA-4-1BBL antigen binding molecules and CEA TCB *in vivo******a) Experimental Procedure***

5 The human CEA targeted 4-1BBL (CEA-4-1BBL, CEA binder T84.66-LCHA or CEACAM5) was tested in a concentration of 10, 3 and 1 mg/kg in combination with the human CEA CD3 TCB in a human gastric MKN45 cancer model. The binder used in the CEA CD3 TCB is CH1A1A 98/99 x 2F1 and not competing with the binder used in the CEA-4-1BBL. MKN45 cells were co-grafted subcutaneously with a mouse fibroblast cell line (3T3) in
10 NSG humanized mice.

 Human MKN45 cells (human gastric carcinoma) were originally obtained from DSMZ and after expansion deposited in the Glycart internal cell bank. Cells were cultured in DMEM containing 10% FCS at 37 °C in a water-saturated atmosphere at 5 % CO₂. In vitro passage 13 was used for subcutaneous injection at a viability of 99.1%. Human fibroblasts NIH-3T3
15 were originally obtained from ATCC, engineered at Roche Nutley to express human FAP and cultured in DMEM containing 10% Calf serum, 1x Sodium Pyruvate and 1.5ug/ml Puromycin. Clone 39 was used at in vitro passage number 8 and at a viability of 97.6%.

 50 microliters cell suspension (1×10^6 MKN45 cells + 1×10^6 3T3-huFAP) mixed with 50 microliters Matrigel were injected subcutaneously in the flank of anaesthetized mice with a
20 22G to 30G needle.

 NSG female mice (purchased from Charles River), age 5 weeks at start of the experiment, were maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). Experimental study protocol was reviewed and approved by local government authorities.
25 After arrival, animals were maintained for one week to get accustomed to the new environment and for observation. Continuous health monitoring was carried out on a daily basis.

 For humanization, mice were injected with Busulfan (20mg/kg) followed 24 hours later by injection of 100,000 human HSC (purchased from StemCell Technologies).

30 7-14 days before cell injection mice were bled and screened for the amount of human t-cells in the blood. Mice were randomized for human T cells with an average T cell count/ul blood of 131. Mice were injected sub cutaneously on study day 0 with 1×10^6 MKN45 cells mixed with 1×10^6 3T3 fibroblasts. Tumors were measured 2 to 3 times per week during the

whole experiment by Caliper. On day 17 mice were randomized for tumor size with an average tumor size of 205 mm³. On day of randomization mice were injected weekly i.v. with Vehicle, CEA CD3 TCB and the combinations of CEA CD3 TCB with CEA-4-1BBL at a dose of 10mg/kg, 3mg/kg or 1mg/kg for up to 4 weeks. All mice were injected i.v. with 200 μ l of the appropriate solution. The mice in the vehicle group were injected with Histidine Buffer and the treatment groups with the CEA CD3 TCB and the combinations of CEA CD3 TCB plus CEA-4-1BBL. To obtain the proper amount of compound per 200 μ l, the stock solutions were diluted with Histidine Buffer when necessary. The dose and schedule used for CEA CD3 TCB was 2.5mg/kg twice/week whereas CEA-4-1BBL was given at a dose of 10 mg/kg, 3 mg/kg or 1 mg/kg once/week.

The experiment was terminated at study day 44.

Table 16: Mice alive on day 44

Group	Vehicle	CEA CD3 TCB	CEA CD3 TCB + CEA-4-1BBL 10mg/kg	CEA CD3 TCB + CEA-4-1BBL 3mg/kg	CEA CD3 TCB + CEA-4-1BBL 1mg/kg
mice alive day 44	7/9	5/9	5/10	7/9	7/9

Some mice had to be sacrificed due to bad health status during the experiment.

3-4 Tumors from remaining mice per group were analysed by flow cytometry and histology at termination day 44 if tumors provided enough material. Single cell suspensions were stained for CD45, CD3, CD4 and CD8 and the amount of cells was analysed. Parts of tumors at termination and from animals during the experiment were formalin fixed and afterwards embedded in Paraffin. Samples were cut and stained for CD3 and CD8.

b) Results

In this study we wanted to prove for the first time that a tumor targeted 4-1BBL (CEA) can improve efficacy of a T-cell bispecific (TCB). We choose as a target for the TCB as well as for the 4-1BBL CEA although using different and not competing binders. The CEA-4-1BBL was given weekly at 3 different doses (10, 3 and 1 mg/kg) to evaluate dose dependency whereas the CEA CD3 TCB was given at the optimal does of 2.5 mg/kg twice per week.

To test our human constructs human immune cells and specifically T cells have to be present in the mouse system. For this reason we used humanized mice meaning mice

transferred with human stem cells. These mice develop over time a partially human immune system consisting mainly of T and B cells.

We coinjected MKN45, a CEA expressing human gastric cancer cell line, and 3T3 fibroblasts which improve the stroma component in the tumor. CEA is targeted by the CEA CD3 TCB, crosslinking T cells with tumor cells and inducing T cell mediated killing of tumor cells and T cell activation. Upon T cell activation 4-1BB is upregulated mainly on CD8 positive T cells. CEA-4-1BBL crosslinks CEA expressing tumor cells and 4-1BB expressing T cells and is therefore inducing 4-1BB signaling. This leads to improved killing capacity, survival and proliferation of the T cells.

We could prove in this study that the combination of CEA-4-1BBL in a dose of 10 mg/kg, 3 mg/kg or 1 mg/kg and CEA CD3 TCB leads to improved efficacy compared to the monotherapy of CEA CD3 TCB (see **Figure 16**). The combination with CEA-4-1BBL at 10 mg/kg or 3 mg/kg leads to stronger tumor growth inhibition than the combination with 1 mg/kg. T-cell infiltration in the tumor at termination on day 44 is significantly increased in the combination groups compared to vehicle and CEA CD3 TCB monotherapy shown by flow cytometry (see **Figures 18A-18C**). The analysis by histology shows only a significant increase of CD8 and CD3 positive T-cells (see **Figures 19A and 19B**, respectively) in the combination groups with 10 and 3 mg/kg but not with 1 mg/kg.

Table 17: Compositions used in the *in vivo* experiments

Compound	Dose	Formulation buffer	Concentration (mg/mL)
CEA-4-1BBL	10 mg/kg, 3 mg/kg and 1 mg/kg	20 mM Histidine, 140 mM NaCl, pH6.0 0.01% Tween20	3.78 (= stock solution)
CEA CD3 TCB	2.5 mg/kg	20 mM Histidine, 140 mM NaCl, 0.01% Tween20, pH 6.0	4.82 (= stock solution)

Tumor growth inhibition based on medians was calculated at study day 36, 38, 41 and 43. The Group treated with CEA CD3 TCB + CEA-4-1BBL 10mg/kg shows the strongest inhibition of tumor growth.

Table 18: Tumor growth inhibition (TGI) at study day 36, 38, 41 and 43

Group	Day 36	Day 38	Day 41	Day 43
CEA CD3 TCB	62.8	55.1	47.3	32.7
CEA CD3 TCB +	93.8	93.0	92.7	112.5

CEA-4-1BBL 10mg/kg				
CEA CD3 TCB + CEA-4-1BBL 3mg/kg	67.6	85.0	86.2	102.1
CEA CD3 TCB + CEA-4-1BBL 1mg/kg	51.6	66.2	73.1	83.5

To test for significant differences in group means for multiple comparisons, the standard analysis of variance (ANOVA) is automatically produced, using the Dunnett's method. On day 43 the combination of CEA CD3 TCB + CEA-4-1BBL 10 mg/kg is significantly different from CEA CD3 TCB monotherapy (see Table 19).

Table 19: One Way Analysis of tumor volumes on day 43, comparison with CEA CD3 TCB

Means Comparisons with a control using Dunnett's Method (d43) (Control Group = CEA CD3 TCB)	p-Value
Vehicle	0.1197
CEA CD3 TCB	1.0000
CEA CD3 TCB + CEA-4-1BBL 1 mg/kg	0.3451
CEA CD3 TCB + CEA-4-1BBL 3 mg/kg	0.0713
CEA CD3 TCB + CEA-4-1BBL 10 mg/kg	0.0167

The combination of CEA CD3 TCB + CEA-4-1BBL 10 mg/kg and 3 mg/kg are significantly different from vehicle considering the area under the curve (AUC) until day 43 as shown in Table 20.

Table 20: One Way Analysis of sAUC until day 43, comparison with Vehicle (AUC=area under the curve)

Comparisons with a control using Dunnett's Method (sAUC) (Control Group = vehicle)	p-Value
Vehicle	1.0000
CEA CD3 TCB	0.0563
CEA CD3 TCB + CEA-4-1BBL 1 mg/kg	0.0527
CEA CD3 TCB + CEA-4-1BBL 3 mg/kg	0.0084
CEA CD3 TCB + CEA-4-1BBL 10 mg/kg	0.0005

In **Figures 17A and 17B** the pharmacokinetic profile of the injected compounds during the first week is shown. In order to study the pharmacokinetic profile of injected compounds

during the first week, 2 mice per Group were bled 1h and 72h after 1st and 2nd therapy. Injected compounds were analysed by ELISA.

(A) Biotinylated human 4-1BB, test sample, Digoxigenin labelled anti-huCH1 antibody and anti-Digoxigenin detection antibody (POD) are added stepwise to a 96-well streptavidin-coated microtiter plate and incubated after every step for 1h at room temperature. The plate is washed three times after each step to remove unbound substances. Finally, the peroxidase-bound complex is visualized by adding ABTS substrate solution to form a colored reaction product. The reaction product intensity which is photometrically determined at 405 nm (with reference wavelength at 490 nm) is proportional to the analyte concentration in the serum sample.

(B) Biotinylated anti-huCD3 -CDR antibody, test sample, Digoxigenin labelled anti-huFc antibody and anti-Digoxigenin detection antibody (POD) are added stepwise to a 96-well streptavidin-coated microtiter plate and incubated after every step for 1h at room temperature. The plate is washed three times after each step to remove unbound substances. Finally, the peroxidase-bound complex is visualized by adding ABTS substrate solution to form a colored reaction product. The reaction product intensity which is photometrically determined at 405 nm (with reference wavelength at 490 nm) is proportional to the analyte concentration in the serum sample.

4-1BBL was detected via 4-1BB binding (A), whereas CEA CD3 TCB was detected via binding to anti-CD3 CDR antibody (B).

All groups injected with compounds show comparable exposure of the molecules between the different groups (dose dependent), either CEA-4-1BBL or CEA CD3 TCB.

The T cell infiltration in tumor at study termination was analysed by FACS. Tumors from 3-4 mice/group were analysed by flow cytometry at day 44. Single cell suspensions were stained for CD45, CD3, CD4 and CD8 and the amount of cells was analysed. One Way ANOVA and Tukey's multiple comparison test (with $p < 0.05$) was used as statistical methods. The T cell infiltration in tumor at study termination is illustrated in **Figures 18A, 18B and 18C**.

Combining CEA-4-1BBL in doses 10, 3 or 1 mg/kg with CEA CD3 TCB leads to a statistically increased infiltration of CD3, CD8 and CD4 (in particular for combination with 10 mg/kg CEA-4-1BBL) positive T-cells in the tumor at day 44 compared to CEA CD3 TCB monotherapy.

Immunohistochemical images of human MKN45 gastric subcutaneous tumors cografted with 3T3 murine fibroblasts derived from the indicated treatment groups in humanized NOG mice were generated. Tissue samples were prepared for immunohistochemical staining. Subcutaneous tumors were harvested from animals at the end of the study, day 44 (vehicle, CEA CD3 TCB and the combinations). Tumors were fixed in formalin 10% (Sigma, Germany) and later processed for FFPET (Leica 1020, Germany). 4 µm paraffin sections were subsequently cut in a microtome (Leica RM2235, Germany). HuCD8 and HuCD3 immunohistochemistry was performed with anti-human CD8 (Cell Marque Corporation, California) and anti-human CD3 (ThermoFischer Scientific, USA) in the Leica autostainer (Leica ST5010, Germany) following the manufacture's protocols. Quantification of huCD8 and CD3 positive T cells was performed with Definiens software (Definiens, Germany). Statistics were analyzed by one way ANOVA with multiple comparison tests. Results showed very low number of T cells in the MNK45/3T3 sc tumors from untreated mice at termination day 44. There is a significant increase of CD3 positive T cells (**Figure 19A**) and CD8 positive T cells (**Figure 19B**) in the groups treated with CEA CD3 TCB + CEA-4-1BBL 10mg/kg and 3 mg/kg group compared to CEA CD3 TCB monotherapy and vehicle (statistics One Way ANOVA, Tukey's multiple comparison test, $p < 0.05$).

Example 8

***In vitro* co-culture assays with human immune effector cells**

The immune functions of T cells were tested in *in vitro* co-culture assays with human immune effector cells (resting PBMC, CD4 or CD8 T cells, NLV specific CD8 T effector memory cells), target antigen positive tumor cells and FAP positive fibroblasts in the presence of TCBs (CEA CD3 TCB (2), CEA TCB CD3) and FAP 4-1BBL). The evaluated tumor cell line was the gastric cancer cell line MKN-45. The mouse embryonic fibroblast cell line NIH/3T3 transduced to express human FAP was used as FAP positive fibroblast. Effector cells were resting human PBMC, isolated resting CD4 or CD8 T cells. In some cases also antigen specific effector memory CD8 T cells were used. In some assays, TNF- α sensor cells were added to monitor TNF- α induction. Tumor cell lysis (kinetic high content life imaging, endpoint flow cytometry), expression of cell surface activation and maturation markers (endpoint flow cytometry) and cytokine secretion (kinetic high content life imaging, endpoint cytometric bead array) was used to monitor the extent of T cell function induced by TCBs and modulated by FAP targeted immune modulators.

a) Target cell lines and fibroblasts

The MKN45 NucLight Red (NLR) cells naturally express CEA. MKN-45 (ATCC, Cat. No. TCP-1008) were transduced with the Essen CellPlayer NucLight Red Lentivirus

(Essenbioscience, Cat. No. 4476; EF1 α , puromycin)) at an MOI of 3 (TU/cell) in the presence of 8 μ g/ml polybrene following the manufacturers instructions to stable express the NucLight Red fluorescent protein restricted to the nucleus. This enables easy separation from non-fluorescent effector T cells or fibroblasts and monitoring of the tumor cell growth by high
5 through put life fluorescence microscopy. Quantification of red events or mena integrated red fluorescence per well over time allows thus real-time assessment of tumor cell lysis or proliferation.

MKN45 NucLight Red cells were cultured in DMEM (GIBCO, Cat.No42430-082) containing 10 % Fetal Bovine Serum (FBS, Gibco by Life Technology, Cat. No. 16000-044,
10 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) and 0.5 μ g/mL Puromycin (Sigma-Aldrich, Cat. No. ant-pr-1).

The crosslinking of FAP-binding antibodies by cell surface FAP was provided by human fibroblast activating protein (huFAP) expressing NIH/3T3-huFAP clone 19. This cell
15 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. Cells were cultured in DMEM (GIBCO, Cat.No. 42430-082) containing 10 % calf serum (Sigma-Aldrich, Cat. No. C8056-500ml, gamma-irradiated, mycoplasma-free and heat inactivated at 56 °C for 35 min) and 1.5 μ g/mL Puromycin (Sigma-Aldrich, Cat. No. ant-pr-1).

20 *b) Preparation of Effector cells*

Buffy coats were obtained from the Zürich blood donation center. 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
25 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 RPMI 1640 medium (Gibco by Life
30 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 μ M β -Mercaptoethanol
35 (SIGMA, M3148). In some cases, RPMI1640 was replaced by FluoroBrite DMEM media

(GIBCO, Invitrogen, Cat No A18967-01) for improved high content live microscopy with reduced background fluorescence.

PBMCs were used as effector cells directly after isolation (resting human PBMCs) or certain subfractions, as resting CD4 T cells or CD8 T cells, were isolated using the untouched
 5 human CD4+ T cell isolation kit (Miltenyi, Ca.No. 130-096-533) and untouched human CD8+ T cell isolation kit (Miltenyi, Ca.No. 130-096-495) according to manufacturers instructions, respectively. Briefly, human PBMC were centrifuged for 8 min at 400 x g, 4°C and were washed once with MACS buffer (PBS + BSA (0.5 % v/w, Sigma-Aldrich, Cat. No. A9418) + EDTA ([2nM], Ambion, AM9261)). The pellet was resuspended with the
 10 respective provided streptavidin labeled negative antibody cocktail and incubated for 5 minutes at 4 °C (per 1*10⁷ cells 40 µL MACS buffer and 10 µL antibody mix) followed by a subsequent incubation with biotinylated magnetic capture beads (per 1*10⁷ cells 30 µL MACS buffer and 20 µL bead mix) for 10 min at 4 °C. Labeled non-CD4 or non-CD8 T cells were removed by magnetic separation using an LS column (Miltenyi, Ca.No. 130-042-401)
 15 according to manufacturer's instructions. The column flow through, containing unlabeled resting CD4 and CD8 T cells, respectively, was centrifuged and washed once with MACS buffer as described above. Cells were adjusted to 2 mio cells/mL in RPMI1640 or Fuorobright DMEM based T cell media.

c) Isolation and culture of antigen-specific CD8 T cells

20 In some assays antigen specific CD8 T cells were used as effector cells. Fresh blood was obtained from a HLA-A2⁺ CMV-infected volunteer. PBMCs were isolated as described above. CD8 T cells were purified from PBMCs using a negative selection human CD8 T cell isolation Kit according to manufacturer's recommendations (Miltenyi Biotec, Cat. No. 130-094-156) as described above. Ten million of isolated CD8 T cells were resuspended in 1 mL
 25 sterile DPBS supplemented with 1 % (v/v) FBS along with 50 µL of PE-labeled HLA-A2-pentamer containing the CMV-derived NLVPMVATV peptide (ProImmune, Cat. No. F008-2B) and incubated for 10 min at room temperature. Cells were washed twice with 3 mL sterile DPBS supplied with 1 % (v/v) FBS. Cells were resuspended in 1 mL cells DPBS supplied with 1 % (v/v) FBS containing 1 µg/mL anti-human CD8-FITC (clone LT8, Abcam, Cat. No.
 30 Ab28010) and incubated for 30 minutes at 4°C. Cells were washed twice, resuspended to a concentration of 5x10⁶ cells/mL in DPBS supplied with 1 % (v/v) FBS, and filtrated through a 30 µm pre-separation nylon-net cell strainer (Miltenyi Biotec, Cat. No. 130-041-407). NLV-peptide-specific CD8⁺ T cells were isolated by FACS sorting using an ARIA cell sorter (BD Bioscience with DIVA software) with the following settings: 100 µm nozzle and purity sort mask. Sorted cells were collected in a 15 ml polypropylene centrifuge tube (TPP, Cat. No.
 35 91015) containing 5 ml RPMI 1640 medium supplied with 10 % (v/v) FBS, 1 % (v/v)

GlutaMAX-I and 400 U/mL Proleukin. Sorted cells were centrifuged for 7 minutes at 350 x g at room temperature and resuspended in same medium to a concentration of 0.53×10^6 cells/mL. 100 μ L/well of this cell suspension were added to each well of a previously prepared feeder plate.

- 5 PHA-L-activated irradiated allogeneic feeder cells were prepared from PBMCs as previously described (Levitsky et al., 1998) and distributed to 96 well culture plates at 2×10^5 feeder cells per well.

- After one day of culturing 100 μ L medium/well were removed from well containing sorted CD8⁺ T-cells and replaced by new RPMI 1640 medium supplemented with 10 % (v/v) FBS and 1 % (v/v) GlutaMAX-I and 400 U/mL Proleukin, this was repeated during culture on a regular basis (every 2-4 days). As soon as cells start to proliferate, they were transferred to 24-well flat-bottom tissue culture plate (TPP, 92024). Cells were expanded/split and reactivated with new feeder cell preparation on a regular basis.
- 10

d) TNF- α sensor cells

- 15 TNF- α sensor cells were HEK 293T cells (ATCC, Cat. No. xxx) transduced with the reporter plasmid pETR14327 encoding for green fluorescent protein (GFP) under the control of an NF κ B sensitive promotor element. HEK 293T cells express naturally the TNF receptor to which TNF- α secreted by activated T cells can bind. This leads to dose dependent activation of NF κ B and translocation to the nucleus, which in turn switches on dose
- 20 dependent GFP production. The GFP fluorescence can be quantified by high through put life fluorescence microscopy over time and allows thus real-time assessment of TNF- α secretion.

e) Cytotoxicity and T cell activation assay

- Mouse embryonic fibroblast NIH/3T3-huFAP cells, TNF- α sensor cells and MKN45 NLR cells were harvested using cell dissociation buffer (Invitrogen, Cat.-No. 13151-014) for 10 minutes at 37°C. Cells were washed once with DPBS. TNF- α sensor cells or fibroblasts were irradiated in an xRay irradiator using a dose of 4500 RAD to prevent later overgrowth of effector or tumor cell lines. Target cell lines, NIH/3T3-huFAP and in some assays TNF- α sensor cells were cultured at a density of 0.1×10^5 cells per well in T cell media in a sterile 96-well flat bottom adhesion tissue culture plate (TPP, Cat. No. 92097) overnight at 37 °C and in
- 25
- 30 5% CO₂ in an incubator (Hera Cell 150).

Resting human PBMC, human CD4 T cells, human CD8 T cells or NLV-specific T cells were prepared as described above and were added at a density of 0.5×10^5 cells per well. A serial dilution row of TCBs (CEA CD3 TCB or CEA CD3 TCB (2)) and a fixed

concentration of FAP 4-1BBL (2 nM) was added to a total volume of 200 μ L per well. Cells were cocultured for up to 72 hours at 37 °C and 5% CO₂ in an incubator (Hera Cell 150).

In some assays, plates were monitored by fluorescence microscopy high content life imaging using the Incucyte Zoom System (Essenbioscience, HD phase-contrast, green
5 fluorescence and red fluorescence, 10 x objective) in a 3 hours interval for up to 72 hours at 37 °C and 5% CO₂. The integrated red fluorescence of healthy tumor cells (RCUxum2/image), which is proportional to the amount of NLR⁺ cells per well, was quantified using the IncucyteZoom Software to monitor tumor cell growth vs lysis by T cells. Values were plotted for the respective time point and conditions against the used TCB concentration to analyse
10 effects on the cytolytic potential of T cells.

In some assays where TNF- α sensor cells were present, the integrated green RCUxum2/image was quantified using the IncucyteZoom Software to monitor TNF- α induced production of GFP by the TNF- α sensor cells. Values were plotted for the respective time point and conditions against the used TCB concentration to analyze effects on TNF- α
15 secretion by T cells.

After 72 hrs, the supernatant was collected for subsequent analysis of selected cytokine using the cytometric bead array according to manufacturer's instructions. Evaluated cytokines were IL-2 (Human IL-2 CBA Flex- set (Bead A4), BD Bioscience, Ca.No. 558270), IL-17A (Human IL-17A CBA Flex- set (Bead B5), BD Bioscience, Ca.No. 560383), TNF- α (Human
20 TNF- α CBA Flex- set (Bead C4), BD Bioscience, Ca.No. 560112), IFN- γ (IFN- γ CBA Flex- set (Bead E7), BD Bioscience, Ca.No. 558269), IL-4 (Human IL-4 CBA Flex- set (Bead A5), BD Bioscience, Ca.No. 558272), IL-10 (Human IL-10 CBA Flex- set (Bead B7), BD Bioscience, Ca.No. 558274) and IL-9 (Human IL-9 CBA Flex- set (Bead B6), BD Bioscience, Ca.No. 558333).

25 Thereafter, all cells were detached from the wells by incubation with cell dissociation buffer for 10 minutes at 37°C followed by centrifugation at 400 x g at 4°C. Pellets were washed with ice cold FACS buffer (DPBS (Gibco by Life Technologies, Cat. No. 14190 326) w/ BSA (0.1 % v/w, Sigma-Aldrich, Cat. No. A9418). Cells were surface-stained with fluorescent dye-conjugated antibodies anti-human CD4 (clone RPA-T4, BioLegend, Cat.-No.
30 300532), CD8 (clone RPa-T8, BioLegend, Cat.-No. 3010441), CD62L (clone DREG-56, BioLegend, Cat.-No. 304834), CD127 (clone 019D5, BioLegend, Cat.-No. A019D5), CD134 (clone Ber-ACT35, BioLegend, Cat.-No. 350008), CD137 (clone 4B4-1, BioLegend, Cat.-No. 309814), GITR (clone 621, BioLegend, Cat.-No. 3311608) and CD25 (clone M-A251, BioLegend, Cat.-No. 356112) for 20 min at 4°C in FACS buffer. Then, they were washed
35 once with FACS buffer before being resuspended in 85 μ L/well FACS buffer containing

containing 0.2 µg/mL DAPI (Santa Cruz Biotech, Cat. No. Sc-3598) before they were acquired the same day using 5-laser LSR-Fortessa (BD Bioscience with DIVA software). Living CD4 and CD8 T cells were gated (DAPI-, NucLight RED-, CD4 or CD8+) and counts, the mean fluorescence intensity (MFI) of activation marker (CD134, CD137, GITR, CD25) or
5 maturation marker (CD127, CD62L) or the percentage of positive cells were plotted for the respective conditions against the used TCB concentration to analyze effects on T activation.

Results

8.1 T cell bispecific antibodies induce a dose dependent upregulation of 4-1BB on CD8 and CD4 T cells

10 Different human immune effector cell preparations (resting PBMC, CD4 or CD8 T cells, NLV specific CD8 T effector memory cells) were cocultured with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEACAM5 CD3 TCB for 48 hrs. The amount of living tumor cells was quantified by fluorescence
15 microscopy high content life imaging using the Incucyte Zoom System and the integrated red fluorescence of healthy tumor cells was used to calculate the specific lysis (**Figure 20**). The expression of 4-1BB was evaluated by flow cytometry on CD4 and CD8 positive T cells (**Figures 21A-21D**).

CEACAM5 CD3 TCB was able to induce lysis of MKN45 NucLight red cells in all used immune effector cell preparations, as shown in **Figure 20** for the 42 hours time point.
20 The EC₅₀ values and the magnitude of lysis differed slightly between the different effector cell preparations and was highest for isolated CD8 T cells and lowest for CD4 T cells. Concomitant to tumor cell lysis, T cells increased surface expression of activation markers including 4-1BB (**Figures 21A-21D**). Surface expression of 4-1BB was highest on CD8 positive T cells, but was also detected to a lower extent on CD4 positive T cells. The extent of
25 4-1BB expression was not depending on the presence of helper cells (no difference of expression levels in PBMC vs isolated populations for CD4 or CD8 T cells). The extent of 4-1BB expression was less strong upon restimulation of fully differentiated effector memory NLV spec. CD8 T cells compared to stimulation of resting CD8 T cells. However, this might also be due to a single endpoint analysis, as the kinetics of 4-1BB on memory and naïve cells
30 differ slightly.

8.2 The presence of FAP-targeted 4-1BBL does not influence the cytolytic potential of T cells

Next we evaluated the influence of 4-1BB costimulation on TCB mediated tumor cell lysis. As described in 8.1, T cells were cocultured for 48 hours with MKN-45 NucLight Red

cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEACAM5 CD3 TCB with or without a fixed concentration of FAP 4-1BBL, respectively.

The amount of living tumor cells was quantified by fluorescence microscopy high content life imaging using the Incucyte Zoom System in 3 hour intervals and the integrated red fluorescence of healthy tumor cells was used to calculate the specific lysis.

The presence of 4-1BBL costimulation did neither speed up tumor cell lysis nor increase the magnitude of tumor cell lysis nor decrease the TCB concentration necessary to achieve lysis of a certain percentage of tumor cells (e.g. shift in EC₅₀ values). This was true for all evaluated effector cell preparations and is shown exemplary for the 42 hours time point in **Figures 22A-22D**.

8.3 The presence of FAP targeted 4-1BBL does influence the secretion of cytokines

In some assays, TNF- α sensor cells were cultured additionally to the above described setting. TNF- α sensor cells naturally express the TNF- α receptor and were genetically modified with GFP under the control of an NF κ B sensitive promotor element. Binding of TNF- α secreted by activated T cells leads to dose dependent activation of NF κ B and subsequently to expression of GFP. The GFP fluorescence can be quantified by high throughput life fluorescence microscopy over time and allows thus real-time assessment of TNF- α secretion. As described in 8.1 above, CD4 T cells were cocultured for 48 hours with MKN-45 NucLight Red cells as target cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEACAM5 CD3 TCB and CEA CD3 TCB, respectively, with or without a fixed concentration of FAP 4-1BBL.

Activation of T cells by the present TCB led to dose dependent release of TNF- α , which led to a dose dependent increase of GFP fluorescence over time in the TNF- α sensor cells. Additional costimulation with FAP 4-1BBL further increased the GFP fluorescence and thus the TNF- α secretion by activated T cells (**Figures 23A-23D**). This effect increased the magnitude of TCB mediated TNF- α secretion but did not lower the TCB concentration itself at which TNF- α secretion was induced. For an easier comparison over time the area under curve (AUC) was calculated for each time point with and w/o 4-1BB costimulation and was plotted against time (**Figures 24A-24B**).

The supernatants of all samples were evaluated at the end point (48 hours) using the cytometric bead array system (BD Bioscience) to quantify the effect on secretion of several cytokines beyond TNF- α . Evaluated cytokines were IL-2 and TNF- α as marker for general T cell activation, IFN- γ (Th1 cytokine), IL-4 (Th2 cytokine), IL-9 (Th9 cytokine) and IL-17A

(Th17 cytokine) to monitor a differentiation towards a certain Th subclass, and IL-10 as immunosuppressive cytokine.

Activation of T cells by the present TCB led, next to TNF- α , to a dose dependent release of all evaluated cytokines, namely IL-2, IL-4, IFN- γ , IL-17a and IL-10 (**Figures 25A-25D, Figures 26A-26D**). Additional co-stimulation with FAP 4-1BBL modulated the extent of dose-dependent cytokine secretion, but did not lower the TCB threshold concentration needed for cytokine secretion. Thereby, an increase of pro-inflammatory IL-2, TNF- α and IFN- γ secretion was observed, whereby the concentration of immunosuppressive IL-10 was lowered. For an easier comparison, the changes in cytokine concentration in samples with 4-1BB costimulation were calculated relative to those without costimulation for the TCB plateau concentration (**Figure 27**).

We also tested the ability of 4-1BBL costimulation to modulate the cytokine secretion of resting CD8 T cells and fully differentiated effector memory NLV spec. CD8 T cells. As described in 8.1, resting human PBMC, isolated CD4 or CD8 T cells and NLV specific CD8 effector memory CD8 T cells were cocultured for 72 hours with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEACAM5 CD3 TCB with or without a fixed concentration of FAP 4-1BBL. The supernatant was evaluated at 72 hours using the cytometric bead array as described above.

4-1BB costimulation supported the secretion of pro-inflammatory cytokines in resting human PBMC, CD4 and CD8 T cells (dose dependency, see **Figures 28A-28H and Figures 29A-29H**), comparison for top TCB concentration see **Figure 30**). A especially remarkable impact was shown on IL-2 and TNF- α production in resting CD8 T cells. Albeit to a lower extent, 4-1BB costimulation was also able to modulate the cytokine secretion of fully differentiated CD8 T cells.

Thus, costimulation via 4-1BBL does not increase directly the cytolytic potential of T cells in a time range of 48-72 hours in the *in vitro* cytotoxicity assay, but it increases the ability to secrete cytokines and modulated the cytokine microenvironment. A more proinflammatory cytokine milieu in the tumor can shift the tumor microenvironment towards a more immune activating and less immunosuppressive state, e.g. a lower level of IL-10 and increased concentrations of IFN- γ can allow myeloid cells in the tumor to mature to Th1 and cytotoxic T cell supporting antigen presenting cells. A shift to a supportive cytokine network will restore a successfully and sustained tumor cell elimination.

Example 9

In vitro co-culture assays with MKN45 cells expressing CEA and PDL-1

FAP-expressing NIH/3T3-huFAP cells were harvested using PBS-based dissociation buffer (Gibco by Life Technologies, Cat.-No: 13151-014), resuspended in assay medium consisting of RPMI (GIBCO, Invitrogen by Life Technologies, Cat.-No 42401-042) supplied with 10% (v/v) FBS and 1% (v/v) GlutaMAX-I (GIBCO, Invitrogen 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 μ M β -Mercaptoethanol (SIGMA, M3148) and irradiated with 50 Gy using X-Ray Irradiator RS 2000 (Rad source).

CEACAM5-expressing MKN45-huPD-L1 cells harvested, labeled with PKH-26 red (Sigma Cat.-No. PKH26GL-1KT) and resuspended in assay medium and irradiated with 50 Gy. Human PBMCs were isolated from buffy coat using Ficoll density centrifugation and were labeled with 40 nM CFDA-SE in 37°C warm PBS (GIBCO by Life Technologies, Cat.-No. 14190-136) for 15 min. After labeled CFSE-labeled PBMCs were washed and resuspended in assay medium. In a 96-well round bottom TC-treated plate in each well 0.01 x 10⁶ irradiated NIH/3T3-huFAP cells, 0.01 x 10⁶ PKH-26 red labeled and irradiated MKN45-huPDL-1 cells, 0.05 x 10⁶ CFSE-labeled PBMCs, 100 nM CEA CD3 TCB or 20 nM CEACAM5 CD3 TCB and 1 nM FAP-4-1BBL or 1 nM DP47-4-1BBL or no Ab were seeded in 200 μ L assay medium. The cells were incubated for 4 days. Afterwards cells were stained for flow cytometry analysis with LIVE/DEATH Fixable Aqua (Molecular Probes, Cat.-No. L34957), Per-CPCy5.5 conjugated anti-human CD137 mouse IgG1 (BioLegend, Cat.-No. 309814), PE/Cy7-conjugated anti-human CD8 mouse IgG1 (BioLegend, 301012), APC-conjugated anti-human CD25 mouse IgG1 (BioLegend, Cat.-No. 302610), APC/Cy7-conjugated anti-human OX40 (BioLegend, Cat.-No. 350022) and BV421-conjugated anti-human CD4-BV421 (BioLegend, Cat.-No. 300532). Cells were acquired using MACS Quant Analyzer 10 (Mitenyi Biotec) and analyzed using FlowJo and GraphPad Prism. Statistics were analyzed using Significance test: One-way Anova with Tukey's multiple comparison test of twelve technical replicates.

The results are shown in **Figure 31** for CEA CD3 TCB and in **Figure 32** for CEACAM5 CD3 TCB.

Example 10

***In vitro* PBMC activation assay combining FAP-4-1BBL with CEA CD3 TCB and/or Atezolizumab**

In this assay FAP-4-1BBL was tested for its potential to activate human PBMCs (isolated from buffy coat, frozen and stored in liquid nitrogen) in the presence or absence of CEA CD3 TCB and Atezolizumab (Tecentriq, anti-human PD-L1-specific humanized human IgG1 κ antibody) similar as described in Example 9. To mimic the tumor environment PBMCs of five different donors were incubated with FAP-expression NIH/3T3-huFAP fibroblast cell line and with CEA-expressing MKN45-PDL1 gastric cancer cell line for four days in the presence or absence of 1 nM FAP-4-1BBL or 1 nM DP47-4-1BBL and/or 100 nM CEA CD3 TCB and/or 80 nM Atezolizumab. For determining PBMC activation CD4 and CD8 T cells were analyzed by flow cytometry for proliferation (CFSE-dilution), CD25 (IL-2R α), 4-1BB (CD137) and PD-1 expression. Supernatant was analyzed by Multiplex for IFN γ , TNF α , GM-CSF, IL-4, IL-2, IL-6, IL-8 and IL-10.

a) Preparation of PBMCs

Buffy coats were obtained from the Zürich blood donation center. 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 Falcon 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 over-layered on 15 mL Histopaque 1077. The tubes were centrifuged for 30 min at 450 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 freezing medium consisting of RPMI 1640 medium (Gibco by Life Technology, Cat. No. 42401-042) supplied with 20% Dimethyl sulfoxide (Sigma, Cat.-No. D2650), 10 % (v/v) 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). 1-1.5 mL were transferred quickly to sterile Cryovials, transferred to Cryoboxes and stored for at least 48 h at -80°C. Afterwards vials were transferred to liquid nitrogen containers or Vapor phase containers.

Vials from 8 donors were thawed and washed in assay medium consisting of RPMI 1640 medium supplied with 10 % (v/v) Fetal Bovine Serum (FBS), 1 % (v/v) GlutaMAX I, 1 mM Sodium pyruvate (SIGMA, Cat. No. S8636), 1 % (v/v) MEM non-essential amino acids (SIGMA, Cat.-No. M7145) and 50 μ M β -Mercaptoethanol (SIGMA, M3148). Cells were

counted, washed with DPBS and resuspended in 37°C DPBS to 1×10^6 cells/mL. CFDA-SE was added to a final concentration of 40 nM and incubated for 15 min at 37 °C. Afterwards FBS was added, cells were washed and set in assay medium (2×10^6 cells/mL) and rested overnight in 6 well tissue-culture plates at 37 °C and 5% CO₂ in cell incubator. The next day

5 PBMCs were harvested, counted and resuspended in assay medium to 1×10^6 cells/mL.

b) Target cell lines

T150 flasks containing NIH/3T3-huFAP clone 19 were washed with DPBS and incubated with enzyme-free PBS-based dissociation buffer for 8 min at 37 °C. Cells were collected, washed, resuspended in assay medium and irradiated with 50 Gy using X-Ray

10 Irradiator RS 2000. Cells were set in assay medium to 0.4×10^6 cells/mL

T150 flasks containing MKN45-PD-L1 were washed with DPBS and incubated with enzyme-free PBS-based dissociation buffer for 8 min at 37 °C. Cells were collected, washed with DPBS and resuspended in C diluent (at least 250 µL, 8×10^7 cells/mL or lower). The same amount of C diluent was supplied with 4 µL/mL PKH-26 dye and mixed well. This dye

15 solution was added to the cells and mixed well and immediately. Cells were incubated for 5 min at room temperature. Afterwards FBS was added, cells were washed in assay, resuspended in assay medium and irradiated with 50 Gy with the X-Ray Irradiator RS 2000 (Rad source). Cells were set in assay medium to 0.4×10^6 cells/mL.

c) Assay Setup

20 For the test compounds master solutions were prepared of each component in assay medium as follows 4 nM FAP-4-1BBL, 4 nM DP47-4-1BBL, 800 nM CEA CD3 TCB and 640 nM Atezolizumab. Cells and components were combined in 96-well round bottom tissue culture plates (TTP, Cat.-No. 92097) in amounts of 25 µL of PKH-26red labeled MKN45-PD-L1 (10^5 cells/well), 25 µL of NIH/3T3-huFAP clone 19 (10^5 cells/well), 50 µL of

25 PBMC of one donor (50^5 cells/well), 50 µL of 4 nM FAP-4-1BBL or 4 nM DP47-4-1BBL solution or assay medium (final concentration 1 nM), 25 µL of 800 nM CEA CD3 TCB solution or assay medium (final concentration 100 nM), and 25 µL of 640 nM Atezolizumab solution or assay medium (final concentration 80 nM). Plates were then incubated for four days at 37 °C and 5 % CO₂ in a humidified cell incubator.

30 After four days 50 µL supernatant was removed and stored at -80 °C to be later analyzed for cytokine content (see below). To perform a flow cytometry analysis of T cell proliferation and surface expression plates were centrifuged and washed once with cold DPBS. Samples are stained for 30 min at 4 °C in 100 µL/well DPBS supplied with 1:1000 diluted LIVE/DEAD Fixable Aqua Dead Cell Stain. Cells were washed once with 200 µL/well DPBS

(centrifugation 350 x g 4 min 4°C, flick off). Cells were resuspended in 50 µL/well staining solution composed of FACS-buffer containing 1 µg/mL anti-human CD137-PerCP/Cy5.5 and 0.67 µg/mL anti-human PD-1-PE/Cy7, 0.5 µg/mL anti-human CD25-APC, 0.67 µg/mL anti-human CD8-APC-Cy7 and 0.67 µg/mL anti-human CD4-BV421 and incubated for 30 min at 4°C. Cells were washed twice with 200 µL/well DPBS (centrifugation 350 x g 4 min 4°C, flick off) and resuspended in 40 µL/well 1% PFA in PBS and incubated at 4°C to fix the staining. Cells were centrifuged and resuspended in 100 µL FACS-buffer before cells were acquired using the MACS Quant Analyzer 10 (Miltenyi Biotec) and an automated Cytomat (ThermoFischer, set to 4°C) plate-handling system. Data was analyzed using FlowJo v10.3 for PC (FlowJo LLC), Microsoft Excel (professional Plus 2010) and GraphPad Prism v6.07 (GraphPad Software, Inc) for total count and percentage of proliferation CD4 and CD8 T cells (CFSE dilution), as well as CD25 (IL-2R α), CD137 (4-1BB) and PD-1 expressing CD4 and CD8 T cells.

To analyze the released cytokines in the supernatant, the previous frozen samples were taken and analyzed for IFN γ , GM-CSF, TNF α , IL-4, IL-2, IL-6, IL-8 and IL-10 using Bio-RAD Bio-Plex ProTM Human Cytokine 8 plex assay (Bio-Rad Laboratories AG, Switzerland). Thawed samples were analyzed as following: The Bio-Plex system was calibrated according to the manufacturer's instructions. The assay buffer, wash buffer and sample diluent of the Bio-RAD Bio-Plex Pro Human Cytokine 8 plex were brought to RT. The Vacuum manifold (Milipore) for 96-well filter plates was set to -1 to -3" Hg. The standard vial was diluted with 500 µL Standard Diluent, vortexed and stored on ice. A four-fold standard diluent was performed in T cell activation assay medium (same medium as the supernatant, see above) including a blank control (medium only). Thawed supernatant were diluted in T cell activation assay medium 1:1.2 and 1:1.5 (two different dilutions were tested). The 10x Bio-Plex coupled beads were vortexed and diluted in Bio-Plex assay buffer and kept protected from light.

A Multi-Screen Filter plate (Millipore) was pre-wetted using Bio-Plex assay buffer and the vacuum manifold. 50 µL/well of the prepared Bio-Plex coupled bead solution was added and filter plates were washed twice with 100 µL/well Bio-Plex assay buffer using the vacuum manifold. Diluted supernatants, standards and blank control were added to the wells (50 µL/well) according to the plate layout. The plate was incubated for 1 h in the dark on a plate shaker (850 rpm) to allow the cytokines to bind to the Bio-Plex coupled beads. In the meanwhile Bio-Plex detection antibody was diluted 1:20 in Bio-Plex detection antibody diluent. The incubated filter plate was washed three times with 100 µL/well Bio-Plex assay buffer using the vacuum manifold. Afterwards the prepared detection antibody solution was vortexed and 25 µL/well were added. The plate was incubated for 30 min in the dark on a plate shaker (850 rpm) to allow the the biotin-labeled detection antibody to bind. In the

meanwhile the SA-PE (PE-conjugated Streptavidin) solution was prepared by dilution it 1:200 in Bio-Plex assay buffer and protected from light. After the incubation the plate was washed three times with 100 μ L/well Bio-Plex assay buffer using the vacuum manifold. 50 μ L/well SA-PE solution were added and the plate was incubated for 10 min in the dark on a plate shaker (850 rpm) to allow the SA-PE to bind to biotin-labeled detection antibodies.

Afterwards plates were washed three times as described above. Finally 100 μ L Bio-Plex assay buffer were added to each well, the plate was shaken for 30 sec at 850 rpm on the plate shaker, then applied to the Bio-Plex system and the analysis was started. The data was analyzed using Bio-Plex Manager Software and GraphPad Prism v6.07 (GraphPad Software, Inc).

10 Results

As shown in **Figures 33, 34 and 35**, the addition of 10 nM CEA CD3 TCB (black chassed bars, opern squares) but not 1 nM FAP-1BBL alone (black and white diagonal striped bar, black down-facing triangles) could increase the proliferation as well as the expression of CD25 and CD137 (4-1BB) on CD8 T cells. The combination of 10 nM CEA CD3 TCB with 1 nM FAP-1BBL (black bare, open diamonds) led to a further increase of CD25 and CD137 (in lesser extend) and increased proliferation of CD8 T cells. Stronger effects were seen when analyzing the cytokines (**Figures 36, 37 and 38**). Fold increase of cytokine release comparing the combination of CEA CD3 TCB and FAP-4-1BBL with CEA CD3 TCB alone is shown in **Figure 39**. The combination of these two components induced synergistic effects mainly for CD8 T cells in proliferation, activation (CD25, CD137) and cytokine release (especially IFN γ , GM-CSF and TNF α). Untargeted DP47-4-1BBL in combination with CEA CD3 TCB (red/white striped bar) could not induce the same effects as the comination of targeted FAP-4-1BBL and CEA-TCB showing that the synergistic effect of FAP-41-BBL is dependent on FAP-targeting and crosslinking.

As also shown in **Figures 33, 34 and 35** the triple combination (grey bar, grey filled up-facing triangles) of CEA-TCB with FAP-4-1BBL and PD-L1 antibody (Atezolizumab) had no additional effect on T cell proliferation or activation (CD137, CD25 expression) but could induce further increased secretion of IFN γ , TNF α and GMC-CSF if compared to single treatments or treatment with the combination of CEA CD3 TCB and FAP-4-1-BBL (see **Figures 36, 37 and 38**). Fold increase of this cytokines comparing the triple combination with the combination of CEA CD3 TCB and FAP-4-1BBL is shown in **Figure 40**. The triple combination showed a very strong synergistic effect on cytokine release (mainly IFN γ , TNF α and GM-CSF).

Example 11**Preparation, purification and characterization of FAP targeted mouse 4-1BBL antigen binding molecule (Hybrid surrogate)**

A bispecific antigen binding molecule comprising an agonistic mouse 4-1BB ligand
 5 with monovalent binding for FAP, also termed hybrid surrogate or FAP-mu4-1BBL, was prepared as described in International Patent Appl. Publ. No. WO 2016/075278 A1. The targeted mouse 4-1BBL was prepared as described for the human ligand by replacing the human ligand with the mouse ectodomain.

The DNA sequence encoding part of the ectodomain (amino acid 104-309, including the
 10 C160S mutation) of mouse 4-1BB ligand was synthesized according to the Q3U1Z9-1 sequence of Uniprot database. The FAP binder used to target the 4-1BB ligand was clone 4B9. The amino acid sequences for the hybrid surrogate FAP-mu4-1BBL can be found in Table 21.

Table 21: Amino acid sequences of mature hybrid surrogate FAP-mu4-1BBL

SEQ ID NO:	Description	Sequence
136	di-mu4-1BBL-CL Fc knob chain	RTEPRPALTTTSPNLGTRENNADQVTPVSHIGCPNTTQQGSPVFAKLLAKNQASLSNTTLNWHSQDGAGSSYLSQGLRYEEDKKELVVDSPGLYYVFLELKLSPFTNTGHKVVQGWVSLVLQAKPQVDDFDNLALTVELFPCSMENKLVDRSWSQLLLLKAGHRLSVGLRAYLHGAQDAYRDWELSYPNNTTSFGLFLVKPDNPWEGGGGSGGGGSRTTEPRPALTTTSPNLGTRENNADQVTPVSHIGCPNTTQQGSPVFAKLLAKNQASLSNTTLNWHSQDGAGSSYLSQGLRYEEDKKELVVDSPGLYYVFLELKLSPFTNTGHKVVQGWVSLVLQAKPQVDDFDNLALTVELFPCSMENKLVDRSWSQLLLLKAGHRLSVGLRAYLHGAQDAYRDWELSYPNNTTSFGLFLVKPDNPWEGGGGSGGGGSRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMH EALHNHYTQKSLSLSPGK
137	mono-mu4-1BBL-CH1 chain	RTEPRPALTTTSPNLGTRENNADQVTPVSHIGCPNTTQQGSPVFAKLLAKNQASLSNTTLNWHSQDGAGSSYLSQGLRYEEDKKELVVDSPGLYYVFLELKLSPFTNTGHKVVQGWVSLVLQAKPQVDDFDNLALTVELFPCSMENKLVDRSWSQLLLLKAGHRLSVGLRAYLHGAQDAYRDWELSYPNNTTSFGLFLVKPDNPWEGGGGSGGGGSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV

		PSSSLGTQTYICNVNHKPSNTKVDEKVEPKSC
138	VHCH1 (4B9) Fc hole chain	EVQLLESGLLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAIIGSGASTYYADSVKGRFTISRDN SKNTLYLQ MNSLRAEDTAVYYCAKGWFGGFNYWGQGT LVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LGAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLT VDKSRWQQGNV FSCSVMEALHNHYTQKSLSLSPGK
139	VLCL(4B9) Light chain	EIVLTQSPGTLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLINVGSRRTATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGIMLPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC

The hybrid surrogate FAP-mu4-1BBL was produced by co-transfecting CHO-K1 cells growing in suspension with the mammalian expression vectors using eviFect (Evitria AG). The cells were transfected with the corresponding expression vectors in a 1:1:1:1 ratio

5 (“vector Fc-hole heavy chain”: “vector FAP light chain” : “vector 4-1BBL Fc-knob heavy chain”: “vector mu4-1BBL light chain”).

For transfection CHO-K1 cells are cultivated in suspension serum free in eviMake culture medium (Evitria AG). After 7 days at 37 °C in an incubator with a 5 % CO₂ atmosphere, cultivation supernatant is collected for purification by centrifugation and the

10 solution is sterile filtered (0.22 mm filter) and kept at 4°C.

Secreted proteins were purified from cell culture supernatants by affinity chromatography using Protein A, followed by size exclusion chromatography. For affinity chromatography, the supernatant was loaded on a Protein A MabSelectSure column (GE Healthcare) equilibrated with 20 mM sodium phosphate, 20 mM sodium citrate pH 7.5.

15 Unbound protein was removed by washing with 20 mM sodium phosphate, 20 mM sodium citrate containing buffer (pH 7.5). The bound protein was eluted using a linear pH-gradient of sodium chloride of 20 mM sodium citrate, 100 mM NaCl, 100 mM Glycine, 0.01% Tween20 pH 3.0. The column was then washed with 20 mM sodium citrate, 100 mM NaCl, 100 mM Glycine, 0.01% Tween20 pH 3.0. The pH of collected fractions was adjusted by adding 1/40

20 (v/v) of 2M Tris, pH8.0. The protein was concentrated and filtered prior to loading on a HiLoad Superdex column (GE Healthcare) equilibrated with 20 mM Histidine, 140 mM NaCl, 0.01% Tween20 pH6.0.

The protein concentration of purified bispecific constructs was determined by measuring the OD at 280 nm, using the molar extinction coefficient calculated on the basis of the amino acid sequence. Purity and molecular weight of the bispecific constructs were analyzed by CE-SDS in the presence and absence of a reducing agent (Invitrogen, USA) using a LabChipGXII (Caliper). The aggregate content of bispecific constructs was analyzed using a TSKgel G3000 SW XL analytical size-exclusion column (Tosoh) equilibrated in a 25 mM K₂HPO₄, 125 mM NaCl, 200mM L-Arginine Monohydrochloride, 0.02 % (w/v) NaN₃, pH 6.7 running buffer at 25°C.

Table 22: Biochemical analysis of hybrid surrogate FAP-mu4-1BBL

Molecule	Monomer [%]	Yield [mg/l]	CE-SDS (non-red)
FAP-mu4-1BBL	95	3.2	92

10

11.1 Functional characterization of hybrid surrogate FAP-mu4-1BBL by surface plasmon resonance

The capacity of binding simultaneously murine 4-1BB Fc(kih) and human or murine FAP was assessed by surface plasmon resonance (SPR) in the manner as described in WO 2016/075278 A1. All SPR experiments were performed on a Biacore T200 at 25 °C with HBS-EP as running buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20, Biacore, Freiburg/Germany). Biotinylated murine 4-1BB Fc(kih) was directly coupled to a flow cell of a streptavidin (SA) sensor chip. Immobilization levels up to 600 resonance units (RU) were used.

The FAP-targeted mu4-1BBL construct was passed at a concentration range of 200 nM with a flow of 30 µL/minute through the flow cells over 90 seconds and dissociation was set to zero sec. Human or murine FAP was injected as second analyte with a flow of 30 µL/minute through the flow cells over 90 seconds at a concentration of 500 nM. The dissociation was monitored for 120 sec. Bulk refractive index differences were corrected for by subtracting the response obtained in a reference flow cell, where no protein was immobilized.

As can be seen in the graphs of **Figure 41A** and **41B**, the hybrid surrogate FAP-mu4-1BBL can bind simultaneously murine 4-1BB and murine/human FAP.

Example 12

Preparation, purification and characterization of bispecific antigen binding molecules with bivalent binding to mouse 4-1BB and monovalent binding to FAP

Bispecific agonistic mouse 4-1BB antibodies with bivalent binding for 4-1BB and monovalent binding for FAP, also termed 2+1, have been prepared in analogy to **Figures 1A** and **1B**. In this example, the first heavy chain HC1 of the construct was comprised of the following components: VHCH1 of an anti-4-1BB (clone MU137-1) followed by Fc containing the mutations Lys392Asp and Lys409Asp (termed Fc-DD), at which C-terminus a VL, or VH, of anti-FAP binder (clone 28H1) was fused. The second heavy chain HC2 was comprised of VHCH1 of anti-4-1BB (clone MU137-1) followed by Fc containing the mutation Glu356Lys and Asp399Lys (termed Fc-KK), at which C-terminus a VH, or VL, of anti-FAP binder (clone 28H1) was fused. The DDKK mutations for enhancing antibody Fc heterodimer formation are inter alia described by Gunasekaran et al., J. Biol. Chem. 2010,19637-19646. Combination of the targeted anti-FAP-Fc DD with the anti-4-1BB-Fc KK chain allows generation of a heterodimer, which includes a FAP binding moiety and two murine mouse 4-1BB binding Fabs. DAPG mutations were introduced in the constant regions of the heavy chains to abrogate binding to mouse Fc gamma receptors according to the method described e.g. in Baudino et al. J. Immunol. (2008), 181, 6664-6669, or in WO 2016/030350 A1. Briefly, the Asp265Ala and Pro329Gly mutations have been introduced in the constant region of the Fc-DD and Fc-KK heavy chains to abrogate binding to Fc gamma receptors (numbering according to Kabat EU index; i.e. D265A, P329G).

The amino acid sequences for 2+1 anti-4-1BB(MU137-1), anti-FAP(28H1) construct with a-FAP(28H1) VH fused to Fc-KK and VL fused to Fc-DD chain can be found respectively in **Table 23**. The amino acid sequences for 2+1 anti-4-1BB(MU137-1), anti-FAP(28H1) construct with a-FAP(28H1) VL fused to Fc-KK and VH fused to Fc-DD chain can be found respectively in **Table 24**.

Table 23: Sequences of bispecific, bivalent anti-**4-1BB** (MU137-1)/anti-FAP (28H1) mouse IgG1 DAPG antigen binding molecules (Constructs with FAP VL fused to Fc-DD chain and VH fused to Fc-KK chain, also termed below Fc-DD-VL)

SEQ ID NO:	Description	Sequence
140	VHCH1 (MU137-1)-Heavy chain Fc-DD-VL (28H1)	DVQLVESGGGLVQPGRSLKLSAASGFIFSFDMAWVRQAP TKGLEWVASISPSGDIPYYRDSVKGRFTVSRENAKSSLYLQM DSLRS EDTATYYCARRSYGGYSELDYWGQGVMVTVSSAKT TPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSQTVTCNVAH PASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTI TLTPKVTCVVVAISKDDPEVQFSWFVDDVEVHTAQTTPREE

		QINSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIEKTIS KTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITNFFPEDITV EWQWNGQPAENYDNTQPIMDTDGSYFVYSDLNQKSNWE AGNTFTCSVLHEGLHNHHTTEKSLSHSPGGGGGSGGGGSGGG GSGGGGSEIVLTQSPGTLSPGERATLSCRASQSRSYLA WYQQKPGQAPRLLIIGASTRATGIPDRFSGSGSGTDFTLTISR LEPEDFAVYYCQQGQVIPPTFGQGTKVEIK
141	VHCH1 (20H4.9)-Heavy chain Fc-KK-VH (28H1)	DVQLVESGGGLVQPGRSLKLSAASGFIFSYFDMAWVRQAP TKGLEWVASISPSGDIPYYRDSVKGRFTVSRENAKSSLYLQM DSLRS EDTATYYCARRSYGGYSELDYWGQGMVTVSSAKT TPPSVYPLAPGSAQAQNSMVTLGCLVKGYFPEPVTVTWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSQTVTCNVAH PASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTI TLTPKVTCVVVAISKDDPEVQFSWFVDDVEVHTAQTTPREE QINSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIEKTIS KTKGRPKAPQVYTIPPPKQMAKDKVSLTCMITNFFPEDITV EWQWNGQPAENYKNTQPIMKTDGSYFVYSLNVAQKSNWE AGNTFTCSVLHEGLHNHHTTEKSLSHSPGGGGGSGGGGSGGG GSGGGGSEVQLLES GGGLVQPGGSLRLSCAASGFTFSSHAM SWVRQAPGKGLEWVSAIWASGEQYYADSVKGRFTISRDN KNTLYLQMNSLRAEDTAVYYCAKGWLG NFDYWGQGLTVT VSS
142	VLCL-Light chain (MU137-1)	DIQMTQSPASLSASLEEIVTITCQASQDIGNWLAWYHQKPGK SPQLLIYGTSSLADGVPSRFGSSSGSQYSLKISRLQVEDIGIY YCLQAYGAPWTFGGGKLELKRADAAPTVSIFPPSSEQLTSG GASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDS KDSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPIVKSFN RNEC

Table 24: Sequences of bispecific, bivalent anti-**4-1BB** (MU137-1)/monovalent anti-FAP (28H1) mouse IgG1 DAPG antigen binding molecules (Constructs with FAP VH fused to Fc DD chain and VL fused to Fc KK chain, termed Fc-DD-VH)

SEQ ID NO:	Description	Sequence
143	VHCH1 (MU137-1)-Heavy chain Fc-DD-VH (28H1)	DVQLVESGGGLVQPGRSLKLSAASGFIFSYFDMAWVRQAP TKGLEWVASISPSGDIPYYRDSVKGRFTVSRENAKSSLYLQM DSLRS EDTATYYCARRSYGGYSELDYWGQGMVTVSSAKT TPPSVYPLAPGSAQAQNSMVTLGCLVKGYFPEPVTVTWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSQTVTCNVAH PASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTI TLTPKVTCVVVAISKDDPEVQFSWFVDDVEVHTAQTTPREE QINSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIEKTIS KTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITNFFPEDITV EWQWNGQPAENYDNTQPIMDTDGSYFVYSDLNQKSNWE AGNTFTCSVLHEGLHNHHTTEKSLSHSPGGGGGSGGGGSGGG GSGGGGSEVQLLES GGGLVQPGGSLRLSCAASGFTFSSHAM SWVRQAPGKGLEWVSAIWASGEQYYADSVKGRFTISRDN KNTLYLQMNSLRAEDTAVYYCAKGWLG NFDYWGQGLTVT VSS
144	VHCH1 (MU137-1)-Heavy chain	DVQLVESGGGLVQPGRSLKLSAASGFIFSYFDMAWVRQAP TKGLEWVASISPSGDIPYYRDSVKGRFTVSRENAKSSLYLQM DSLRS EDTATYYCARRSYGGYSELDYWGQGMVTVSSAKT

	Fc-KK-VL (28H1)	TPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSQTVTCNVAH PASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTI TLTPKVTCVVVAISKDDPEVQFSWFVDDVEVHTAQTKEPREE QINSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIEKTIS KTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITNFFPEDITV EWQWNGQPAENYKNTQPIPKTDGSYFVYSKLVNQKSNWE AGNTFTCSVLHEGLHNHHTTEKSLSHSPGGGGGSGGGGSGGG GSGGGGSEIVLTQSPGTLSPGERATLSCRASQSVSRSYLA WYQQKPGQAPRLLIIGASTRATGIPDRFSGSGSGTDFLTISR LEPEDFAVYYCQQGQVIPPTFGQGTKVEIK
142	VLCL-Light chain (MU137-1)	see Table 23

The bispecific 2+1 anti-4-1BB anti-FAP muIgG1 DAPG was produced by co-transfecting CHO-K1 cells growing in suspension with the mammalian expression vectors using eviFect (Evitria AG). The cells were transfected with the corresponding expression
5 vectors in a 1:1:1 ratio (“vector Fc-DD heavy chain”: “vector light chain” : “vector Fc-KK heavy chain”).

For transfection CHO-K1 cells are cultivated in suspension serum free in eviMake (Evitria AG) culture medium. After 7 days at 37 °C in an incubator with a 5 % CO₂ atmosphere, cultivation supernatant is collected for purification by centrifugation and the
10 solution is sterile filtered (0.22 µm filter) and kept at 4°C.

Secreted proteins were purified from cell culture supernatants by affinity chromatography using Protein A, followed by size exclusion chromatography. For affinity chromatography, the supernatant was loaded on a Protein A MabSelectSure column (CV = 5 mL, GE Healthcare) equilibrated with 40 mL 20 mM sodium phosphate, 20 mM sodium
15 citrate pH 7.5. Unbound protein was removed by washing with at least 10 column volumes of 20 mM sodium phosphate, 20 mM sodium citrate containing buffer (pH 7.5). The bound protein was eluted using a linear pH-gradient of sodium chloride (from 20 to 100 mM) created over 15 column volumes of 20 mM sodium citrate, 100 mM NaCl, 100 mM Glycine, pH 3.0. The column was then washed with 10 column volumes of 20 mM sodium citrate, 100
20 mM NaCl, 100 mM Glycine, pH 3.0. The pH of collected fractions was adjusted by adding 1/40 (v/v) of 2M Tris, pH8.0. The protein was concentrated and filtered prior to loading on a HiLoad Superdex 16/600 S200 column (GE Healthcare) equilibrated with 20 mM Histidine, 140 mM NaCl, pH6.0.

The protein concentration of purified bispecific constructs was determined by
25 measuring the OD at 280 nm, using the molar extinction coefficient calculated on the basis of the amino acid sequence. Purity and molecular weight of the bispecific constructs were

analyzed by CE-SDS in the presence and absence of a reducing agent (Invitrogen, USA) using a LabChipGXII (Caliper). The aggregate content of bispecific constructs was analyzed using a TSKgel G3000 SW XL analytical size-exclusion column (Tosoh) equilibrated in a 25 mM K_2HPO_4 , 125 mM NaCl, 200mM L-Arginine Monohydrochloride, 0.02 % (w/v) NaN₃, pH 6.7 running buffer at 25°C.

Table 25 - Biochemical analysis of bispecific antigen binding molecules with a bivalent binding to 4-1BB and a monovalent binding to FAP (2+1) anti-4-1BB (MU137-1), anti-FAP(28H1) mouse IgG1 DAPG

Molecule	Monomer [%]	Yield [mg/l]	CE-SDS (non-red)
4-1BB (MU137-1)/ FAP(28H1) DAPG IgG1 2+1 (Fc-DD-VL), in the following named muFAP-4-1BB	98	3.6	92

12.1 Functional characterization of mouse surrogate muFAP-4-1BB by surface plasmon resonance

The capacity of binding simultaneously murine 4-1BB Fc(kih) and murine FAP was assessed by surface plasmon resonance (SPR) in the manner as described in WO 2016/075278 A1. All SPR experiments were performed on a Biacore T200 at 25 °C with HBS-EP as running buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20, Biacore, Freiburg/Germany). Biotinylated murine 4-1BB Fc(kih) was directly coupled to a flow cell of a streptavidin (SA) sensor chip. Immobilization levels up to 600 resonance units (RU) were used.

The FAP-targeted 4-1BB constructs were passed at a concentration range of 200 nM with a flow of 30 μ L/minute through the flow cells over 90 seconds and dissociation was set to zero sec. Murine FAP was injected as second analyte with a flow of 30 μ L/minute through the flow cells over 90 seconds at a concentration of 500 nM. The dissociation was monitored for 120 sec. Bulk refractive index differences were corrected for by subtracting the response obtained in a reference flow cell, where no protein was immobilized. As can be seen in the graph of **Figure 41C**, the mouse surrogate muFAP-4-1BB can bind simultaneously murine 4-1BB and murine FAP.

Example 13

Pharmacokinetic profile of muFAP-4-1BB after single injection in C57BL/6 mice

A single dose of 2.5 mg/kg of muFAP-4-1BB (prepared according to Example 12) was injected into C57BL/6 mice. All mice were injected i.v. with 200 μ l of the appropriate solution. To obtain the proper amount of compounds per 200 μ l, the stock solution (**Table 26**, muFAP-4-1BB) was diluted with histidine buffer. Three mice per time point were bled at 10min, 1hr, 6hr, 24hr, 48hr, 72hr, 96hr, 6 days and 9 days. The injected compound was analyzed in serum samples by ELISA. Biotinylated murine 4-1BB, test serum sample, detection antibody anti-m μ IgG labelled with HRP were added stepwise to a 96-well streptavidin-coated microtiter plate and incubated after every step for 1h at room temperature. The plate was washed three times after each step to remove unbound substances. Finally, the peroxidase-bound complex was visualized by adding ABTS substrate solution to form a colored reaction product. The reaction product intensity was photometrically determined at 405nm (with reference wavelength at 490nm) and was proportional to the analyte concentration in the serum sample. The result is shown in **Figure 42**. muFAP-4-1BB showed a stable PK-behavior which suggested a once weekly schedule for subsequent efficacy studies.

Example 14

Anti-tumor effect by combination therapy of muFAP-4-1BB and anti-PD-L1 antibody *in vivo*

An efficacy study with the combination of muFAP-4-1BB and a PD-L1 antibody was carried out in the MC38-CEA model.

An anti-mouse PD-L1 antibody based on the YW243.55.S70 PD-L1 antibody described in WO 2010/077634 (sequence shown in Figure 11 of said document) was used in the *in vivo* tumor models. This antibody contained a DAPG mutation as described in Example 12 to abolish Fc γ R interaction. The variable region of YW243.55.S70 was attached to a murine IgG1 constant domain with DAPG Fc mutations. The anti-PD-L1 antibody used in this example comprises heavy chains according to SEQ ID NO: 145 and light chains according to SEQ ID NO: 146.

MC38-huCEA (murine colon carcinoma) cells were obtained from the City of Hope and expanded at Roche Innovation Center Zurich and maintained in RPMI medium supplemented with 10% FCS, 4 μ g/ml Puromycin and 50 μ g/ml Hygromycin. Passage 10 at a viability of 96% was used for *in vivo* cell injection. 0.5×10^6 cells were resuspended in 100 μ l of RPMI (w/o) 50% + GFR matrigel 50% and injected s.c. (subcutaneously).

Immunocompetent human CEA transgenic (hCEATg) C57BL/6J mice were obtained under license agreement from Beckmann Research Institute of City of Hope and bred at Charles River Laboratories. Mice at an age of 8-10 weeks at start of the experiment were maintained under specific-pathogen-free condition with daily cycles of 12 h light / 12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). The experimental study protocol was reviewed and approved by local government. After arrival, animals were maintained for one week to get accustomed to the new environment and for observation. Continuous health monitoring was carried out on a regular basis.

According to the protocol (**Figure 43**), female hCEATg mice were injected with tumor cells s.c. as described above and treated once weekly with the compounds or PBS (Vehicle) when tumor size reached appr. 180 mm³ (day 15). All mice were injected i.v. with 200 µl of the appropriate solution once per week. To obtain the proper amount of compounds per 200 µl, the stock solutions (**Table 26**) were diluted with histidine buffer when necessary.

Table 26: Compositions used in the *in vivo* experiments

Compound	Formulation buffer	Concentration (mg/mL)
muFAP-4-1BBL	20 mM Histidine, 140 mM NaCl, pH 6.0	1.81 (= stock solution)
PD-L1 antibody	20 mM Histidine, 140 mM NaCl, pH 6.0	23.4 (= stock solution)

Table 27: Study groups in the *in vivo* experiments

Group	No. of animals	Compound	Dose /mouse (mg/kg)	Route/ Mode of administration	No of treatments
A	10	vehicle	-	i.v.	3
B	10	muFAP-4-1BB	3	i.v.	3
C	10	anti-PD-L1	10	i.v.	3
D	10	anti-PD-L1 + muFAP-4-1BB	10/ 3	i.v.	3

For combination therapy (Group D) with muFAP-4-1BB and anti-PD-L1, therapies were injected concomitant. Tumor growth was measured three times per week using a caliper (Example 2, Figure 2) and tumor volume was calculated as followed:

$$T_v: (W^2/2) \times L \quad (W: \text{Width}, L: \text{Length})$$

- 5 The study was stopped after 3 weeks of treatment (day 37 after tumor cell injection). **Figure 44A** shows the tumor growth kinetics (Mean +/- SEM) in all treatment groups, the individual tumor growth kinetics of each animal for all groups is shown in **Figure 44C** and as a waterfall plot indicating the tumor growth change from start of treatment at day 15 until day 37 in **Figure 44B**. Monotherapy of muFAP-4-1BB did not reveal any tumor growth inhibition.
- 10 a-PD-L1 treatment alone induced tumor growth inhibition (TGI: 40 and TCR: 0.37) with one mouse being tumor free at day 37. However, the combination of muFAP-4-1BB and a-PD-L1 induced strong tumor regression in 8 out of 10 mice (TGI: 98 and TCR: 0.05) resulting in 50% tumor free mice by day 37.

Statistical analysis was carried out using JMP software:

$$15 \quad TGI: \frac{100 - \text{Av}(T_{\text{treatment}}^{\text{[day x]}} - T_{\text{treatment}}^{\text{[baseline]}})}{\text{Av}(T_{\text{Vehicle}}^{\text{[day x]}} - T_{\text{Vehicle}}^{\text{[baseline]}})} * 100$$

$$TCR: \frac{\text{Av}(T_{\text{treatment}}^{\text{[day x]}})}{\text{Av}(T_{\text{Vehicle}}^{\text{[day x]}})}$$

- The resulting TGI and TCR values are shown in **Table 28** (TGI means tumor growth inhibition, TGI > 100 means tumor regression and TGI = 100 is defined as tumor stasis, TCR means treatment to control ratio, TCR = 1 means no effect and TCR = 0 is defined as
- 20 complete regression).

Table 28: TGI and TCR at study day 37 (Vehicle as Control Group)

Group	TGI	TCR
muFAP-4-1BB	-28	1.17
anti-PD-L1	40	0.37
muFAP-4-1BB + anti-PD-L1	98	0.05

Example 15

Anti-tumor effect by combination therapy of FAP-4-1BBL and FolR1 CD3 TCB *in vivo*

- 25 a) Experiments with monovalent FAP(28H1)-4-1BBL

The human monovalent FAP-targeted 4-1BBL (mono FAP-4-1BBL, FAP binder 28H1) was tested as single agent and in combination with the human FOLR1 CD3 TCB against the untargeted 4-1BBL (binder DP47) as single agent and in combination. Human ovarian cancer cells (SKOV3) were engrafted subcutaneously in NOG mice from Taconic. 2 days before
5 beginning of therapy mice were injected with human PBMCs.

Human SKOV3 cells (ovarian carcinoma) were originally obtained from ATCC and after expansion deposited in the Roche internal cell bank. Cells were cultured in RPMI containing 10% FCS, 1% Glutamax at 37 °C in a water-saturated atmosphere at 5 % CO₂. In vitro passage 42 was used for subcutaneous injection at a viability of 96.9%. 50 microliters
10 cell suspension (5×10^6 SKOV3 cells) mixed with 50 microliters Matrigel were injected subcutaneously in the flank of anaesthetized mice with a 22G to 30G needle

70 NOG female mice having an age of 6-8 weeks at start of experiment (purchased from Taconic, SOPF facility) were maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa;
15 TierschG). Experimental study protocol was reviewed and approved by local government (P 2005086). After arrival animals were maintained for one week to get accustomed to new environment and for observation. Continuous health monitoring was carried out on regular basis.

Mice were injected subcutaneously on study day 0 with 5×10^6 SKOV3 cells and tumors
20 were measured 2 to 3 times per week during the whole experiment by Caliper. Buffy coats were obtained from Züricher Blutspende and PBMCs purified freshly before injection on day 28. 9.1 Mio PBMCs were injected i.p. in 200 µl volume of RPMI. Therapies were administered intravenously (i.v.) starting at day 29. Subsequently, the animals were treated weekly until study end. On the day of randomization mice were injected i.v. with Vehicle,
25 untargeted 4-1BBL (9.85mg/kg), FOLR1 CD3 TCB, mono FAP-4-1BBL (9.85mg/kg), FOLR1 CD3 TCB + untargeted 4-1BBL (9.85mg/kg), FOLR1 CD3 TCB + FAP-4-1BBL (9.85mg/kg) or FOLR1-TCB +FAP-4-1BBL (0.985 mg/kg).

All mice were injected i.v. with 200 µl of the appropriate solution. The mice in the vehicle group were injected with Histidine Buffer and the treatment groups with the 4-1BBL
30 containing constructs, the FOLR1 CD3 TCB or the combinations. To obtain the proper amount of compound per 200 µl, the stock solutions were diluted with Histidine Buffer when necessary. The dose and schedule used for FOLR1 CD3 TCB was 5 µg/kg, once/week whereas the 4-1BBL constructs were given at a dose of 9.85 mg/kg or 0.985 mg/kg once/week

2-3 mice/group were bled 10 min, 1h, 8h, 24h and 7d after first therapy to determine the
35 pharmacokinetic profile of the compounds during the first week. Mouse serum samples were

analyzed with ELISA method. Biotinylated 4-1BB (Roche Innovation Center Zürich), test sample, Digoxigenin labelled anti-huCH1 antibody (Roche Diagnostics GmbH) and anti-Digoxigenin detection antibody (Roche Diagnostics GmbH) were added stepwise to a 96-well streptavidin-coated microtiter plate. The peroxidase-bound complex was visualized by adding
 5 ABTS substrate solution to form a colored reaction product.

The experiment was terminated at study day 44, 1 day after the 3rd therapy. Tumors were harvested in PBS, single cell suspensions were generated and stained for different immune cell markers and analysed by FACS (staining antibodies Biolegend: Anti-human CD45 AF488, Anti-human CD3 Percp-Cy5.5, Anti-human CD8 PE-Cy7, Anti-human CD4
 10 BV421).

Parts of tumors at termination were formalin fixed and afterwards embedded in Paraffin. Samples were cut and stained for CD3 and CD8.

Table 29: Compositions used in the *in vivo* experiments

Compound	Dose	Formulation buffer	Concentration (mg/mL)
monovalent untargeted 4-1BBL (DP47)-4-1BBL	9.85 mg/kg	20 mM Histidine, 140 mM NaCl, 0.01% Tween-20, pH 6.0	3.07 (=stock concentration)
monovalent FAP (28H1) 4-1BBL	9.85 mg/kg or 0.985 mg/kg	20 mM Histidine, 140 mM NaCl, pH 6.0, 0.01% Tween20	2.3 (=stock concentration)
FolR1 CD3 TCB	5 µg/kg	20 mM Histidine, 140 mM NaCl, pH 6.0, 0.01% Tween20	3.88 (=stock concentration)

15 **Table 30: Study groups in the *in vivo* experiments**

Group	No. of animals	Mouse strain	Compound	Dose /mouse	Route/ Mode of administration	No of treatments
A	9	NOG	vehicle	-	i.v.	3
B	10	NOG	untargeted 4-1BBL	197 µg	i.v.	3
C	9	NOG	FolR1 CD3 TCB	0.1 µg	i.v.	3

D	10	NOG	FAP (28H1) 4-1BBL	197 µg	i.v.	3
E	10	NOG	FolR1 CD3 TCB + untargeted 4-1BBL	0.1 µg 197 µg	i.v.	3
F	10	NOG	FolR1 CD3 TCB + FAP (28H1) 4-1BBL	0.1 µg 197 µg	i.v.	3
G	9	NOG	FolR1 CD3 TCB + FAP (28H1) 4-1BBL	0.1 µg 19.7 µg	i.v.	3

Table 31: Study Plan

<i>Study Day</i>	<i>Experimental Procedure</i>
Day 0	Harvesting and preparation of SKOV3
Day 0	SKOV3 injection s.c.
Day 27	Injection of PBMCs i.p.
Day 29	Injection of 1 st therapy
Day 29-36	Bleed 2 mice/group 10 min, 1 h, 8 h, 24 h and 7 d after 1 st therapy
Day 36	Injection of 2 nd therapy
Day 43	Injection of 3 rd therapy
Day 44, end of experiment	Termination of experiment

Figure 45 shows that the combination FolR1 CD3 TCB + mono FAP (28H1)-4-1BBL mediated superior efficacy in terms of Tumor growth inhibition compared to the vehicle group. All combination groups (FolR1 CD3 TCB + FAP(28H1)-4-1BBL and FolR1 CD3 TCB + untargeted 4-1BBL) are significantly different from Vehicle control.

Table 32: Tumor growth inhibition (TGI) at study days 35, 40 and 42

Group	TGI day 35 [%]	TGI day 40 [%]	TGI day 42 [%]
B (monovalent untarg.4-1BBL)	41.2	28.1	19.3
C (FolR1 CD3 TCB)	58.8	75.5	73.0
D (monovalent FAP 4-1BBL)	79.5	56.4	51.6
E (FolR1 CD3 TCB + monovalent untarg. 4-	79.2	86.9	90.5

1BBL)			
F (FolR1 CD3 TCB + monovalent FAP 4-1BBL)	73.2	89.1	90.9
G (FolR1 CD3 TCB + monovalent FAP 4-1BBL, low dose)	51.9	75.5	70.0

b) Experiments with mono- and bivalent FAP(28H1)-4-1BBL

The human monovalent and bivalent FAP-targeted 4-1BBL (mono FAP(28H1)-4-1BBL and bi FAP(28H1)-4-1BBL) were tested as single agent and in combination with the human FolR1 CD3 TCB against the mono- and bivalent untargeted 4-1BBL (mono untarg-4-1BBL and bi untarg-4-1BBL). Human ovarian SKOV3 cells were injected subcutaneously in NOG mice from Taconic. 2 Days before the first therapy mice were injected with human PBMCs isolated from buffy coat.

Human SKOV3 cells (ovarian carcinoma) were originally obtained from ATCC and after expansion deposited in the Roche internal cell bank. Cells were cultured in RPMI containing 10% FCS, 1% Glutamax at 37 °C in a water-saturated atmosphere at 5 % CO₂. In vitro passage 44 was used for subcutaneous injection at a viability of 99.3%. 50 microliters cell suspension (5x10⁶ SKOV3 cells) mixed with 50 microliters Matrigel were injected subcutaneously in the flank of anaesthetized mice with a 22G to 30G needle.

Buffy coats were obtained from Züricher Blutspende and PBMCs purified freshly before injection on day 28. 10 Mio PBMCs were injected i.p. in 200ul volume of RPMI.

80 NOG female mice were delivered by Taconic. Mice were maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). Experimental study protocol was reviewed and approved by local government (P 2005086). After arrival animals were maintained for one week to get accustomed to new environment and for observation. Continuous health monitoring was carried out on regular basis.

Mice were injected sub cutaneously on study day 0 with 1x10⁶ SKOV3 cells. Tumors were measured 2 to 3 times per week during the whole experiment by Caliper. Freshly purified PBMCs were injected on day 27, 2 days before the first therapy. One day after randomization, on day 29, mice were injected i.v. with Vehicle, FolR1 CD3 TCB, mono untarg-4-1BBL, bi-untarg-4-1BBL, mono FAP-4-1BBL, bi FAP-4-1BBL, mono untarg-4-1BBL + FolR1 CD3 TCB, mono FAP-4-1BBL + FolR1 CD3 TCB or bi FAP-4-1BBL + FolR1 CD3 TCB up to 2 weeks.

All mice were injected i.v. with 200 μ l of the appropriate solution. The mice in the vehicle group were injected with Histidine Buffer and the treatment groups with the 4-1BBL containing constructs, the FOLR1-TCB or the combinations. To obtain the proper amount of compound per 200 μ l, the stock solutions were diluted with Histidine Buffer when necessary.

- 5 The dose and schedule used for FolR1 CD3 TCB was 5 μ g/kg, once/week whereas the 4-1BBL constructs were given at a dose of 10 mg/kg once/week.

Table 33: Compositions used in the *in vivo* experiments

Compound	Dose	Formulation buffer	Concentration (mg/mL)
monovalent untargeted 4-1BBL (DP47)-4-1BBL	10 mg/kg	20 mM Histidine, 140 mM NaCl, 0.01% Tween-20, pH 6.0	3.07 (=stock concentration)
bivalent untargeted 4-1BBL (DP47)-4-1BBL	10 mg/kg	20 mM Histidine, 140 mM NaCl, 0.01% Tween-20, pH 6.0	2.57 (=stock concentration)
monovalent FAP (28H1) 4-1BBL (mono FAP-4-1BBL)	10 mg/kg	20 mM Histidine, 140 mM NaCl, pH 6.0	4.5 (=stock concentration)
bivalent FAP (28H1) 4-1BBL (bi FAP-4-1BBL)	10 mg/kg	20 mM Histidine, 140 mM NaCl, pH 6.0	3.07 (=stock concentration)
FolR1 CD3 TCB	5 μ g/kg	20 mM Histidine, 140 mM NaCl, pH 6.0, 0.01% Tween20	0.76 (=stock concentration)

- 2 mice/group were bled 10 min, 1 h, 7 h, 72 h and 7d after first therapy to determine the pharmacokinetic profile of the compounds during the first week. Mouse serum samples were analyzed with ELISA method. Biotinylated hu 4-1BB (Roche Innovation Center Zürich), test sample, Digoxigenin labelled anti-huCH1 antibody (Roche Diagnostics GmbH) and anti-Digoxigenin detection antibody (Roche Diagnostics GmbH) were added stepwise to a 96-well streptavidin-coated microtiter plate. The peroxidase-bound complex was visualized by adding ABTS substrate solution to form a colored reaction product.

- The experiment was terminated at study day 42, 6 days after the second therapy. Tumors were harvested in PBS, single cell suspensions were generated and stained for different immune cell markers and analysed by FACS (staining antibodies Biolegend: Anti-human CD45 AF488, Anti-human CD3 Percp-Cy5.5, Anti-human CD8 PE-Cy7, Anti-human CD4 BV421).

Table 34: Study groups in the *in vivo* experiments

Group	No. of animals	Mouse strain	Compound	Dose (mg/kg)	Route/ Mode of administration	No of treatments
A	8	NOG	vehicle	-	i.v.	2
B	9	NOG	FolR1 CD3 TCB	5 µg/kg	i.v.	2
C	9	NOG	mono untargeted 4-1BBL	10 mg/kg	i.v.	2
D	9	NOG	bi untargeted 4-1BBL	10 mg/kg	i.v.	2
E	9	NOG	mono FAP (28H1) 4-1BBL	10 mg/kg	i.v.	2
F	9	NOG	bi FAP (28H1) 4-1BBL	10 mg/kg	i.v.	2
G	9	NOG	FolR1 CD3 TCB + mono untargeted 4-1BBL	5 µg/kg + 10 mg/kg	i.v.	2
H	9	NOG	FolR1 CD3 TCB + mono FAP (28H1) 4-1BBL	5 µg/kg + 10 mg/kg	i.v.	2
I	9	NOG	FolR1 CD3 TCB + bi FAP (28H1) 4-1BBL	5 µg/kg + 10 mg/kg	i.v.	2

Table 35: Study Plan

<i>Study Day</i>	<i>Experimental Procedure</i>
Day 0	Harvesting and preparation of SKOV3
Day 0	SKOV3 injection s.c.
Day 27	Injection of PBMCs i.p.
Day 29	Injection of 1 st therapy
Day 29-36	Bleed 2mice/group 10min, 1h, 8h, 24h and 7d after 1 st therapy
Day 36	Injection of 2 nd therapy
Day42	Termination of experiment

Figure 49 shows that all combinations with FolR1 CD3 TCB showed slightly improved efficacy in terms of Tumor growth inhibition compared to the vehicle group and FolR1 CD3 TCB alone. No statistical differences could be observed between the different combinations.

Table 36: Tumor growth inhibition (TGI) at study days 39 and 41

Group	TGI day 39 [%]	TGI day 41 [%]
FolR1 CD3 TCB	31.9	39.5
monovalent untarg. 4-1BBL	5.2	-9.1
bivalent untarg. 4-1BBL	-15.2	-23.6
monovalent FAP 4-1BBL	10.6	12.4
bivalent FAP 4-1BBL	17.7	4.4
FolR1 CD3 TCB + monovalent untarg. 4-1BBL	59.8	73.1
FolR1 CD3 TCB + monovalent FAP 4-1BBL	52.4	66.8
FolR1 CD3 TCB + bivalent FAP 4-1BBL	59.8	77.1

5

Example 16

Assessing the ability of FAP 4-1BBL to support TCB-mediated target cell killing *in vitro*

To assess the capacity of FAP 4-1BBL to support TCB-mediated tumor cell killing, purified CD8⁺ T cells or pan T cells served as effector cells and RFP-expressing MV3 melanoma cells served as tumor targets. Effector cells were purified by negative selection (Miltenyi) from buffy coats of healthy donors. 105 effector cells per well were co-cultured with 5000 MV3 target cells in flat bottom 96 well plates. Killing of MV3 target cells in presence of 5 pM MCSP CD3 TCB alone or in combination with 1 nM FAP 4-1BBL was monitored over the course of 5 days capturing 4 images per well every 3 hours using an IncuCyte live cell imager (Essen Biosciences), counting RFP⁺ target cells using the IncuCyte ZOOM software (Essen Biosciences). RFP⁺ object counts per image over time served as proxy for target cell death. TCB-mediated target cell killing was distinguished from spontaneous target cell death by monitoring counts of MV3 target cells in presence of effector T cells alone over time (=baseline control). Killing by TCB was calculated as 100 – x, x being %targets relative to the baseline control. Statistical analyses were performed using student's t-test, comparing the areas under the curves (AUC) of %killing over time.

MCSP CD3 TCB mediated target cell killing was significantly increased by FAP 4-1BBL for CD8⁺ T cells (3 out of 3 donors) and pan T cells (3 out of 3 donors). Despite observable target cell killing by MCSP CD3 TCB alone throughout the experiment, killing was not effective at termination of the experiment, with target cell growth achieving baseline

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levels in 3/3 donors using purified pan T cells and 1/3 donors using purified CD8⁺ T cells. In contrast, target cell killing was effective in all donors with the combination of MCSP CD3 TCB and FAP 4-1BBL, achieving average plateaus at 89% killing (CD8⁺ T cells) and 72% killing (pan T cells), indicative of a strong supportive capacity of FAP 4-1BBL for MCSP

5 CD3 TCB mediated target cell killing (see **Figures 52 and 53**).

Claims

1. A T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method for treating or delaying progression of cancer, wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is used in
5 combination with a 4-1BB (CD137) agonist.
2. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of claim 1, wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is an anti-CEA/anti-CD3 bispecific antibody, an anti-FolR1/anti-CD3 bispecific antibody or an anti-MCSP/anti-CD3 bispecific antibody.
- 10 3. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of claims 1 or 2, wherein the T-cell activating anti-CD3 bispecific antibody and the 4-1BB agonist are administered together in a single composition or administered separately in two or more different compositions.
- 15 4. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 3, wherein the 4-1BB agonist acts synergistically with the T-cell activating anti-CD3 bispecific antibody.
5. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 4, wherein the 4-1BB agonist comprises three ectodomains of 4-1BBL or fragments thereof.
- 20 6. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 5, wherein the 4-1BB agonist is a molecule comprising three ectodomains of 4-1BBL or fragments thereof and wherein the ectodomains of 4-1BBL comprise an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:
25 6, SEQ ID NO:7 and SEQ ID NO:8, particularly the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:5.
7. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 6, wherein the 4-1BB agonist is an antigen binding molecule comprising at least one antigen binding domain capable of specific
30 binding to a tumor-associated antigen.

8. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 7, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to a tumor-associated antigen.

5 9. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 8, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain.

10 10. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 9, wherein the 4-1BB agonist is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

15 11. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 10, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to Fibroblast activation protein (FAP).

20 12. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 9, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP, wherein 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:9, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID
25 NO:10, 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, or
(b) a heavy chain variable region (V_H FAP) comprising (i) CDR-H1 comprising the amino
30 acid sequence of SEQ ID NO:15, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:16, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:17, and a light chain variable region (V_L FAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:18, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:19, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:20.

13. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 12, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to FAP, wherein 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:21 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:22 or wherein 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:23 and a light chain variable region (V_LFAP) comprising an amino acid sequence of SEQ ID NO:24.

14. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 13, wherein the 4-1BB agonist is an antigen binding molecule comprising

(a) at least one antigen binding domain capable of specific binding to FAP,

(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL or a fragment thereof.

15. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 14, wherein the 4-1BB agonist is an antigen binding molecule comprising

(a) at least one Fab domain capable of specific binding to FAP comprising a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:21 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:22 or a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:23 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:24, and

(b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

16. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 14, wherein the 4-1BB agonist is an antigen binding molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:67 and a second light chain comprising the amino acid sequence of SEQ ID NO:68.

17. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 4, wherein the 4-1BB agonist is an anti-FAP/anti-4-1BB bispecific antibody.

18. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 10, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA.

19. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 10 or 18, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises (a) a heavy chain variable region (V_{HCEA}) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:49, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:50, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:51, and a light chain variable region (V_{LCEA}) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:54.

20. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 10 or 18 or 19, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to CEA, wherein the antigen binding domain capable of specific binding to CEA comprises a heavy chain variable region (V_{HCEA}) comprising an amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_{LCEA}) comprising an amino acid sequence of SEQ ID NO:56.

21. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 10 or 21 to 25, wherein the 4-1BB

agonist is an antigen binding molecule comprising

- (a) at least one antigen binding domain capable of specific binding to CEA,
(b) a first and a second polypeptide that are linked to each other by a disulfide bond,
wherein the first polypeptide comprises two ectodomains of 4-1BBL or fragments thereof that
5 are connected to each other by a peptide linker and in that the second polypeptide comprises
one ectodomain of 4-1BBL or a fragment thereof.

22. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 9 or 18 to 21, wherein the 4-1BB agonist is an antigen binding molecule comprising

- 10 (a) at least one Fab domain capable of specific binding to CEA comprising a heavy chain variable region (V_{HCEA}) comprising the amino acid sequence of SEQ ID NO:55 and a light chain variable region (V_{LCEA}) comprising the amino acid sequence of SEQ ID NO:56, and
(b) a first and a second polypeptide that are linked to each other by a disulfide bond,
wherein the antigen binding molecule is characterized in that the first polypeptide comprises
15 the amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

- 20 23. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 5, wherein the 4-1BB agonist is an anti-CEA/anti-4-1BB bispecific antibody.

24. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 23, wherein the first antigen binding
25 domain comprises a heavy chain variable region (V_{HCD3}) comprising CDR-H1 sequence of SEQ ID NO:33, CDR-H2 sequence of SEQ ID NO:34, and CDR-H3 sequence of SEQ ID NO:35; and/or a light chain variable region (V_{LCD3}) comprising CDR-L1 sequence of SEQ ID NO:36, CDR-L2 sequence of SEQ ID NO:37, and CDR-L3 sequence of SEQ ID NO:38.

25. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated
30 antigen for use in a method of any one of claims 1 to 24, wherein the first antigen binding domain comprises a heavy chain variable region (V_{HCD3}) comprising the amino acid sequence of SEQ ID NO:39 and/or a light chain variable region (V_{LCD3}) comprising the amino acid sequence of SEQ ID NO:40.

26. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 25, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_H CD3), a light chain variable region (V_L CD3), and a second antigen binding domain comprising a heavy chain variable region (V_H CEA) and a light chain variable region (V_L CEA).

27. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 26, wherein the second antigen binding domain comprises

10 (a) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:41, CDR-H2 sequence of SEQ ID NO:42, and CDR-H3 sequence of SEQ ID NO:43, and/or a light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:44, CDR-L2 sequence of SEQ ID NO:45, and CDR-L3 sequence of SEQ ID NO:46, or

15 (b) a heavy chain variable region (V_H CEA) comprising CDR-H1 sequence of SEQ ID NO:49, CDR-H2 sequence of SEQ ID NO:50, and CDR-H3 sequence of SEQ ID NO:51, and/or a light chain variable region (V_L CEA) comprising CDR-L1 sequence of SEQ ID NO:52, CDR-L2 sequence of SEQ ID NO:53, and CDR-L3 sequence of SEQ ID NO:54.

28. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 27, wherein the second antigen binding domain comprises a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:47 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:48 or wherein the second antigen binding domain comprises a heavy chain variable region (V_H CEA) comprising the amino acid sequence of SEQ ID NO:55 and/or a light chain variable region (V_L CEA) comprising the amino acid sequence of SEQ ID NO:56.

29. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 28, wherein the anti-CEA/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to CEA.

30. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 29, wherein the anti-CEA/anti-CD3 bispecific antibody comprises an Fc domain comprising one or more amino acid substitutions that reduce binding to an Fc receptor and/or effector function.

31. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 25, wherein the T-cell activating anti-CD3 bispecific antibody is an anti-FolR1/anti-CD3 bispecific antibody.

32. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 25 or 31, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V_H CD3), a second antigen binding domain comprising a heavy chain variable region (V_H FolR1) and a common light chain variable region.

33. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 25 or 31 or 32, wherein the first antigen binding domain comprises a heavy chain variable region (V_H CD3) comprising CDR-H1 sequence of SEQ ID NO:121, CDR-H2 sequence of SEQ ID NO:122, and CDR-H3 sequence of SEQ ID NO:123; the second antigen binding domain comprises a heavy chain variable region (V_H FolR1) comprising CDR-H1 sequence of SEQ ID NO:124, CDR-H2 sequence of SEQ ID NO:125, and CDR-H3 sequence of SEQ ID NO:126; and wherein the common light chain comprises a CDR-L1 sequence of SEQ ID NO:127, CDR-L2 sequence of SEQ ID NO:128, and CDR-L3 sequence of SEQ ID NO:129.

34. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 25 or 31 to 33, wherein the the first antigen binding domain comprises a heavy chain variable region (V_H CD3) comprising the sequence of SEQ ID NO:130; the second antigen binding domain comprises a heavy chain variable region (V_H FolR1) comprising the sequence of SEQ ID NO:131; and wherein the common light chain comprises the sequence of SEQ ID NO:132.

35. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 25, wherein the T-cell activating anti-CD3 bispecific antibody is an anti-MCSP/anti-CD3 bispecific antibody.

36. The T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen for use in a method of any one of claims 1 to 35, wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is used in combination with a 4-1BB (CD137) agonist and in combination with an agent blocking PD-L1/PD-1 interaction, in particular an anti-PD-L1 antibody or an anti-PD1 antibody.

37. A pharmaceutical product comprising (A) a first composition comprising as active ingredient a T-cell activating anti-CD3 bispecific antibody and a pharmaceutically acceptable carrier; and (B) a second composition comprising as active ingredient a 4-1BB agonist and a pharmaceutically acceptable carrier, for use in the combined, sequential or simultaneous,
5 treatment of a disease, in particular cancer.

38. A pharmaceutical composition comprising a T-cell activating anti-CD3 bispecific antibody and a 4-1BB agonist.

39. The pharmaceutical composition of claim 38 for use in treating or delaying progression of cancer, in particular for the treatment of advanced and/or metastatic solid
10 tumors.

40. Use of a combination of a T-cell activating anti-CD3 bispecific antibody and a 4-1BB agonist in the manufacture of a medicament for treating or delaying progression of a proliferative disease, in particular cancer.

41. A method for treating or delaying progression of cancer in a subject comprising
15 administering to the subject an effective amount of a T-cell activating anti-CD3 bispecific antibody and an effective amount of a 4-1BB agonist.

42. The method for treating or delaying progression of cancer in a subject of claim 41, wherein the 4-1BB agonist comprises three ectodomains of 4-1BBL or fragments thereof.

43. The method for treating or delaying progression of cancer in a subject of claims 41
20 or 42, wherein the T-cell activating anti-CD3 bispecific antibody and the 4-1BB agonist are administered together in a single composition or administered separately in two or more different compositions.

44. The method for treating or delaying progression of cancer in a subject of claims 41
25 to 43, wherein the T-cell activating anti-CD3 bispecific antibody and the 4-1BB (CD137) agonist are administered intravenously or subcutaneously.

45. The method for treating or delaying progression of cancer in a subject of any one of claims 41 to 44, wherein the T-cell activating anti-CD3 bispecific antibody is administered concurrently with, prior to, or subsequently to the 4-1BB agonist.

46. A 4-1BB agonist comprising at least one antigen binding domain capable of specific
30 binding to a tumor-associated antigen for use in a method for treating or delaying progression of cancer, wherein the 4-1BB agonist comprising at least one antigen binding domain capable

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of specific binding to a tumor-associated antigen is used in combination with an agent blocking PD-L1/PD-1 interaction.

47. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of claim 46, wherein the agent blocking PD-L1/PD-1 interaction is an anti-PD-L1 antibody or an anti-PD1 antibody, in particular atezolizumab.

48. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of claims 46 or 47, wherein the 4-1BB agonist is an antigen binding molecule comprising three ectodomains of 4-1BBL or fragments thereof and at least one antigen binding domain capable of specific binding to Fibroblast activation protein (FAP).

49. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 46 to 48, wherein the 4-1BB agonist is an antigen binding molecule comprising an IgG Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

50. The 4-1BB agonist comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 46 to 49, wherein the 4-1BB agonist is an antigen binding molecule comprising a first heavy chain comprising the amino acid sequence of SEQ ID NO:65, a first light chain comprising the amino acid sequence of SEQ ID NO:66, a second heavy chain comprising the amino acid sequence of SEQ ID NO:67 and a second light chain comprising the amino acid sequence of SEQ ID NO:68.

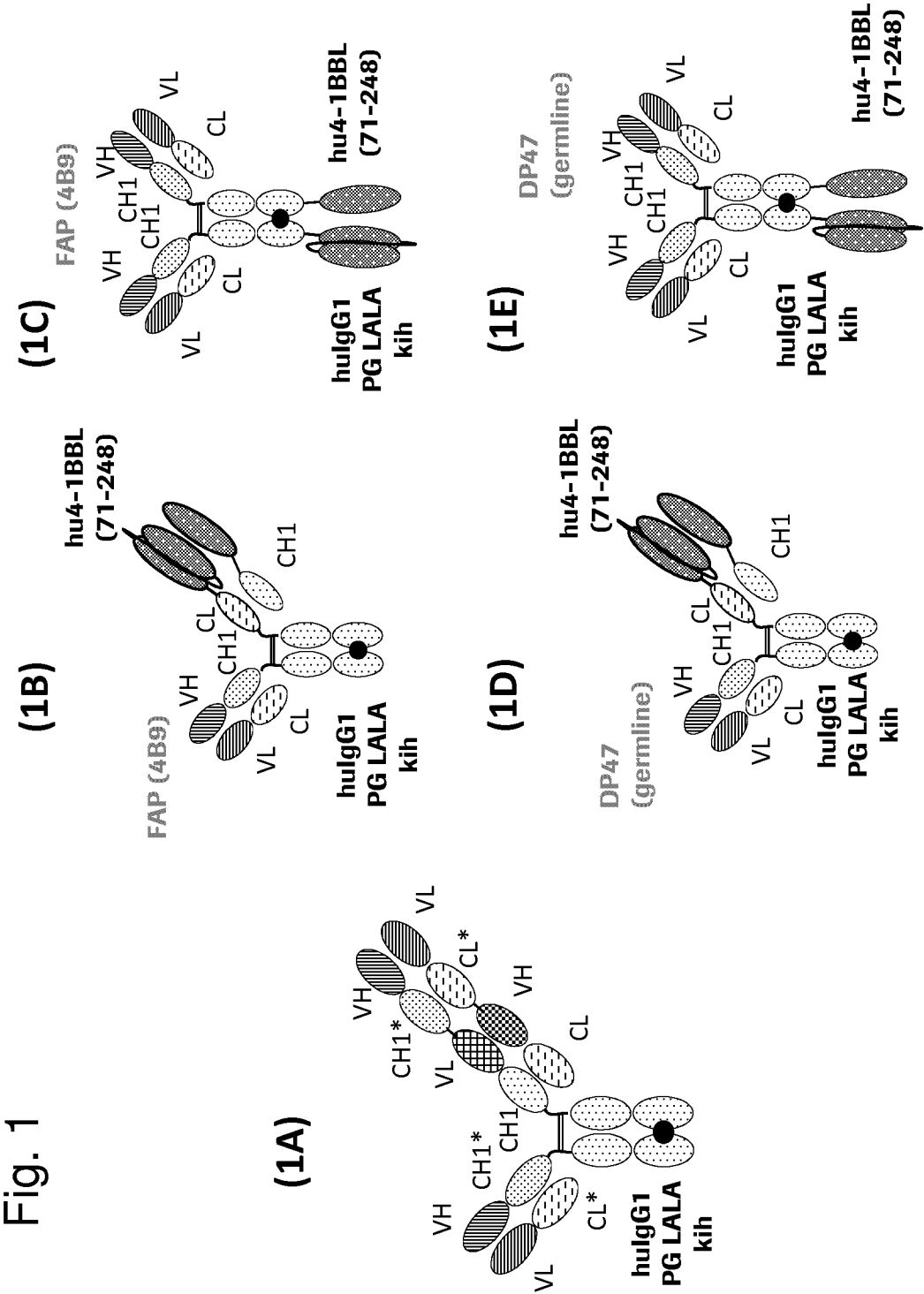


Fig. 2

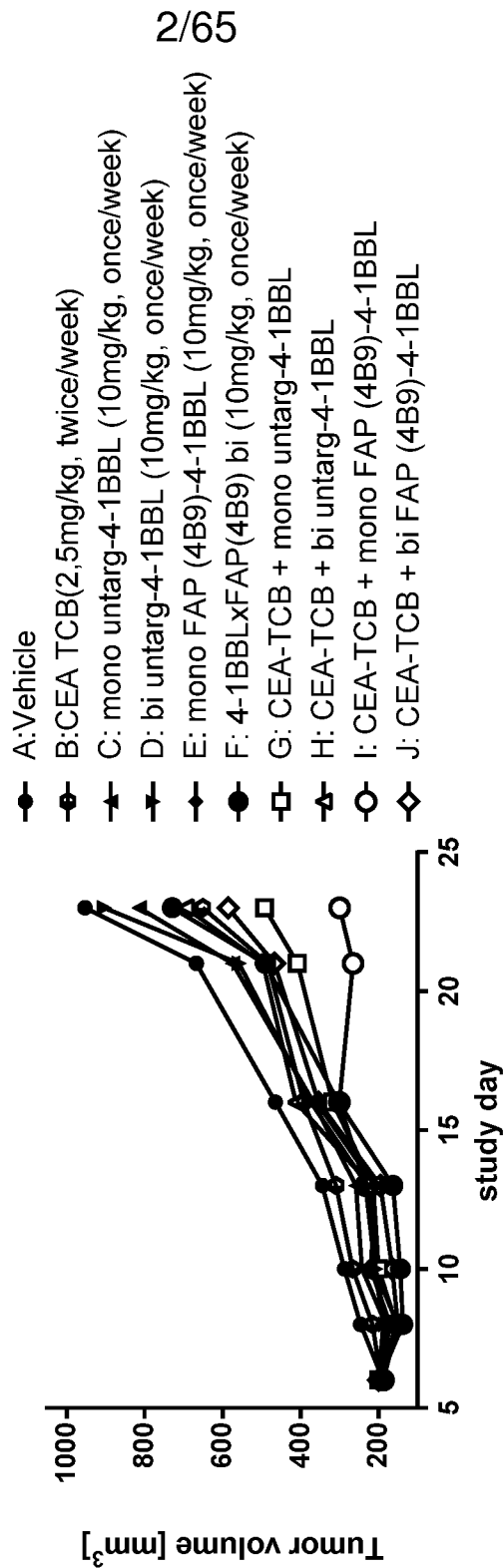


Fig. 3

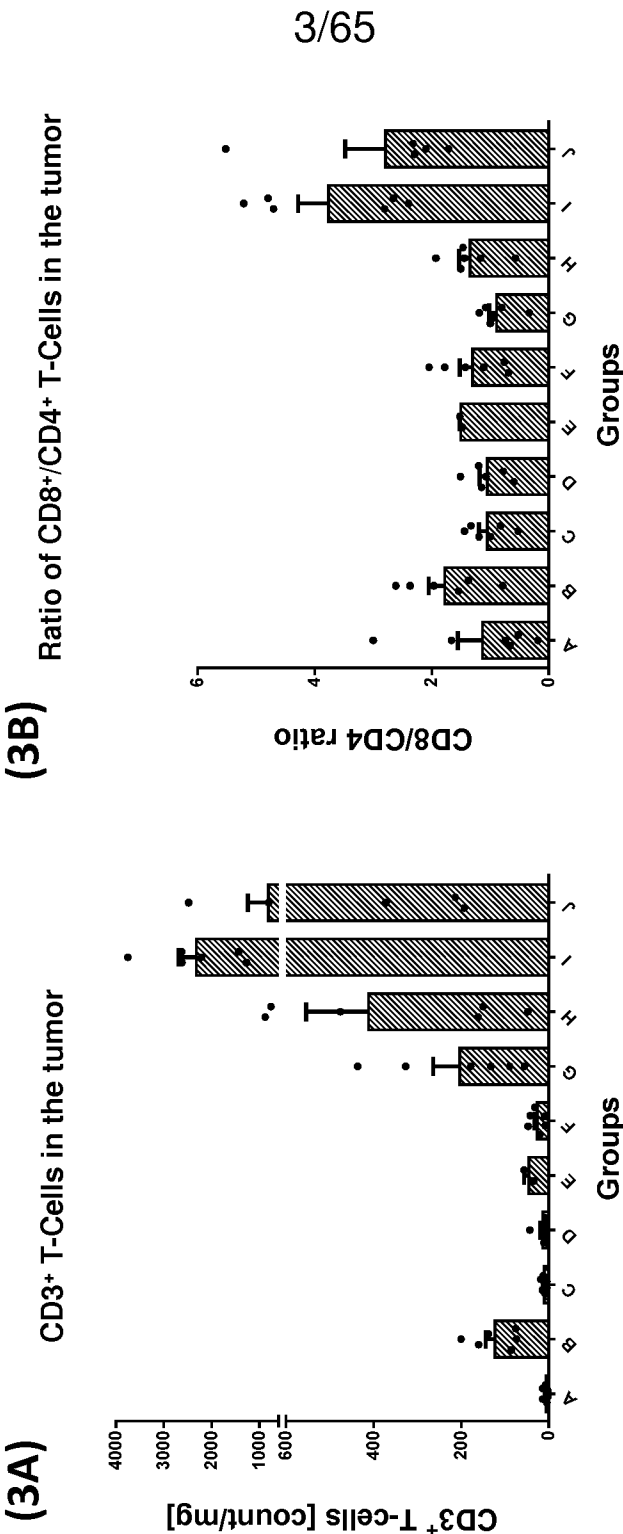


Fig. 3

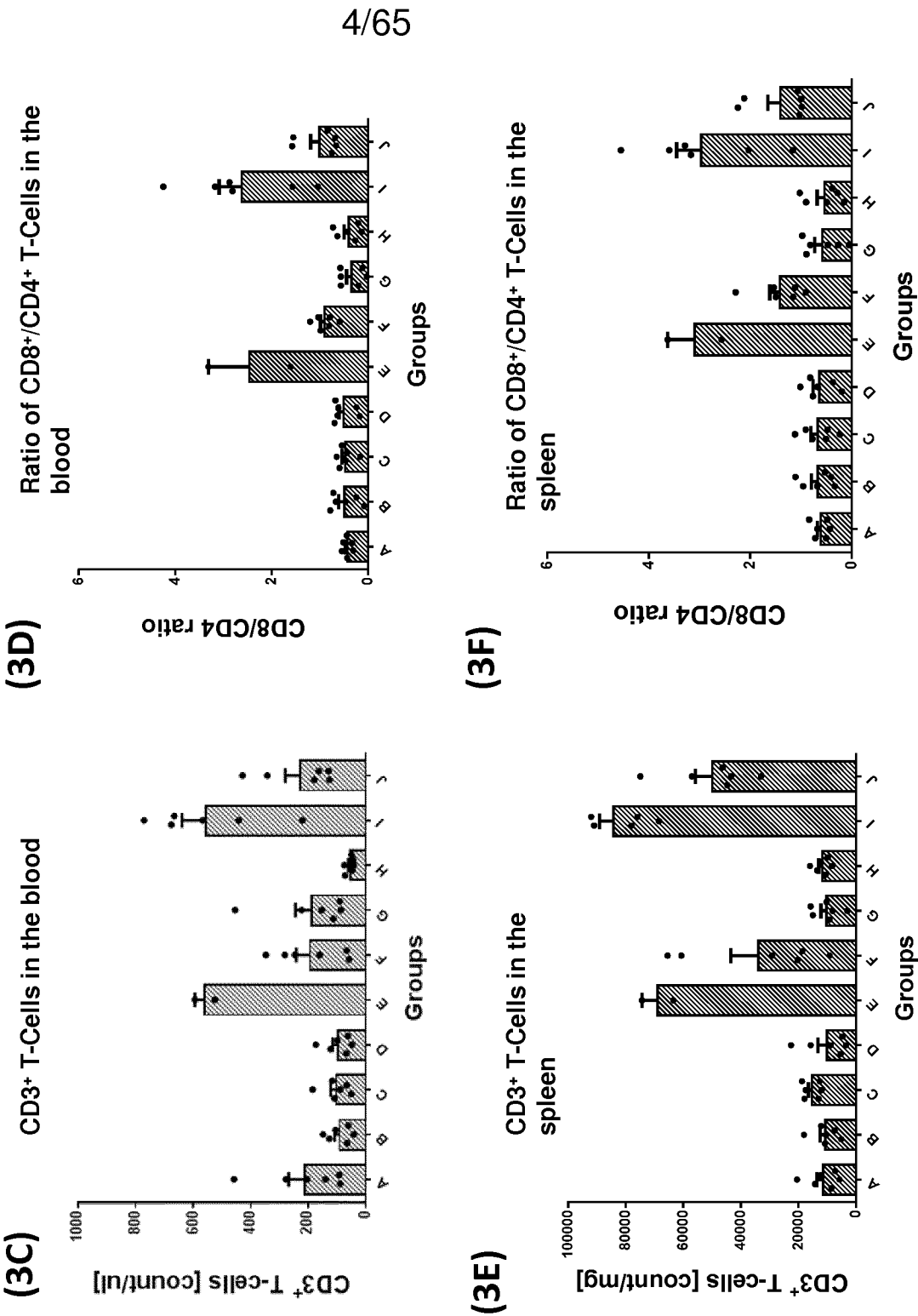


Fig. 4

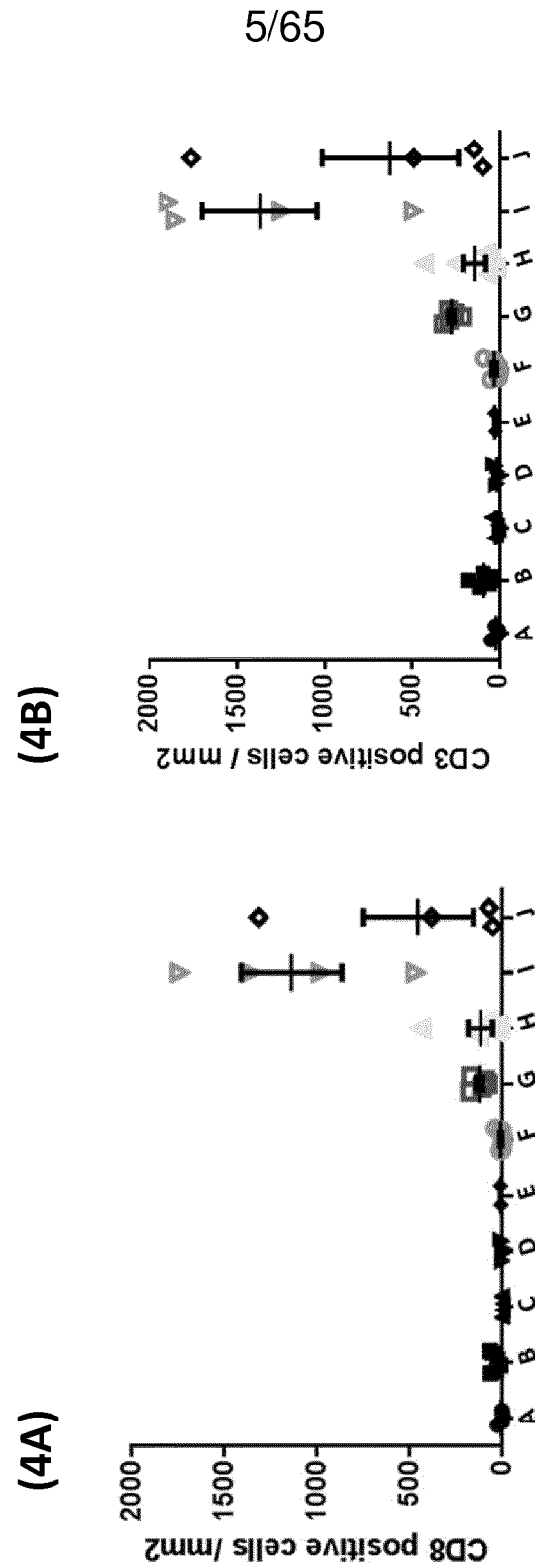


Fig. 5

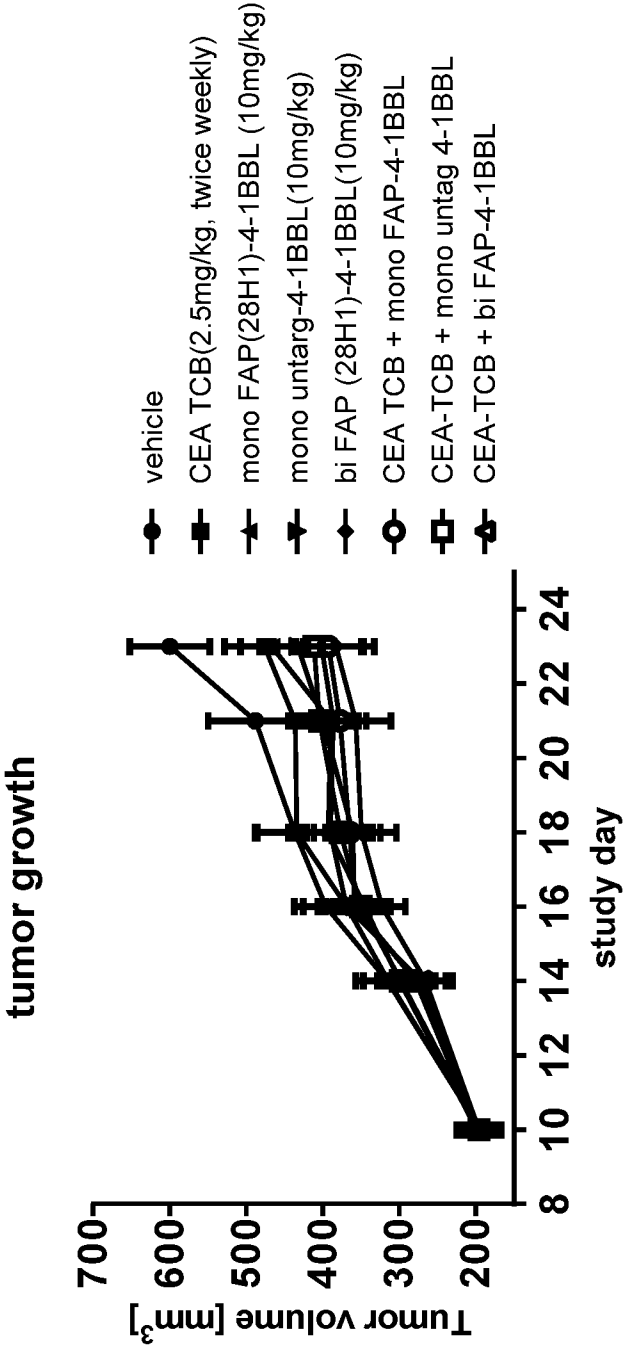


Fig. 6

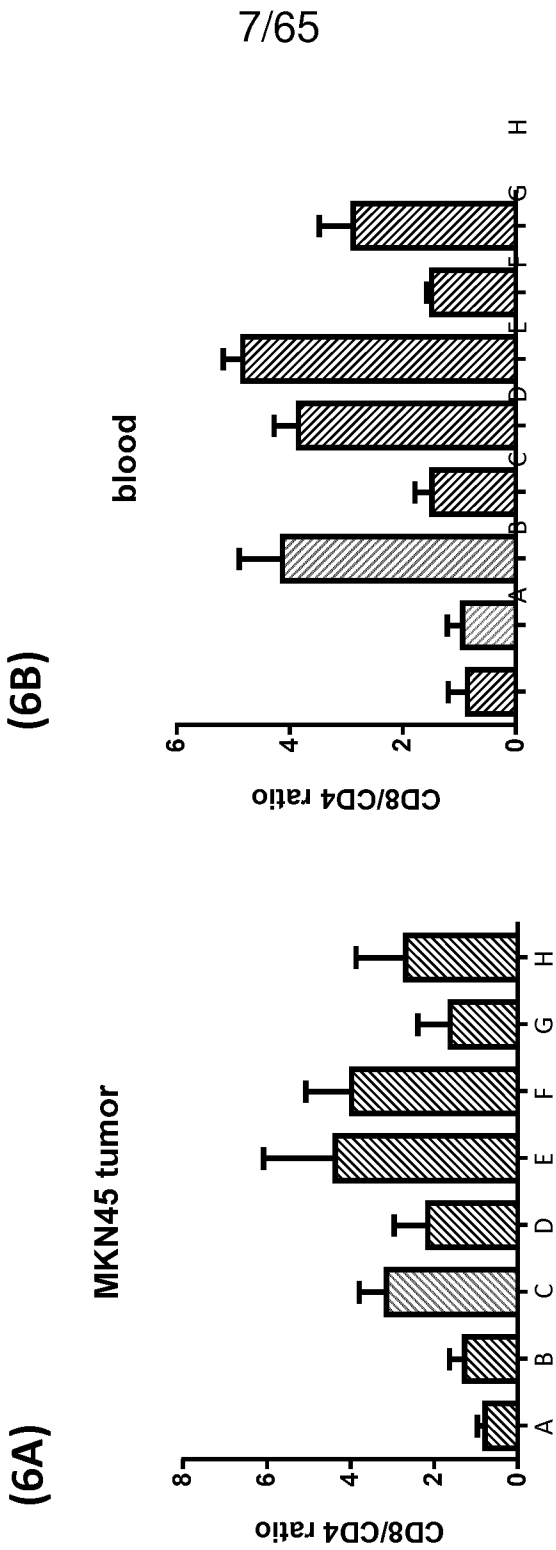


Fig. 7

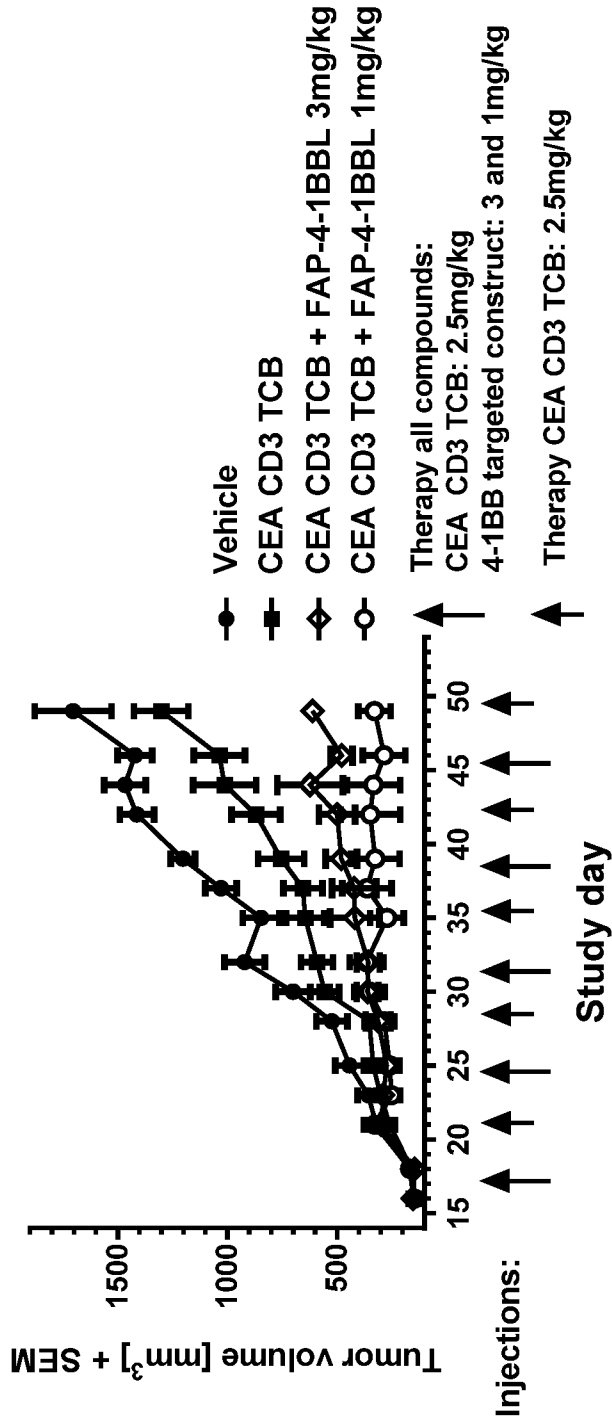


Fig. 8

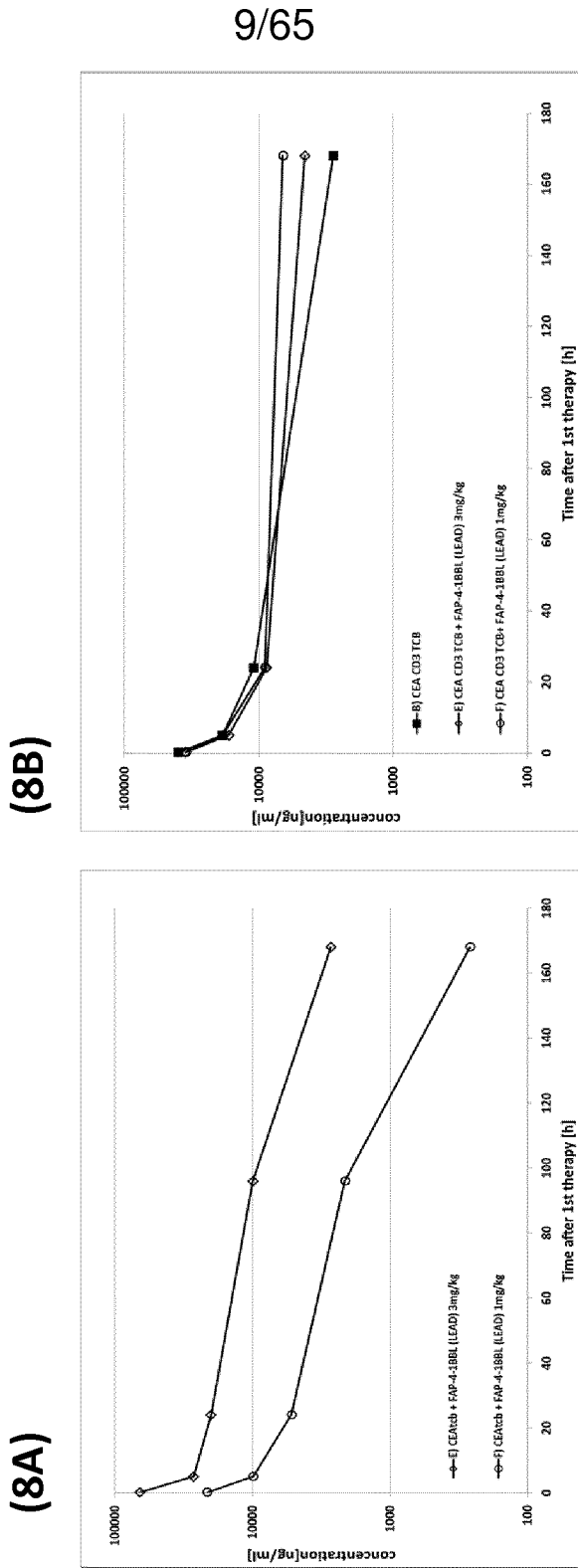
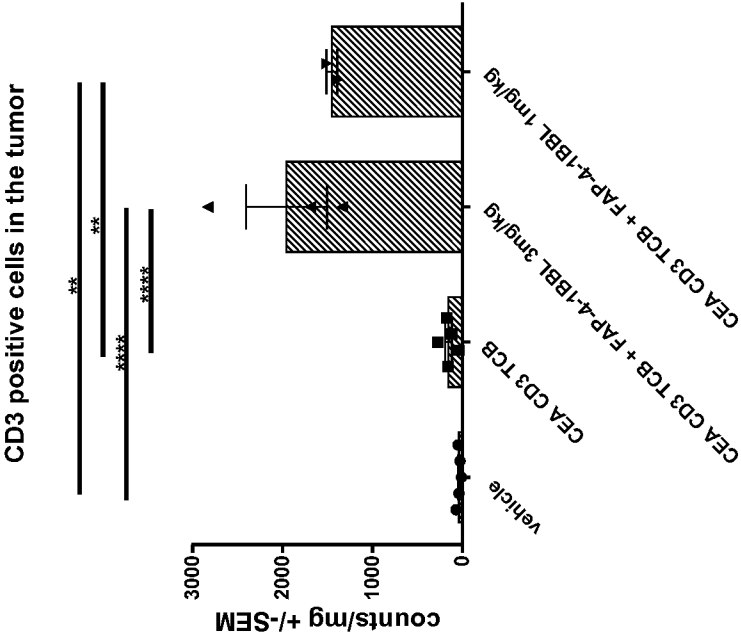


Fig. 9

(9A)



(9B)

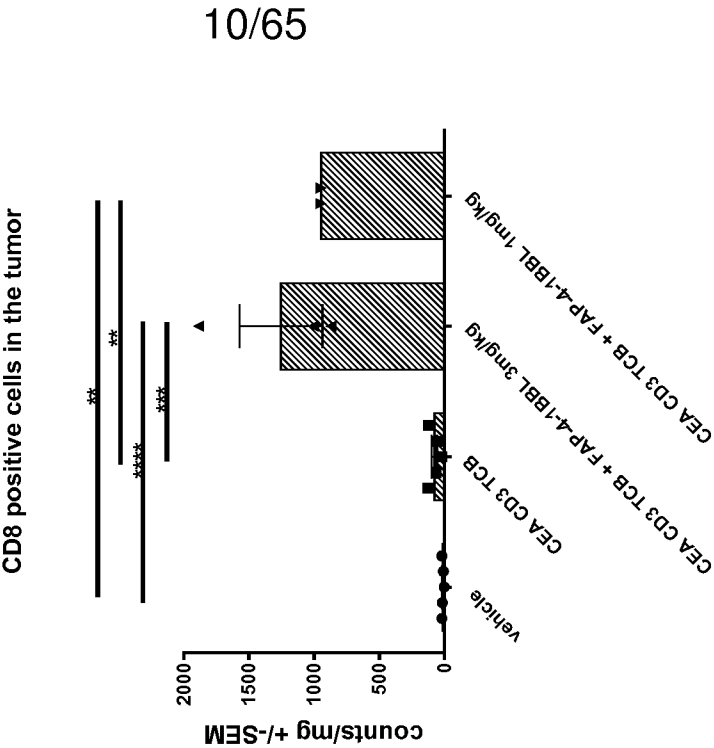
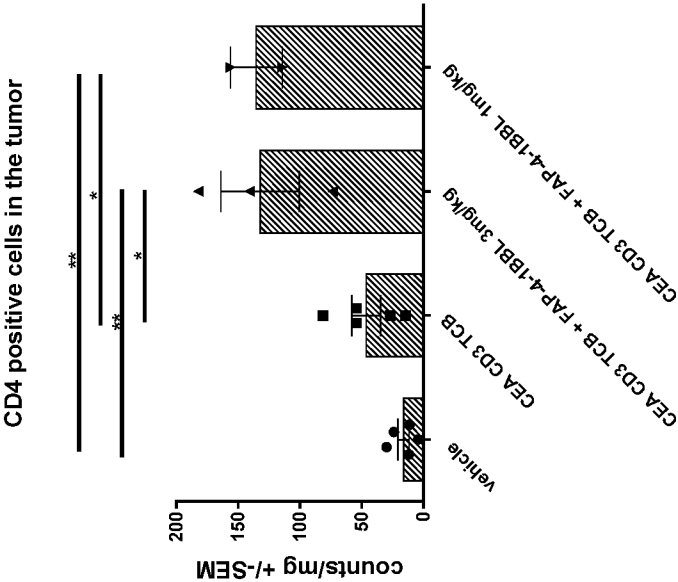


Fig. 9

(9C)



(9D)

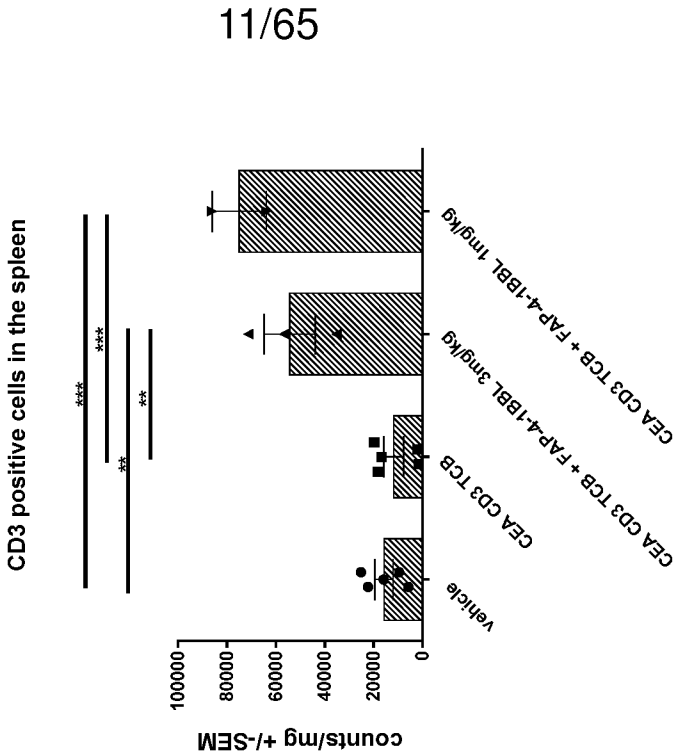


Fig. 10A

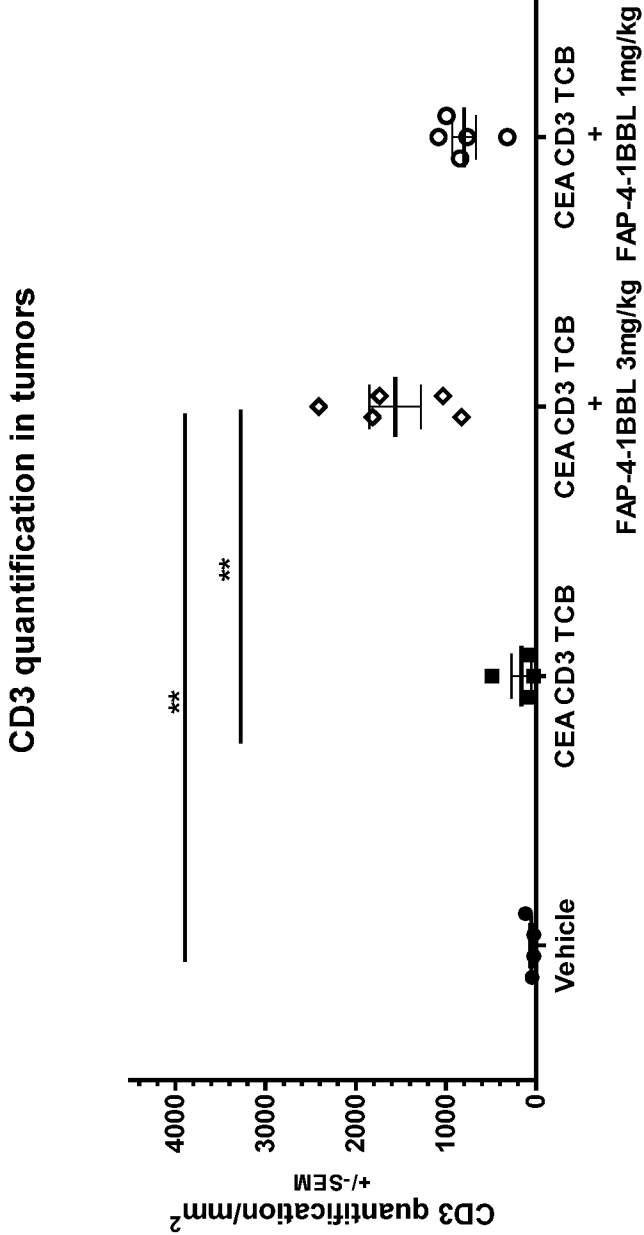


Fig. 10B

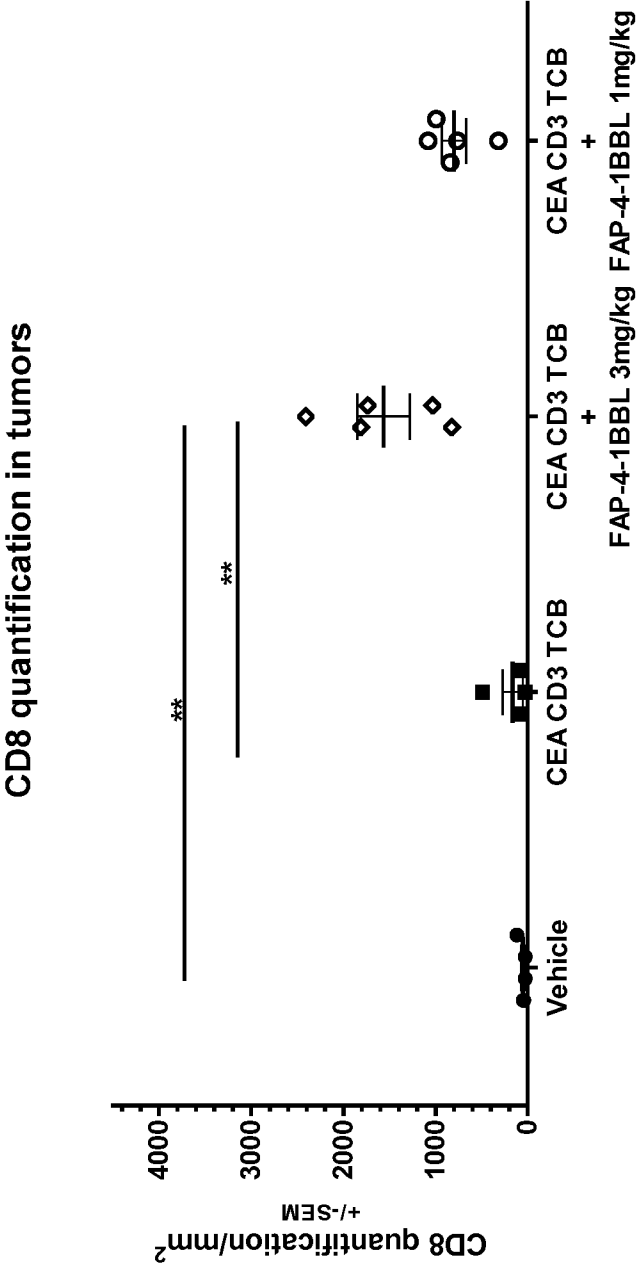


Fig. 11

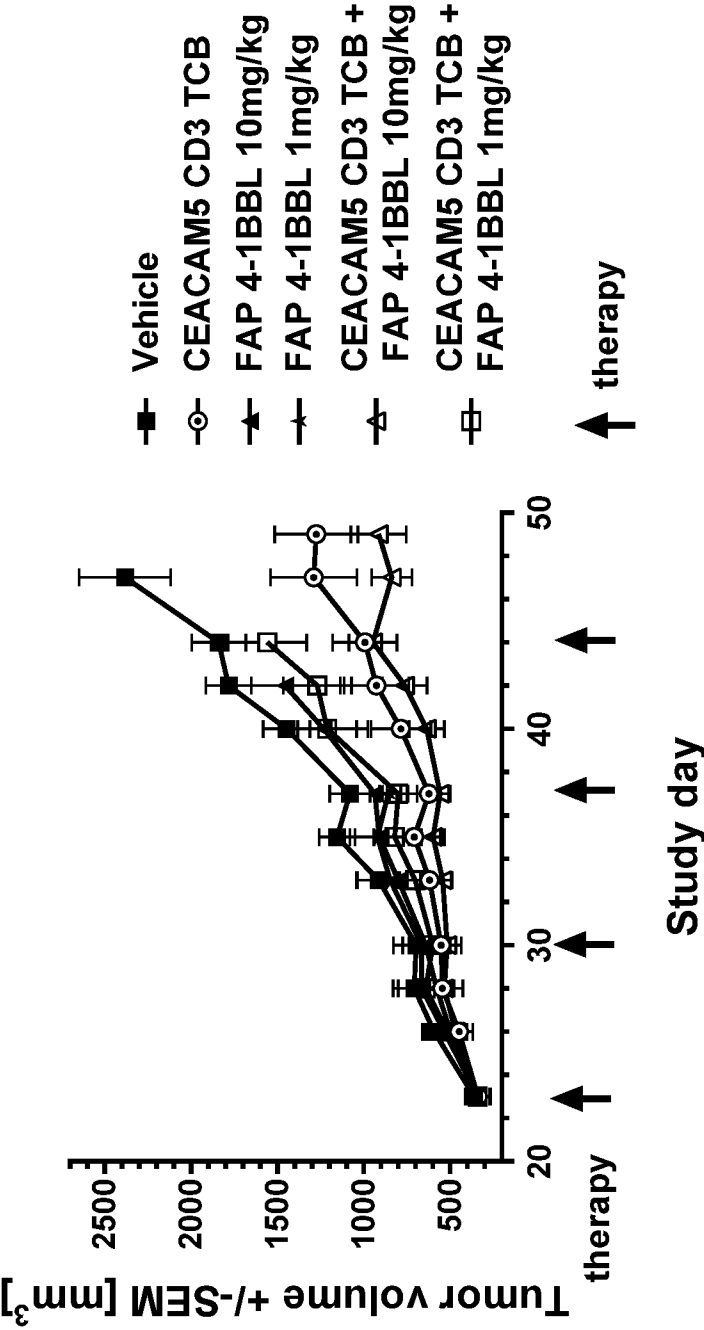


Fig. 12A

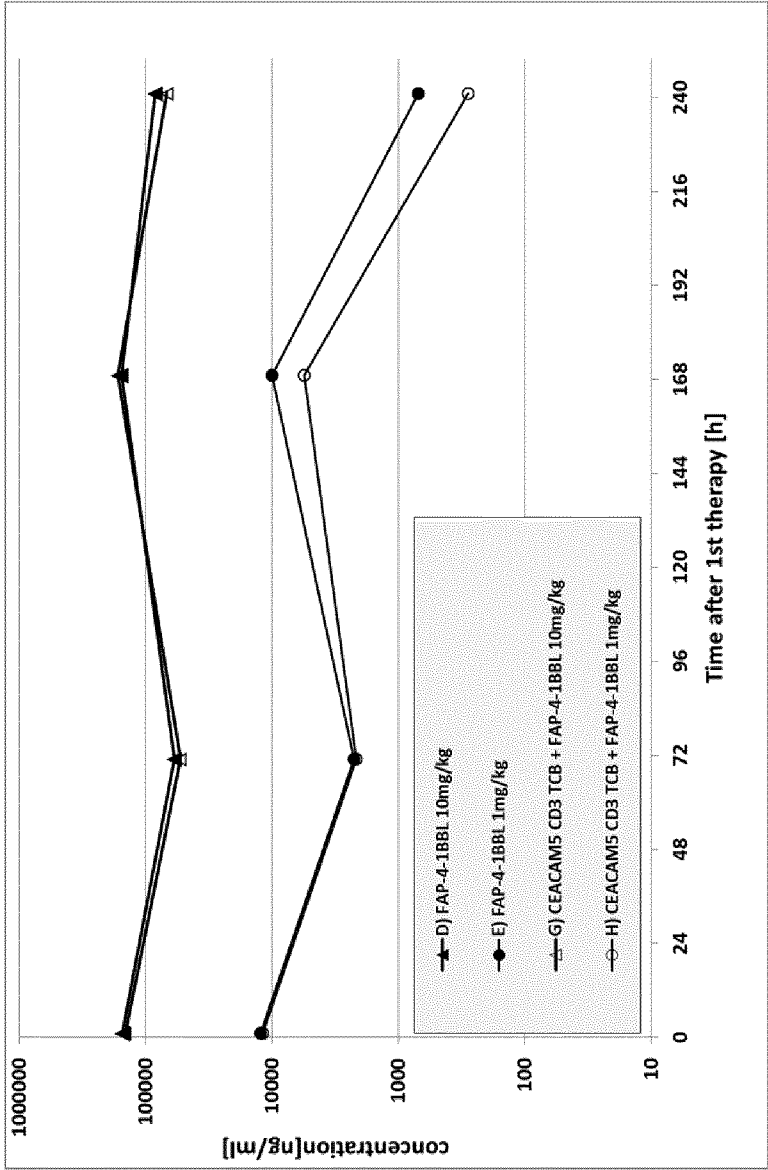


Fig. 12B

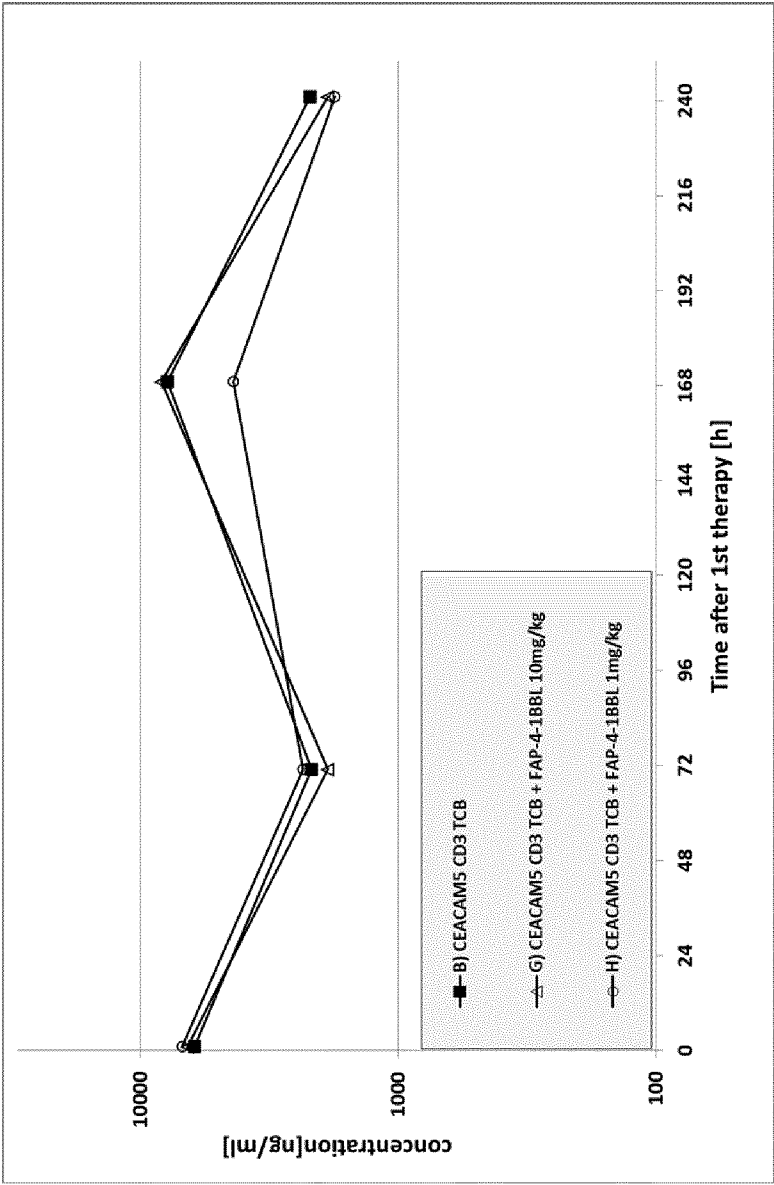


Fig. 13A

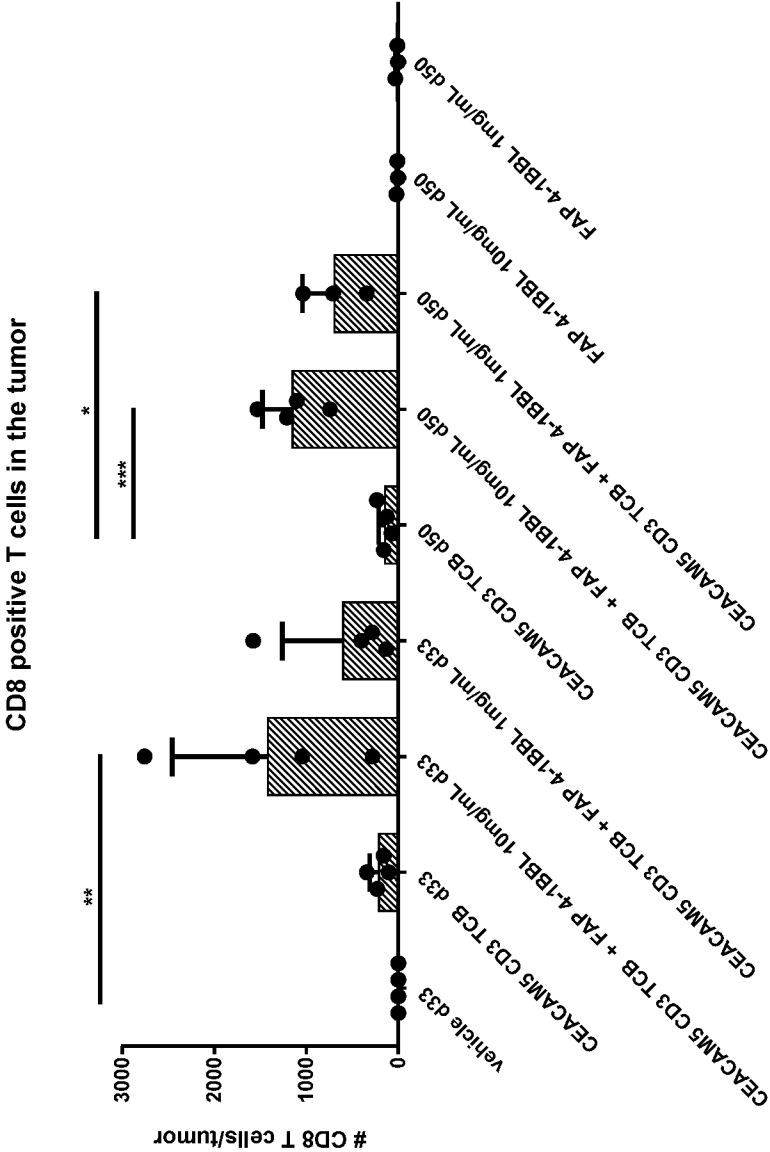


Fig. 13B

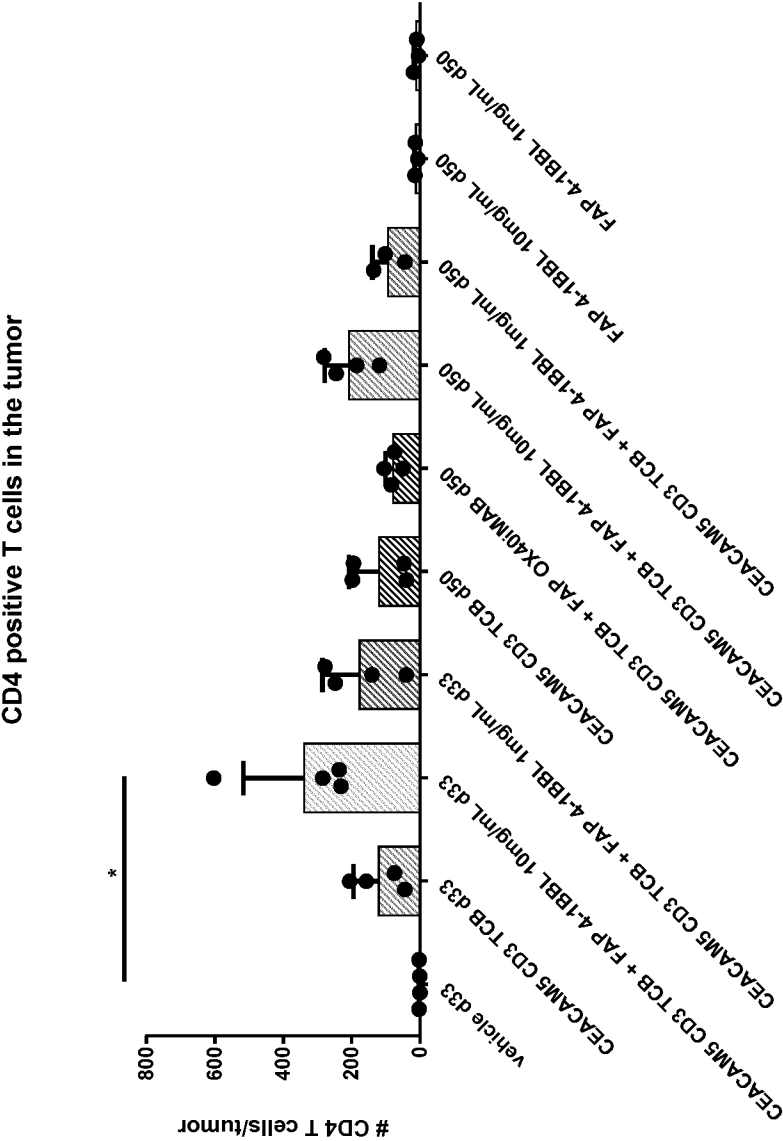


Fig. 14A

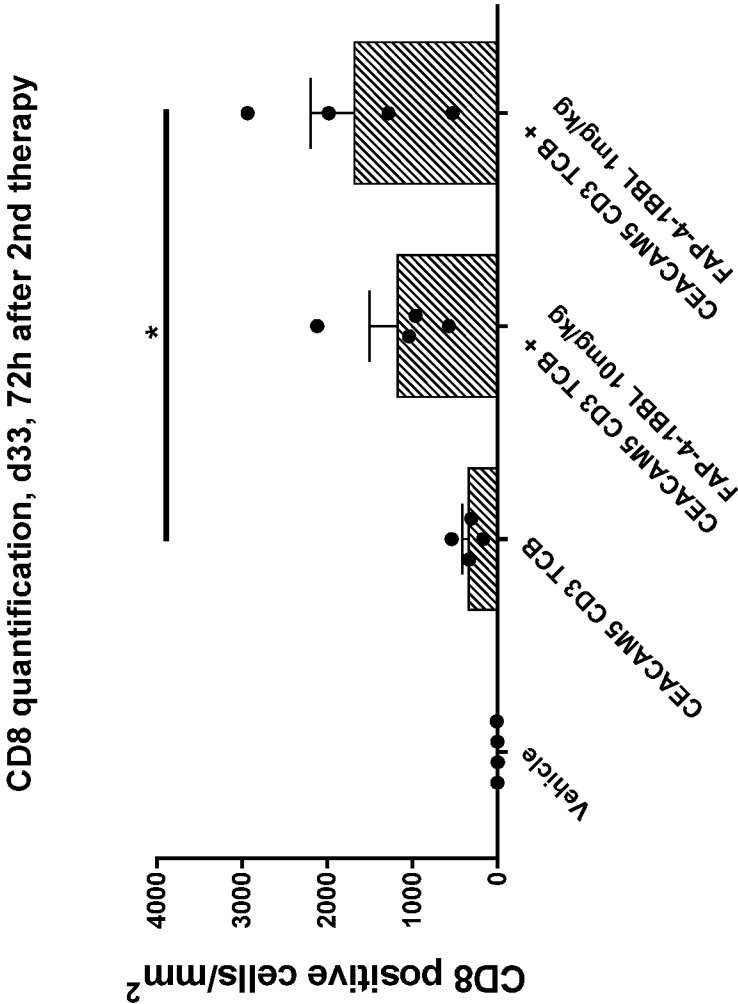


Fig. 14B

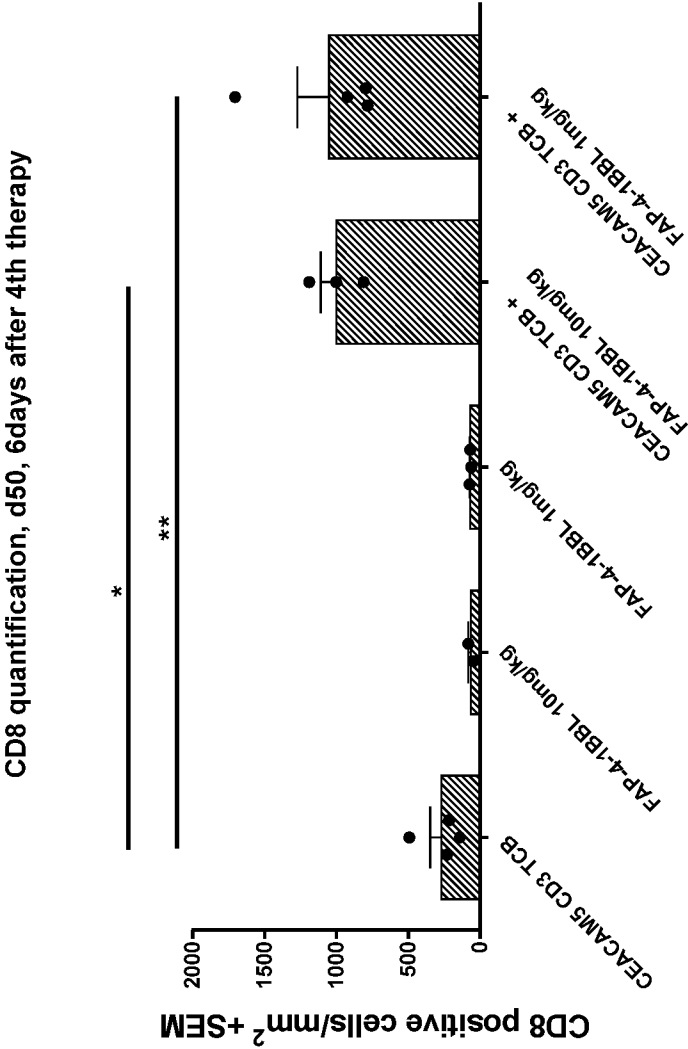


Fig. 15

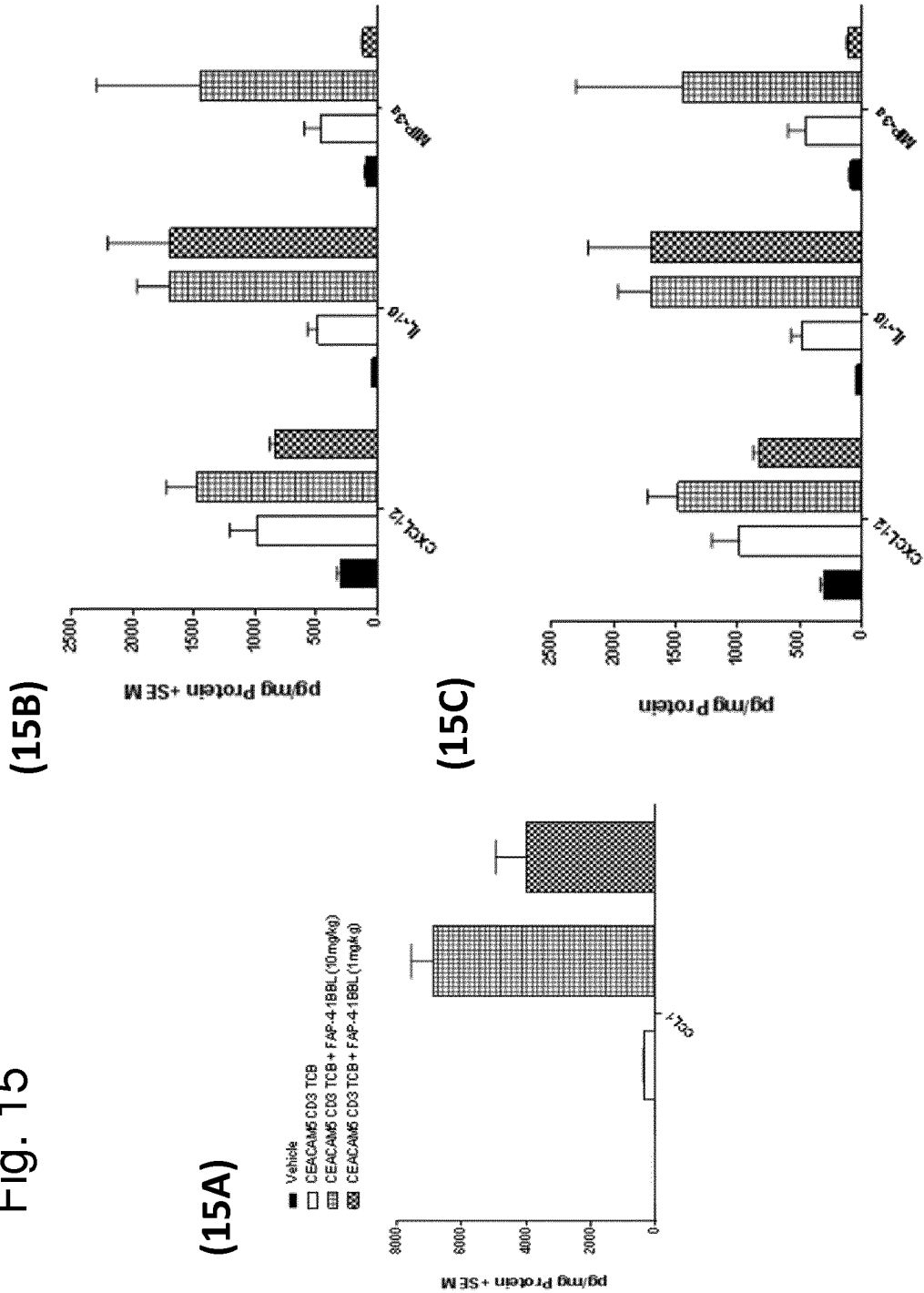


Fig. 16

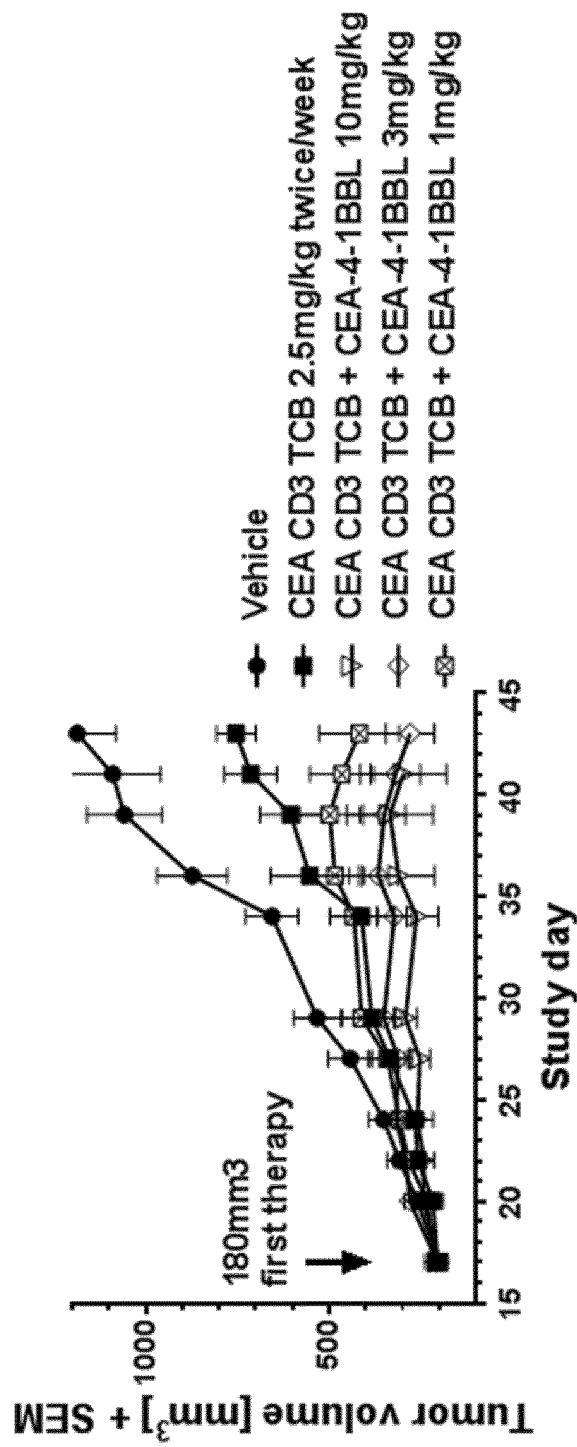


Fig. 17A

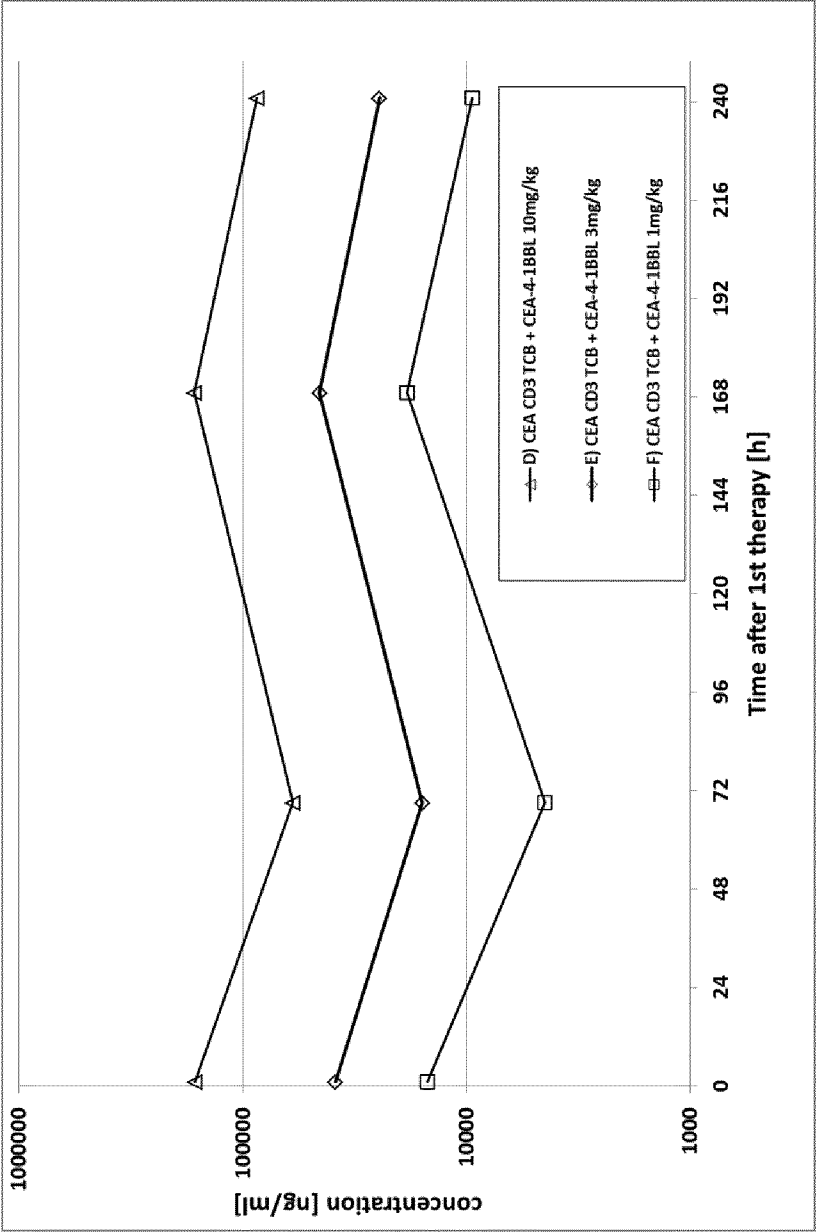


Fig. 17B

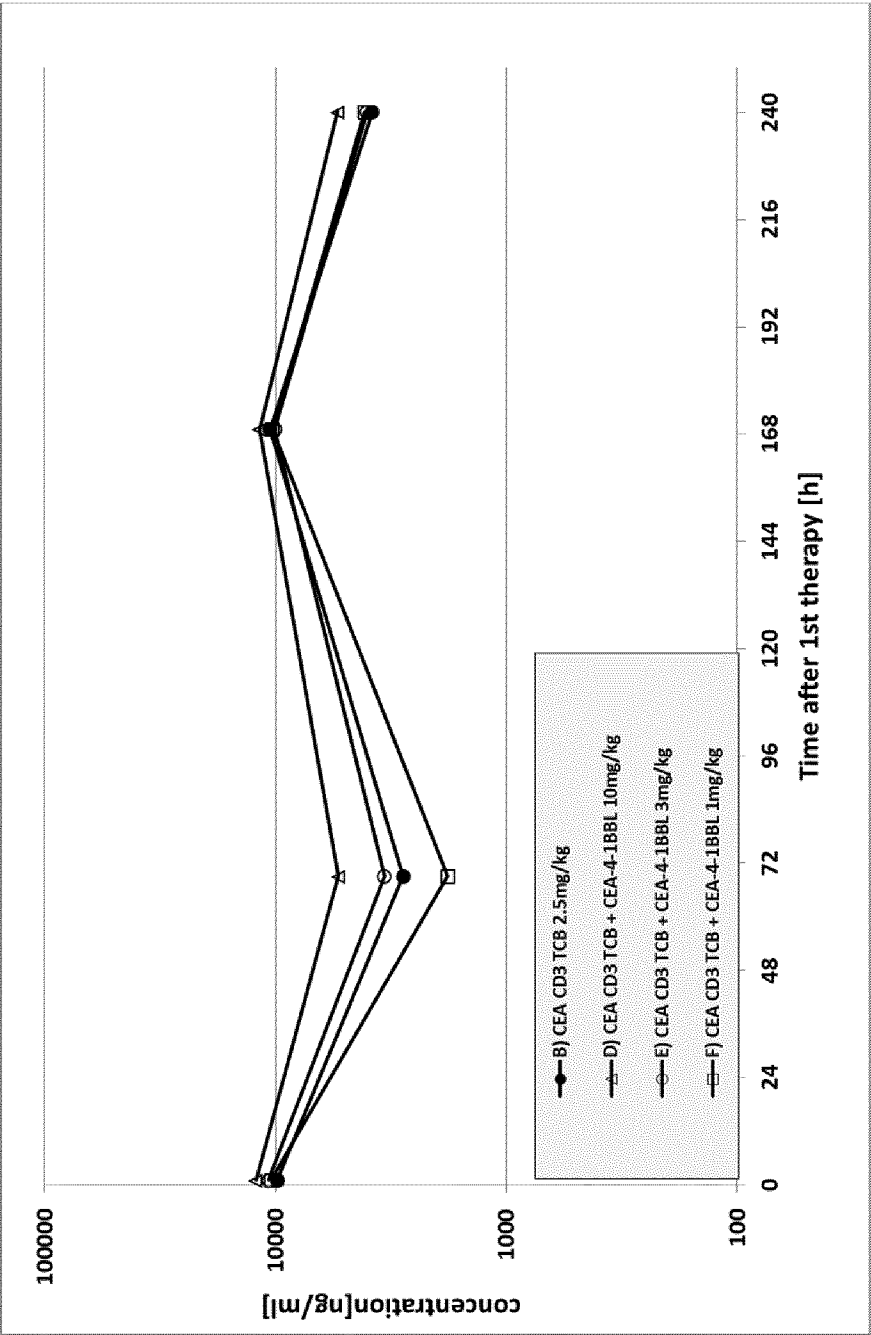


Fig. 18B

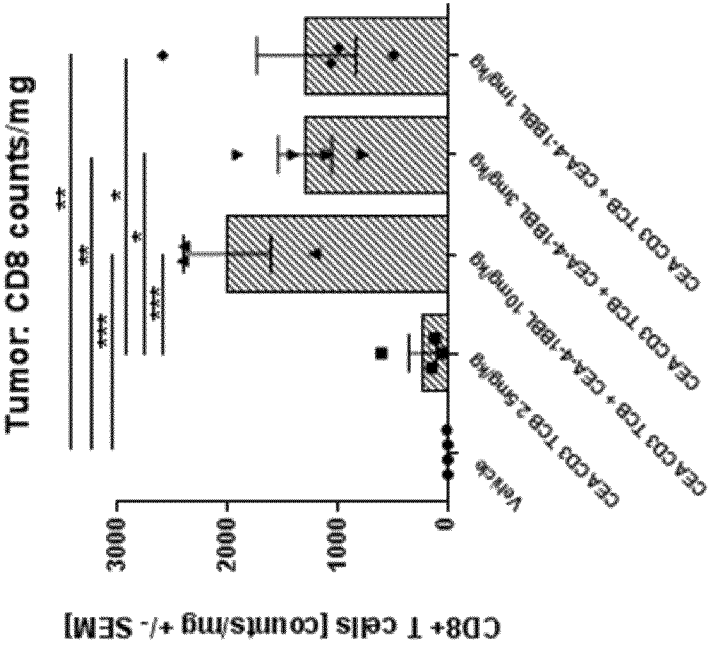
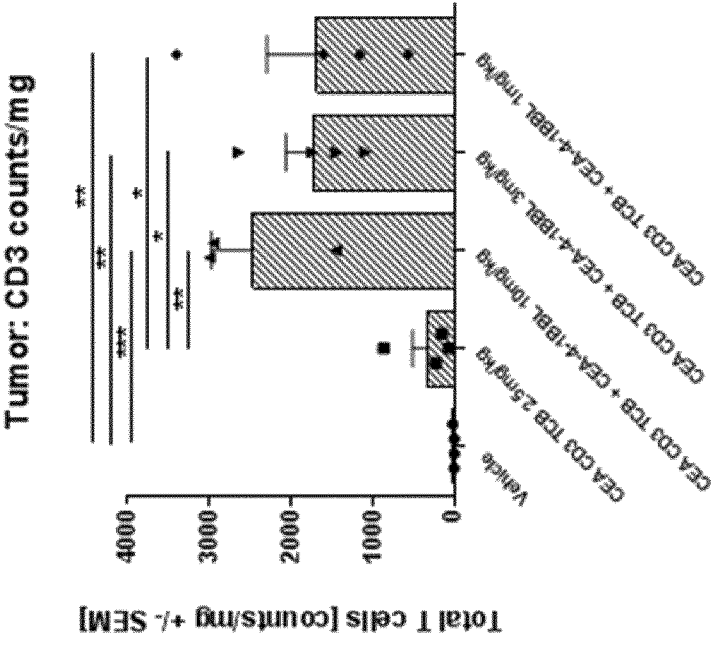


Fig. 18A



26/65

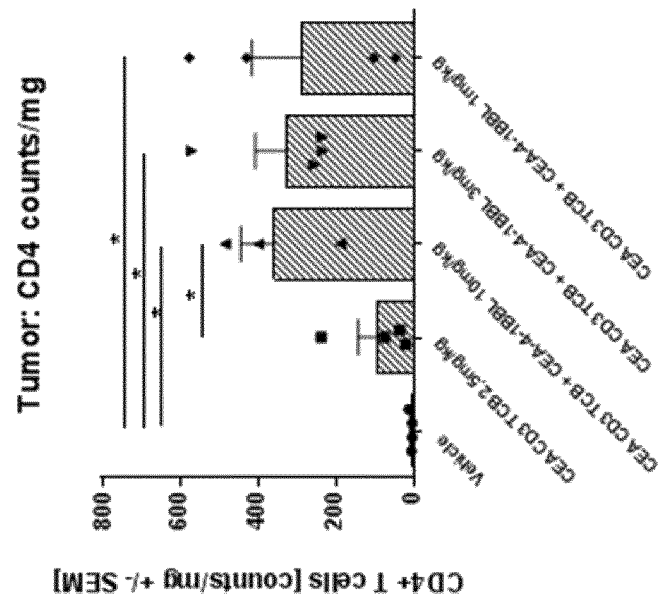


Fig. 18C

Fig. 19A

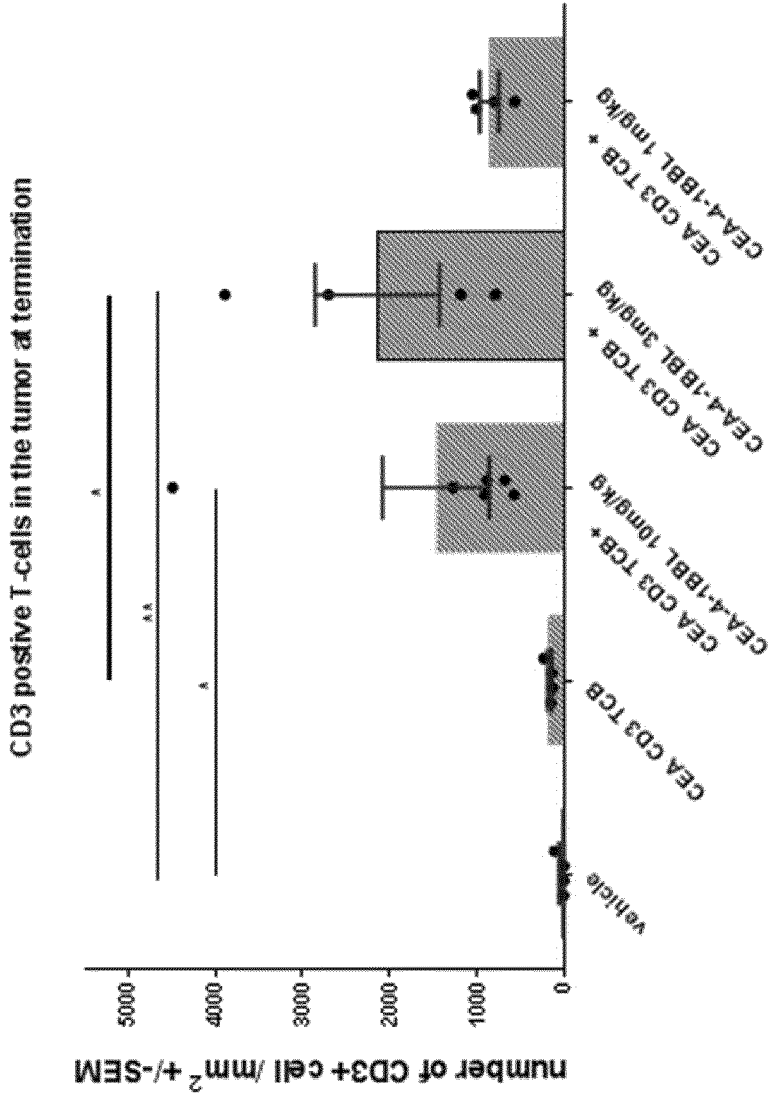


Fig. 19B

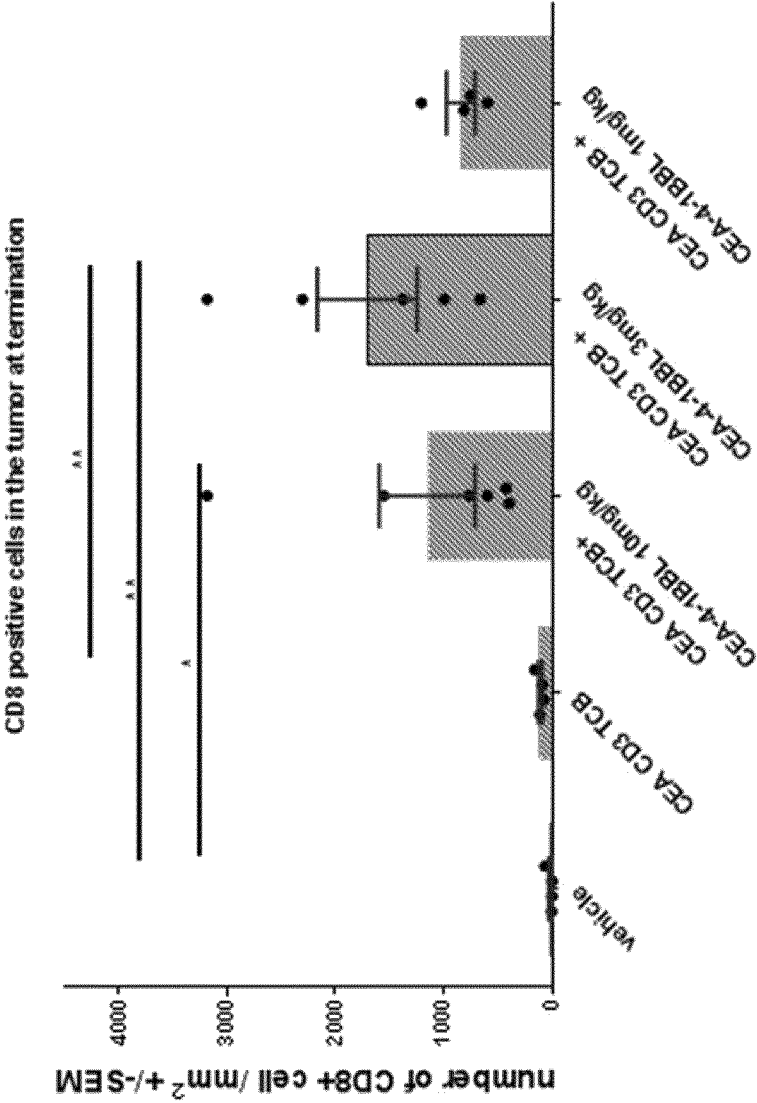


Fig. 20

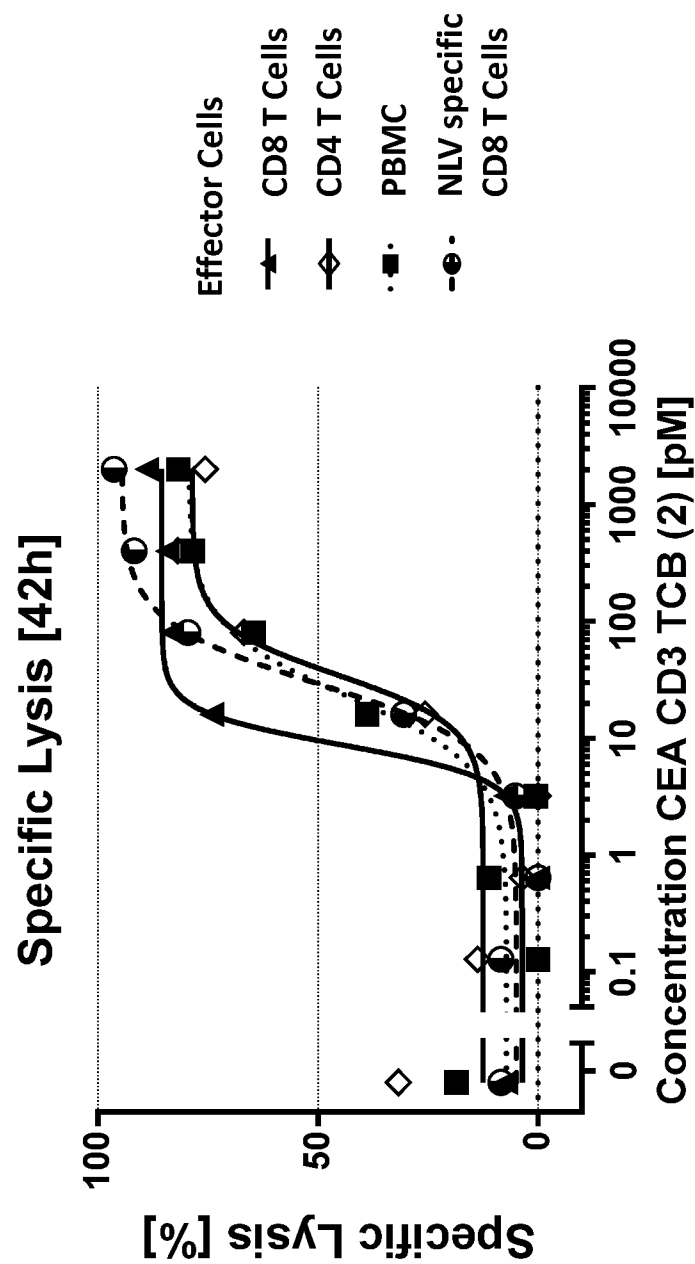


Fig. 22A

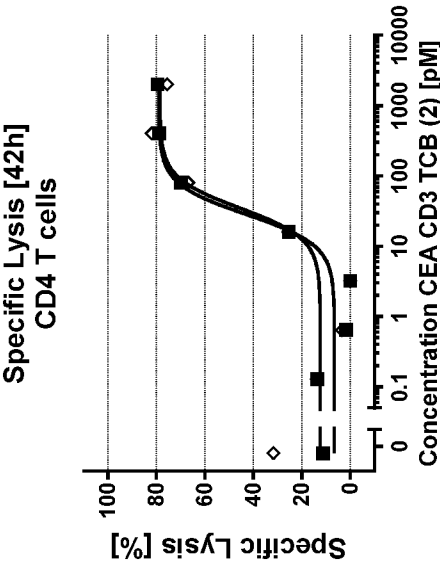


Fig. 22B

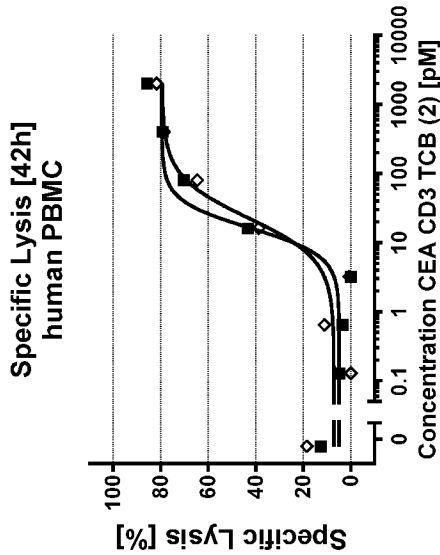


Fig. 22C

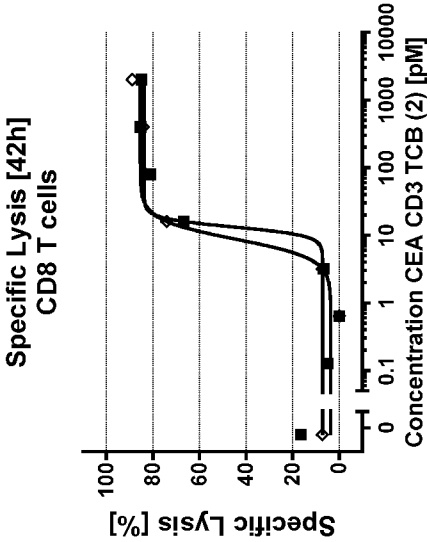
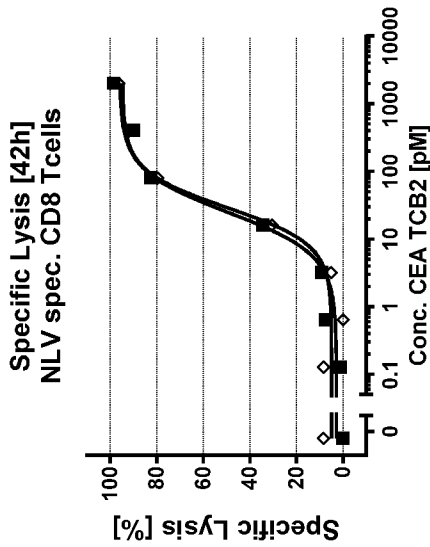


Fig. 22D



◇ w/o costimulation
■ w/ FAP 4-1BBL

Fig. 23A

MKN-45 NuclLight red
CEA CD3 TCB (2)
w/o costimulation

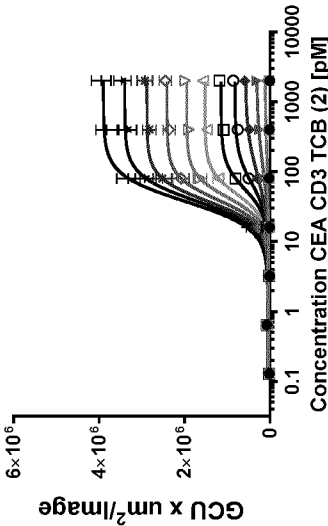


Fig. 23B

MKN-45 NuclLight red
CEACAM5 CD3 TCB
w/ FAP 4-1BBL

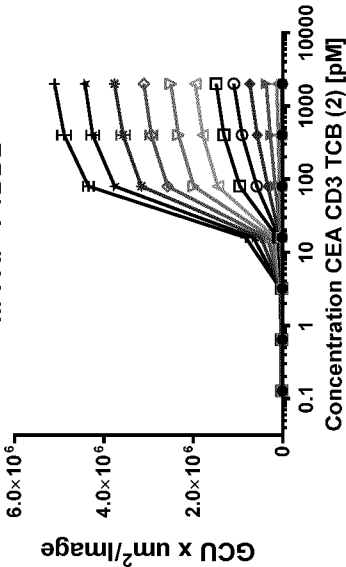


Fig. 23C

MKN-45 NuclLight red
CEA CD3 TCB
w/o costimulation

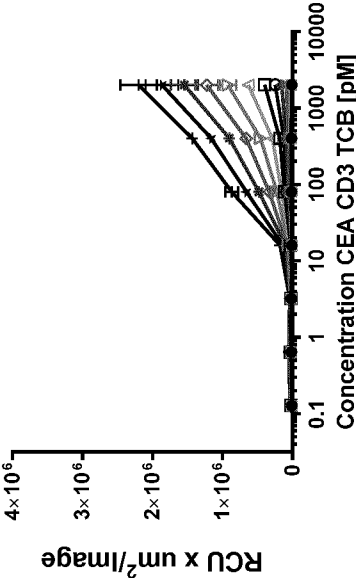
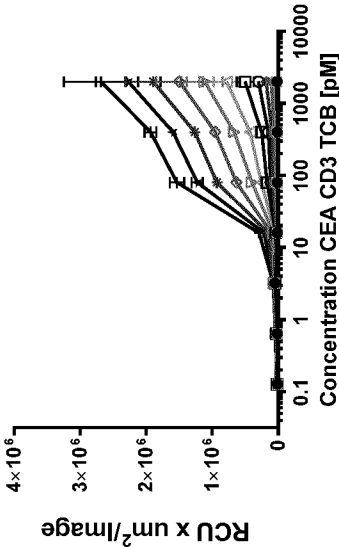


Fig. 23D

MKN-45 NuclLight red
CEA CD3 TCB
w/ FAP 4-1BBL



Time point (hrs)

- 0
- 3
- 6
- 9
- 12
- 15
- 18
- 21
- 24
- 27
- 30
- 33
- 36

Fig. 24A

Fig. 24B

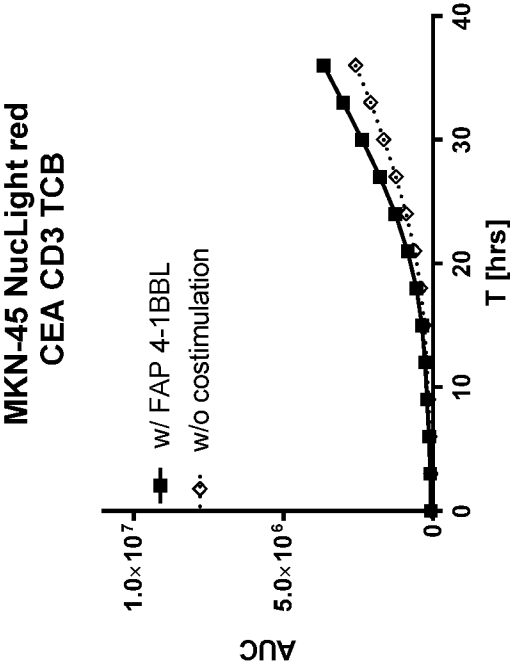
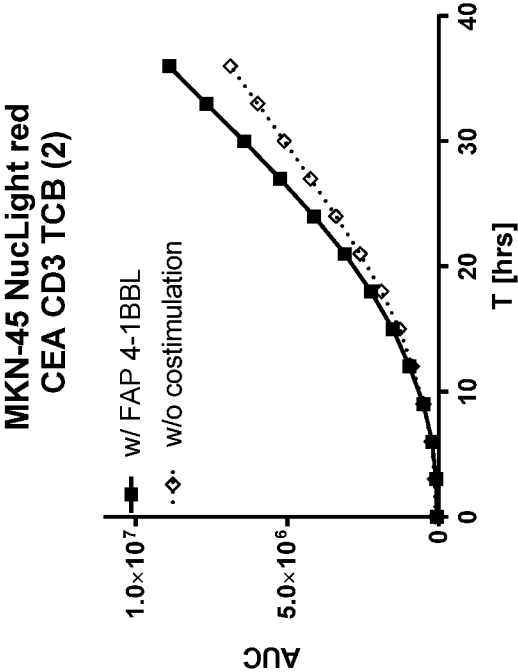


Fig. 25A

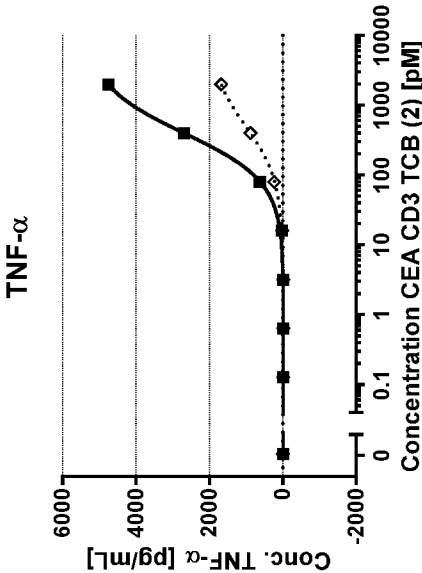


Fig. 25B

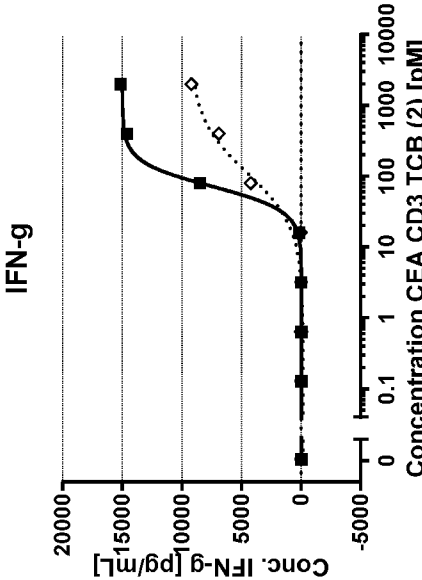


Fig. 25C

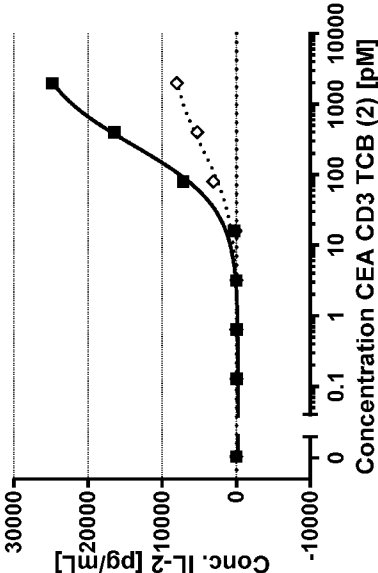


Fig. 25D

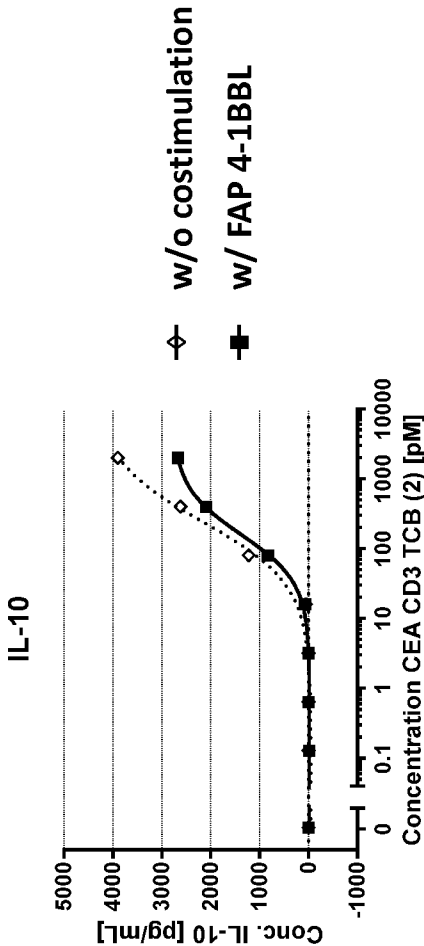


Fig. 26A

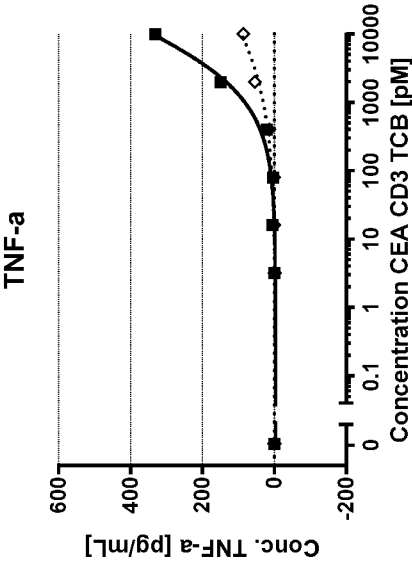


Fig. 26B

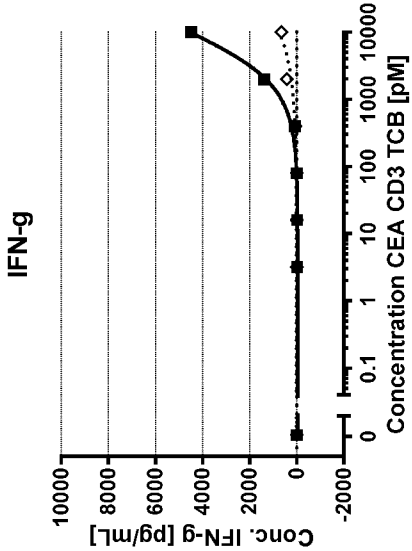


Fig. 26C

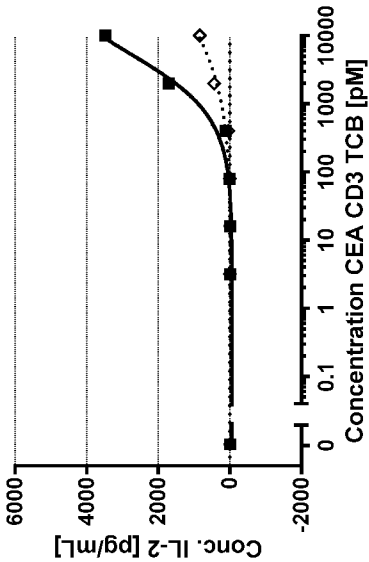
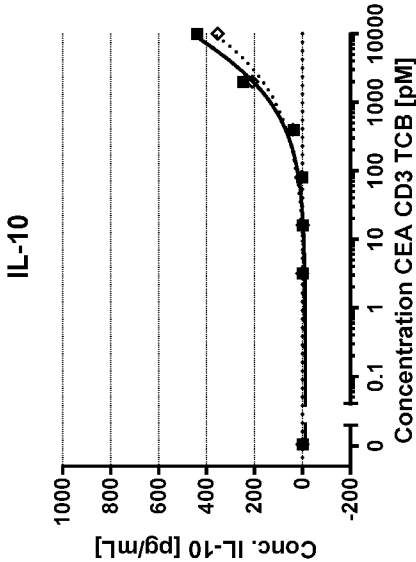


Fig. 26D



◇ w/o costimulation
■ w/ FAP 4-1BBL

Fig. 27

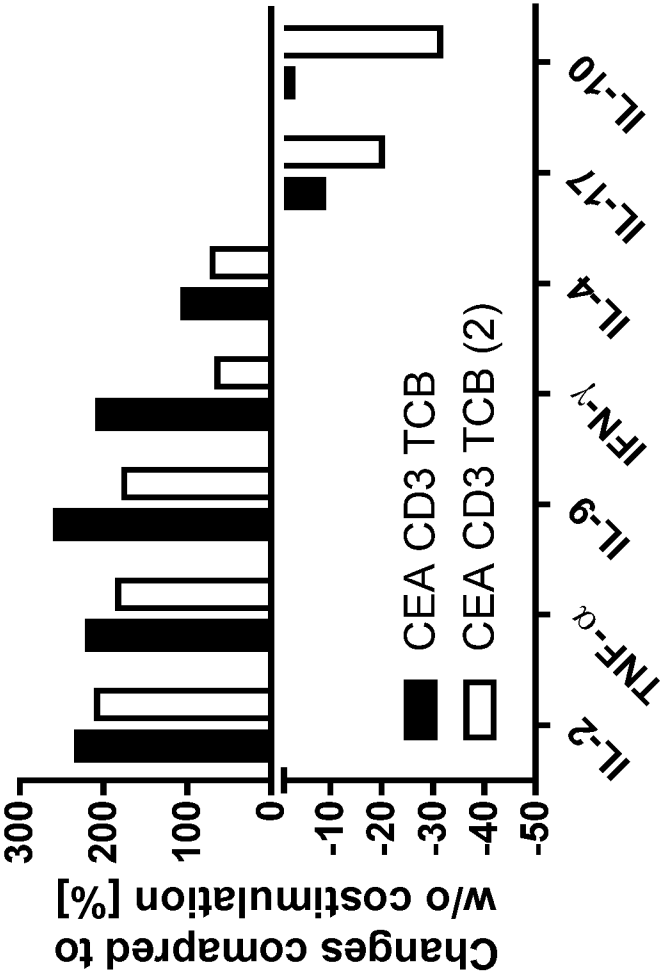


Fig. 28A

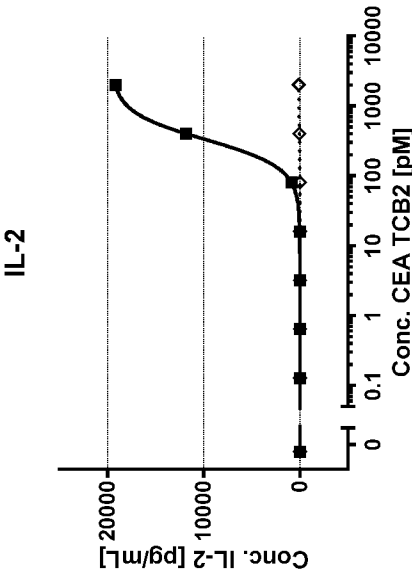


Fig. 28B

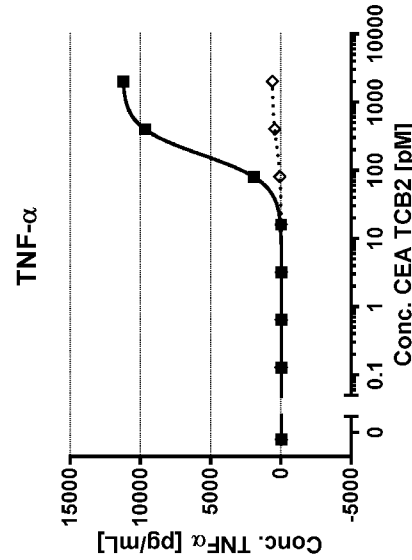


Fig. 28C

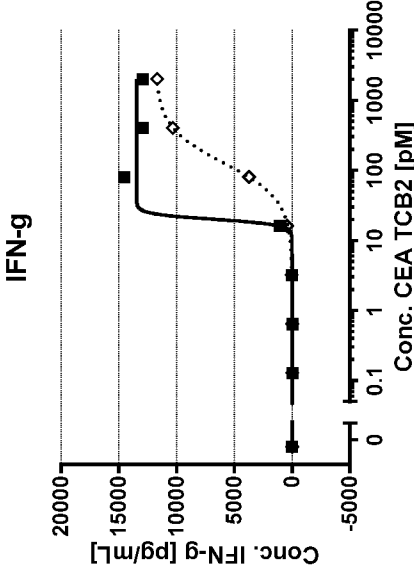
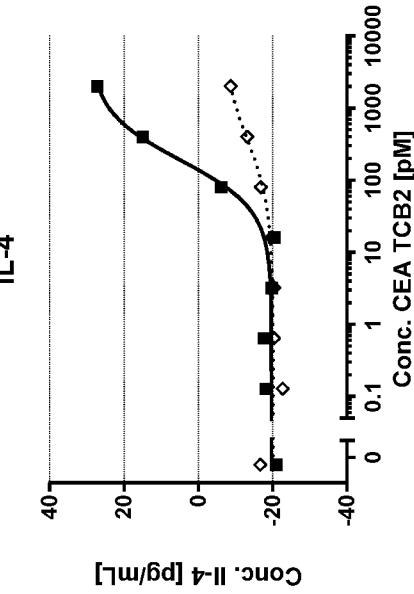


Fig. 28D



w/o costimulation
w/ FAP 4-1BBL

Fig. 28E

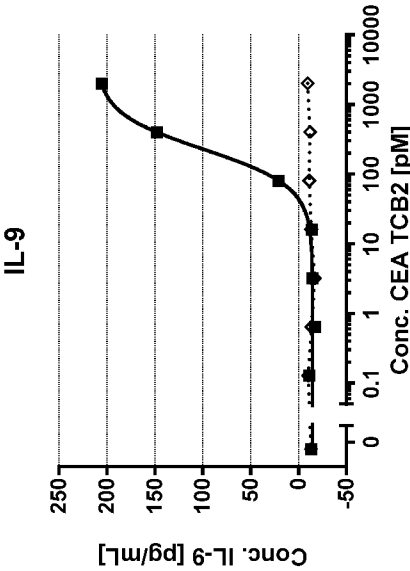


Fig. 28F

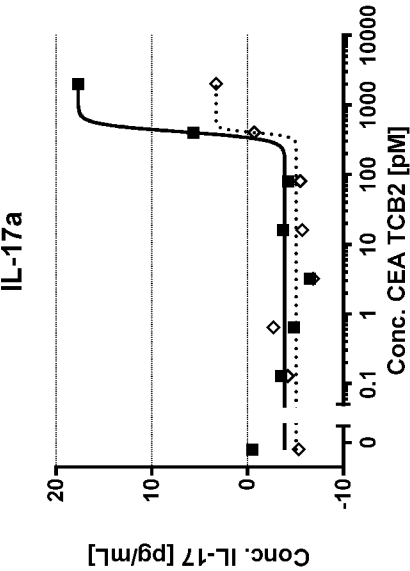


Fig. 28G

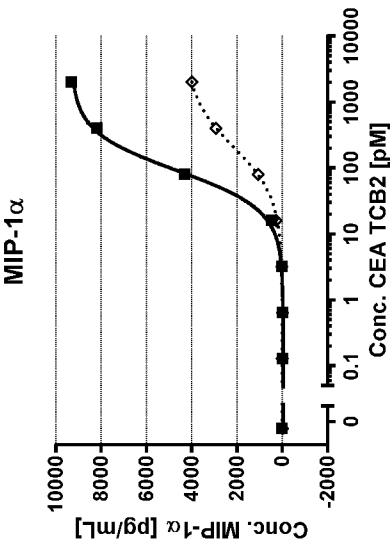
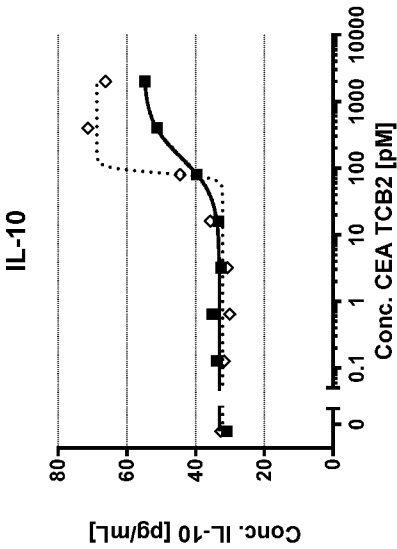


Fig. 28H



◇ w/o costimulation
■ w/ FAP 4-1BBL

Fig. 29A

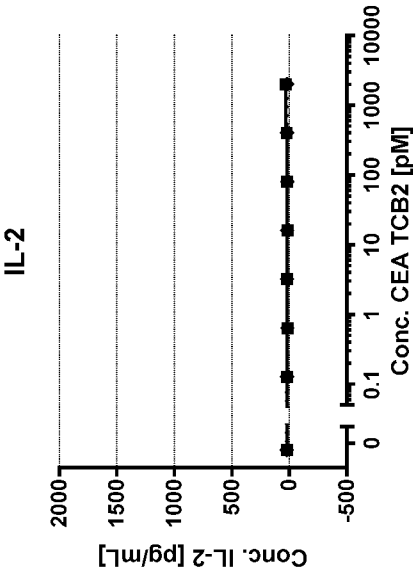


Fig. 29B

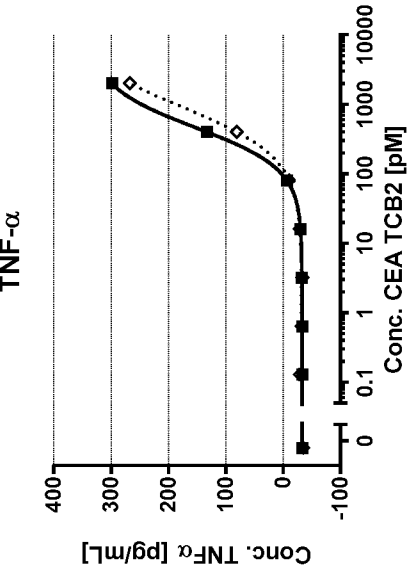


Fig. 29C

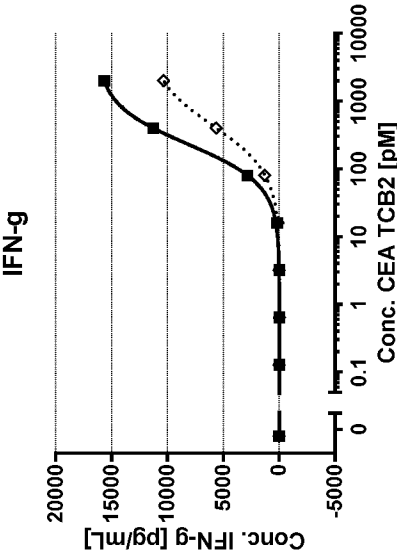


Fig. 29D

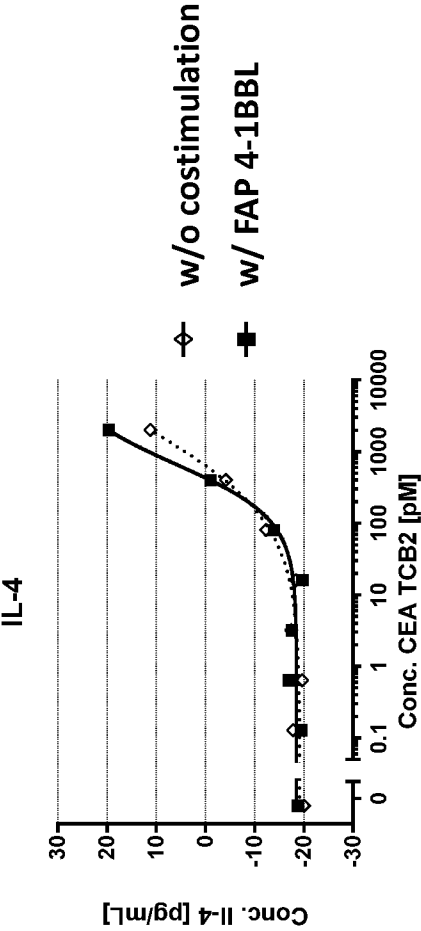


Fig. 29E

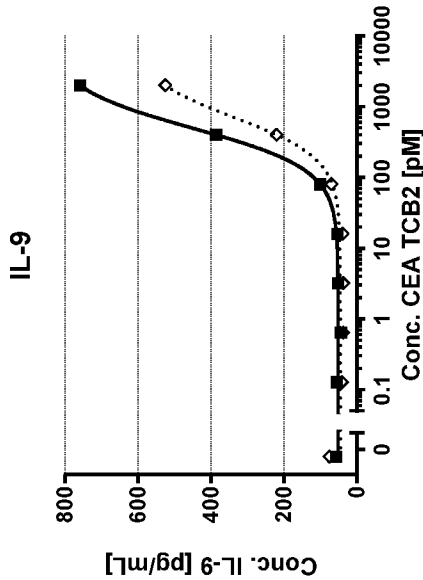


Fig. 29F

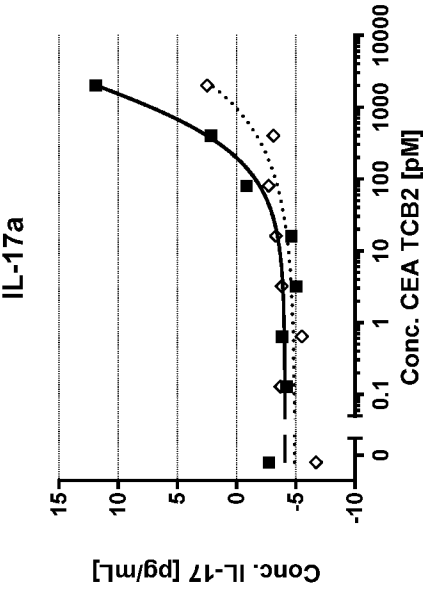


Fig. 29G

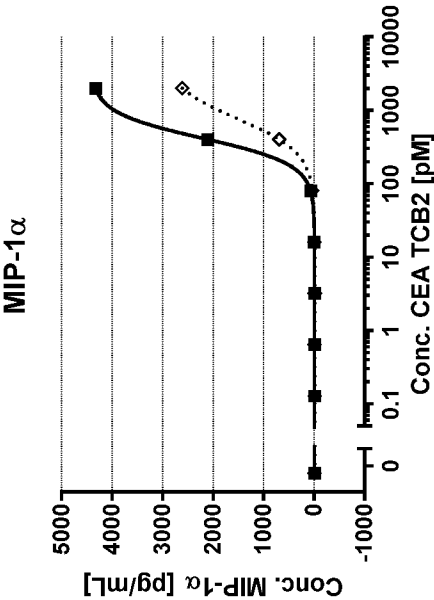
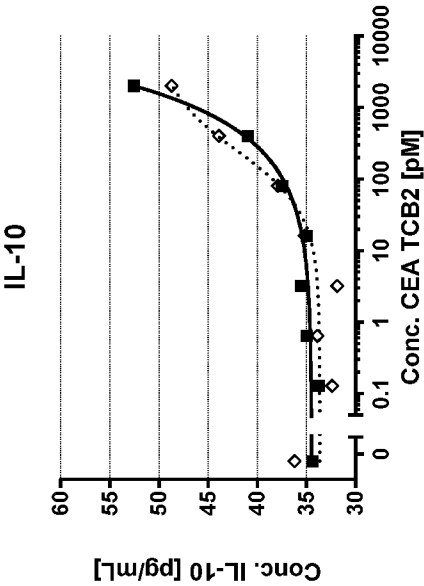


Fig. 29H



◇ w/o costimulation
■ w/ FAP 4-1BBL

Fig. 30

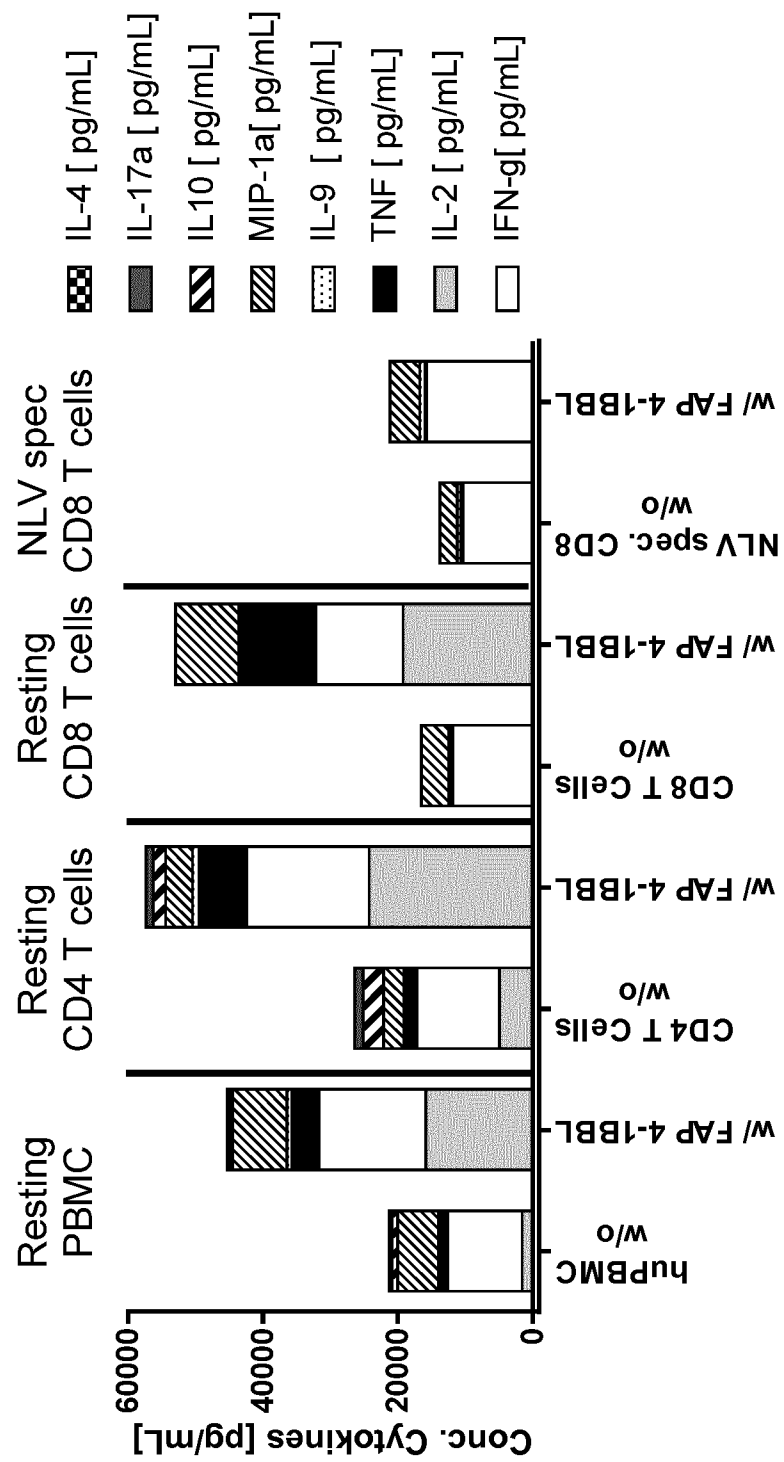


Fig. 31

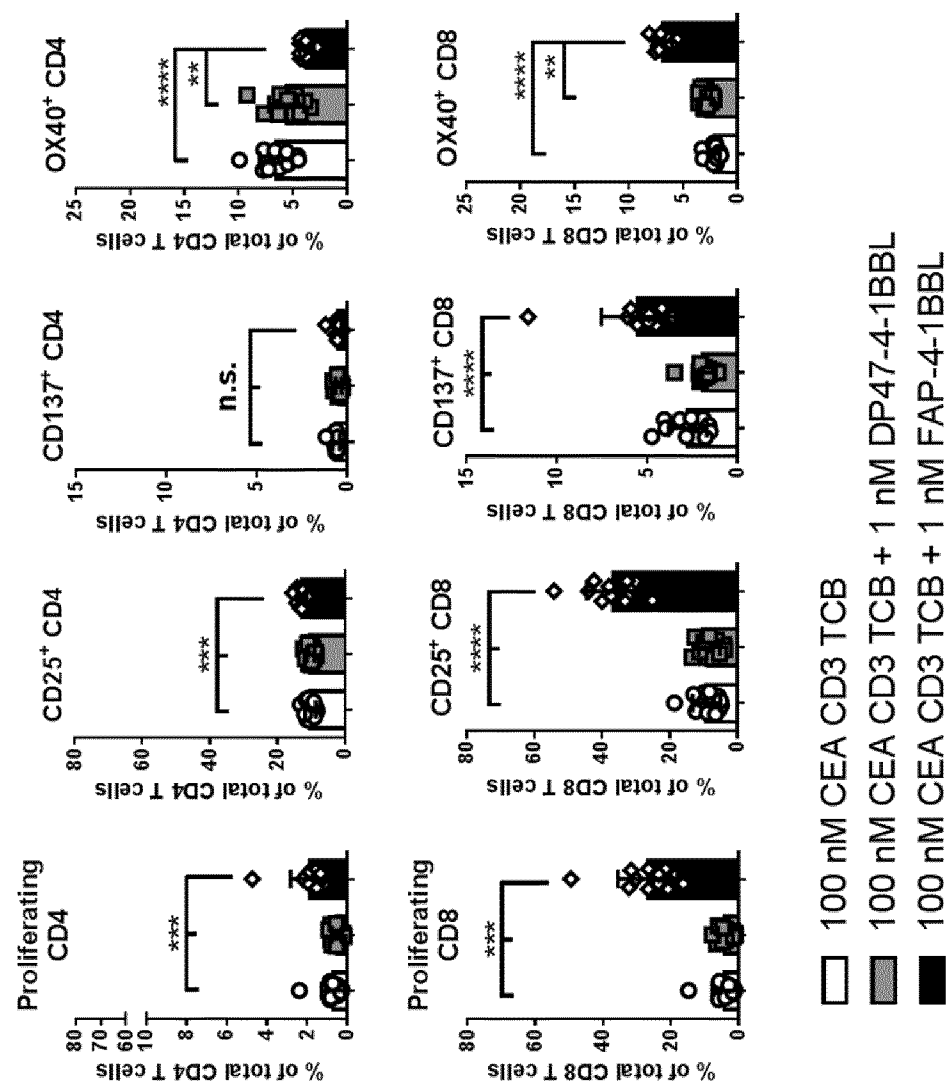


Fig. 32

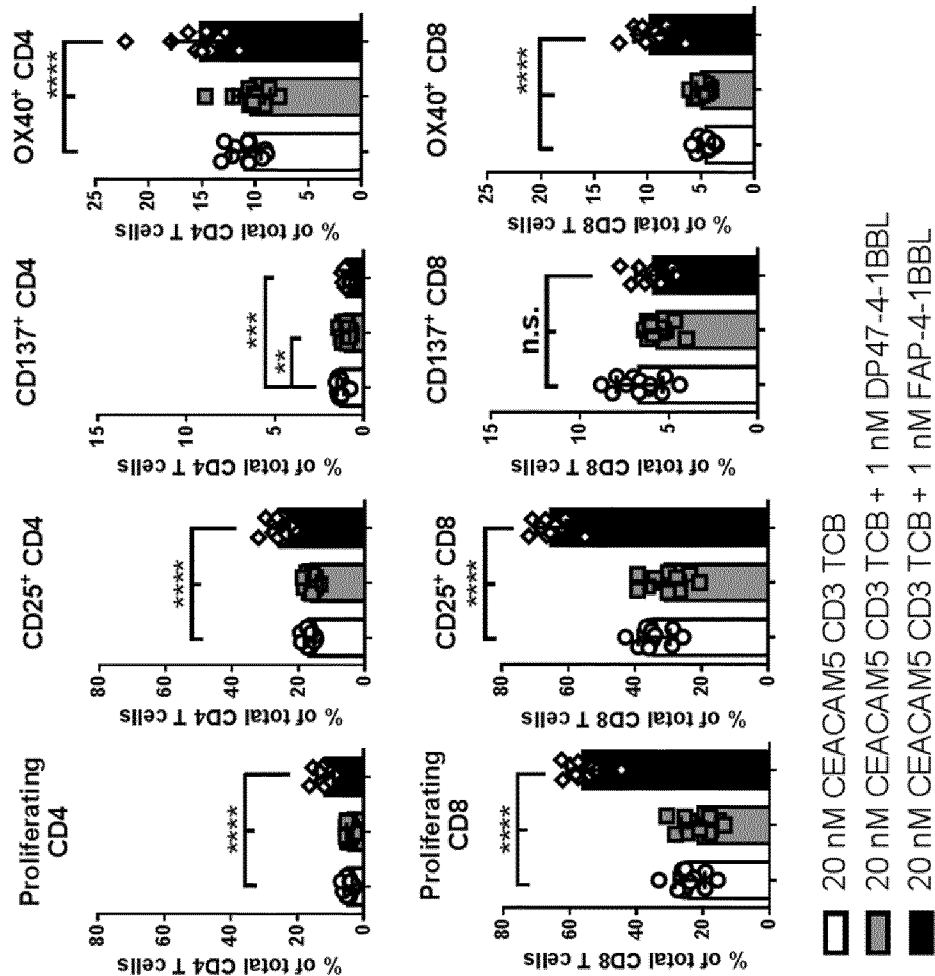


Fig. 33

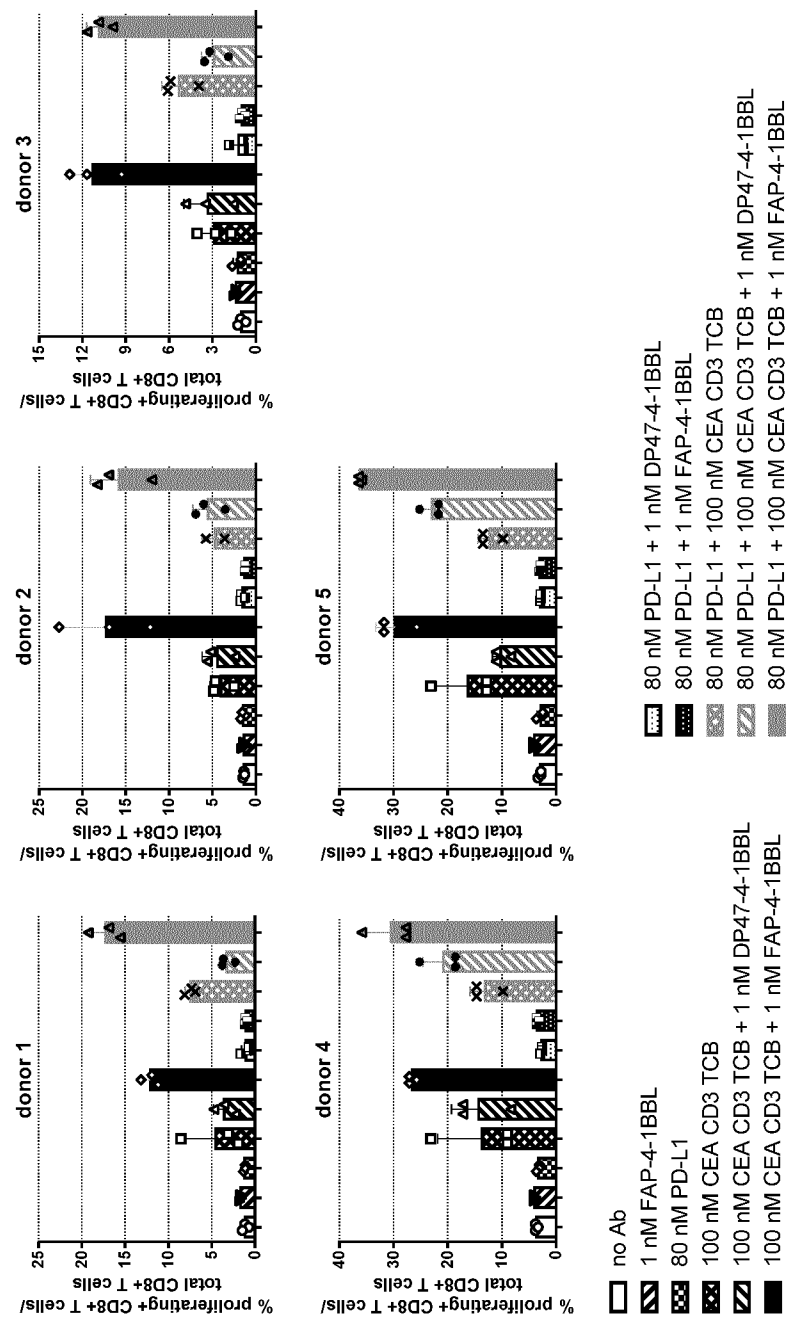


Fig. 34

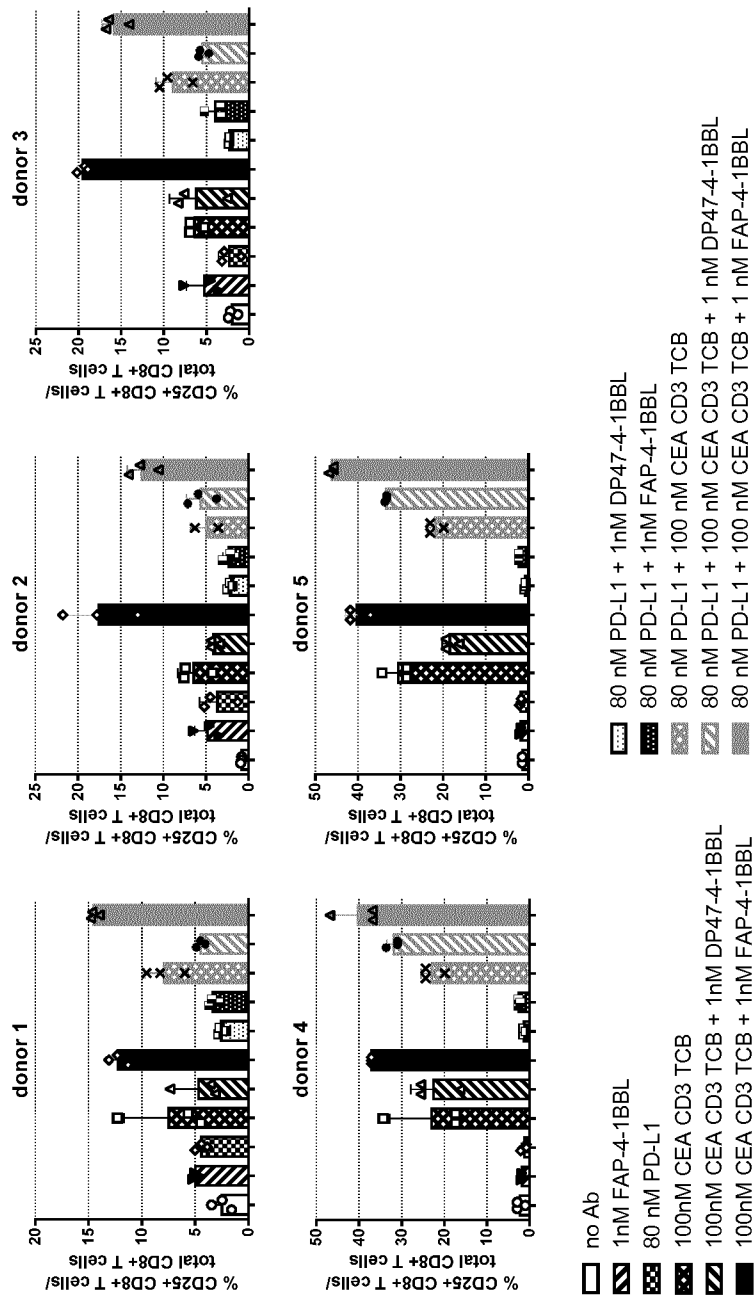


Fig. 35

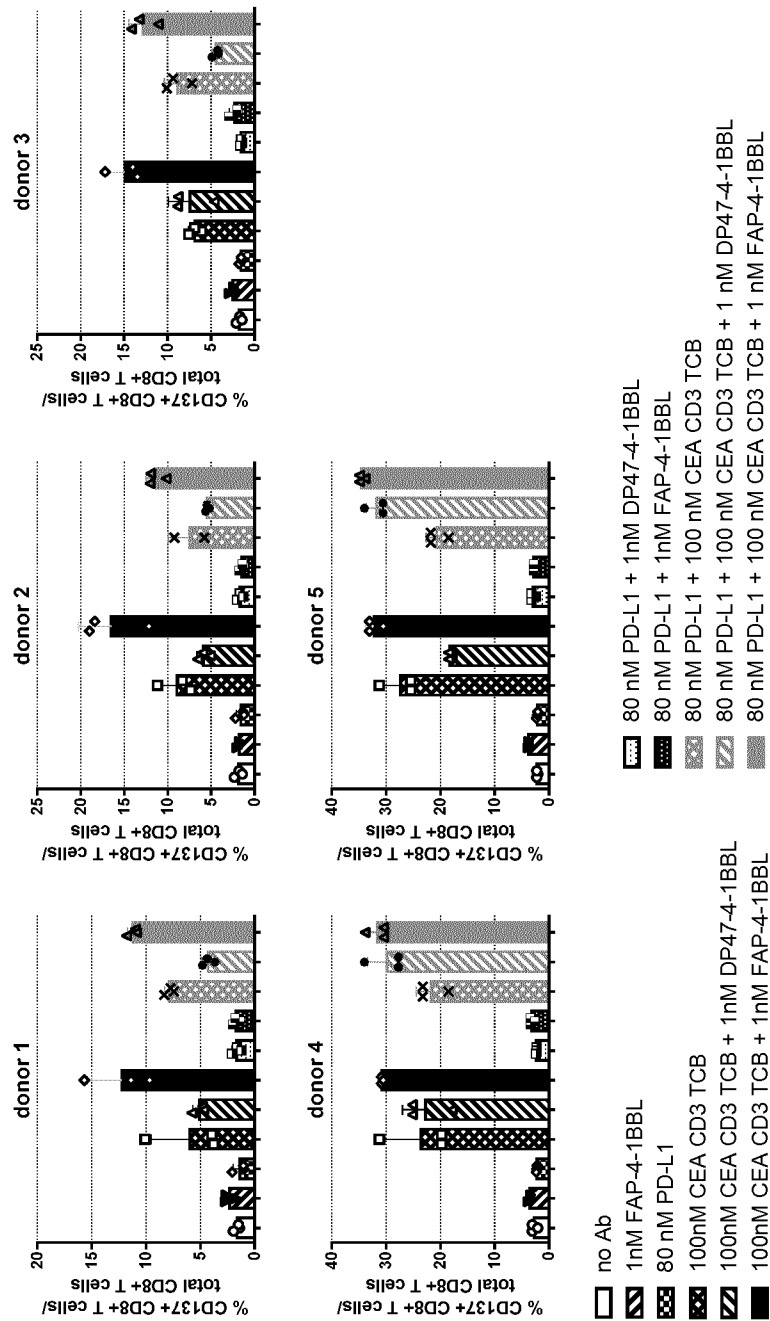


Fig. 36

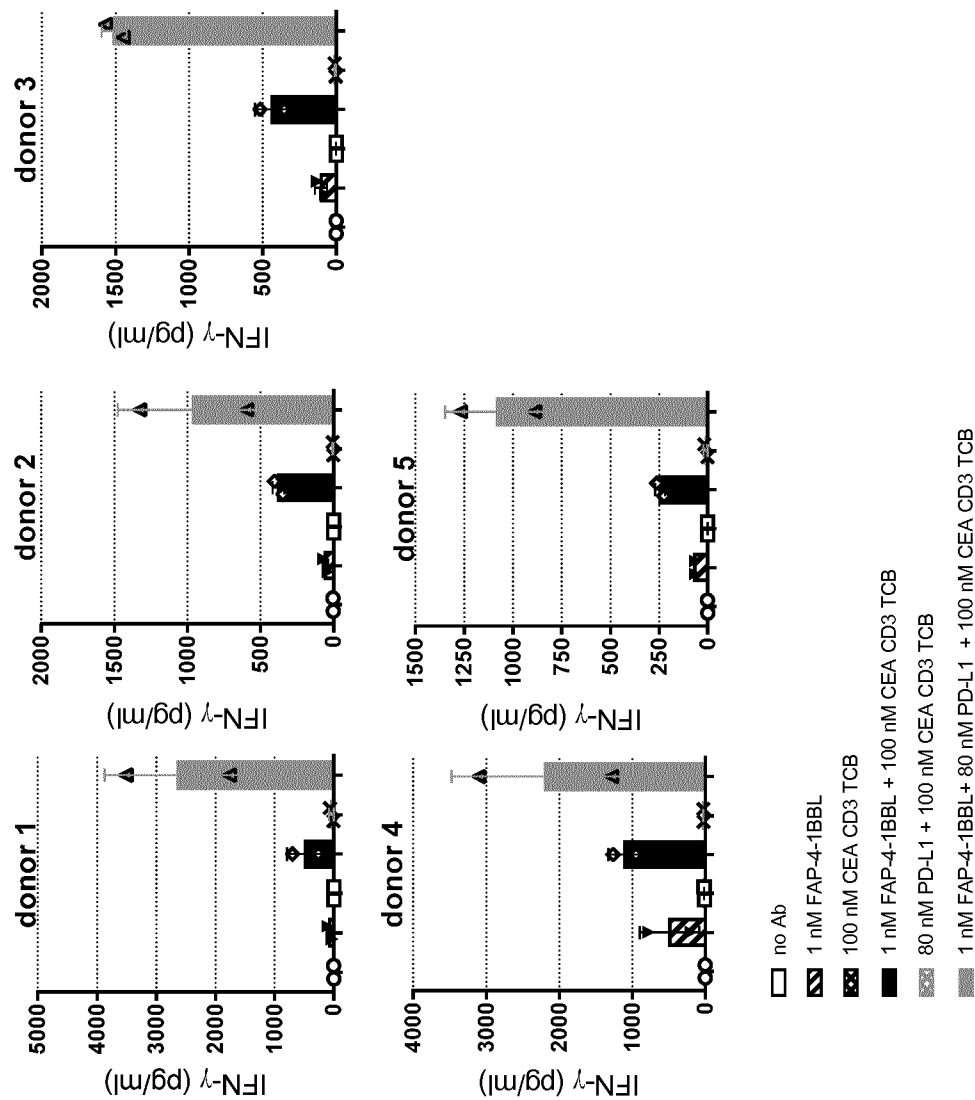


Fig. 37

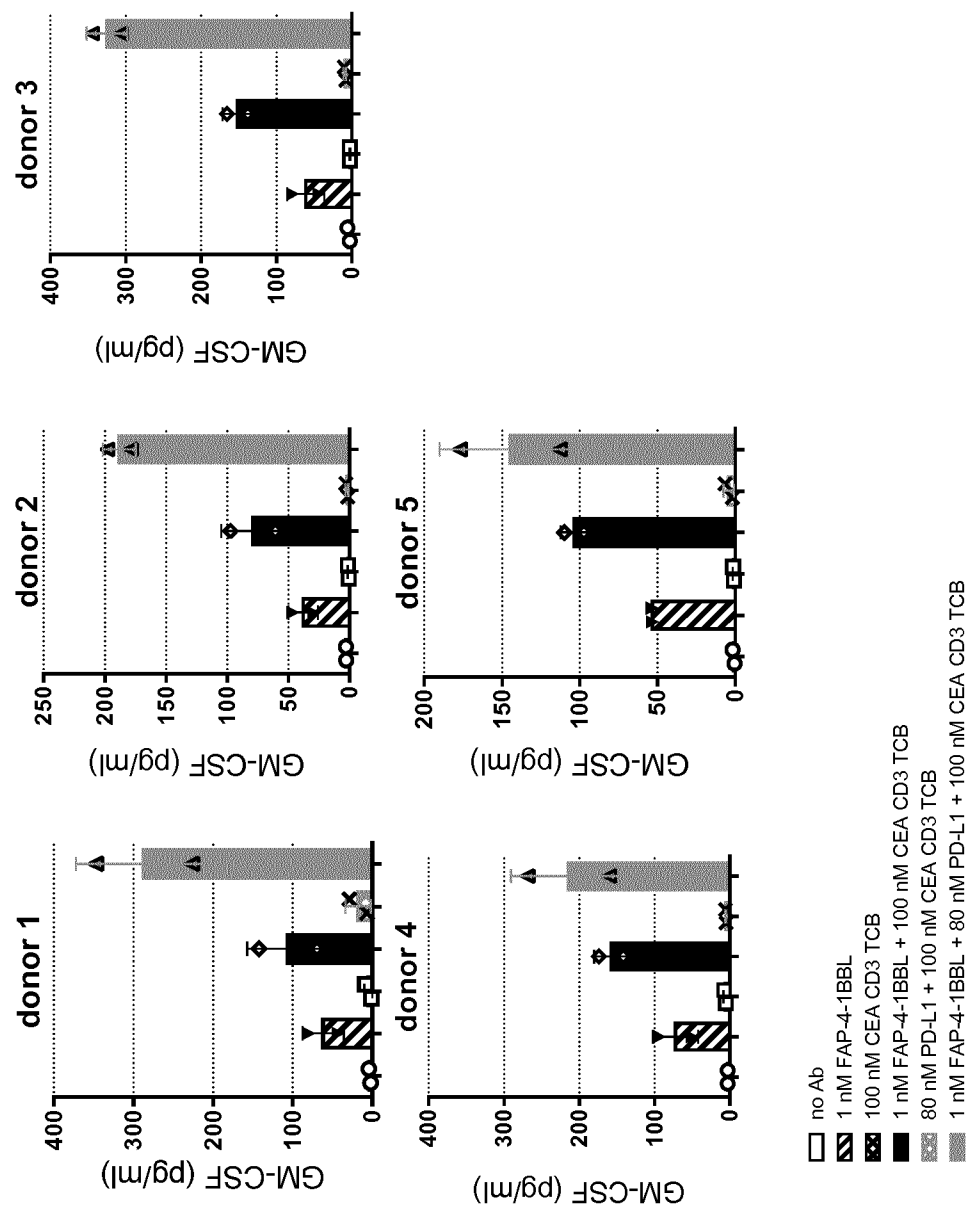


Fig. 38

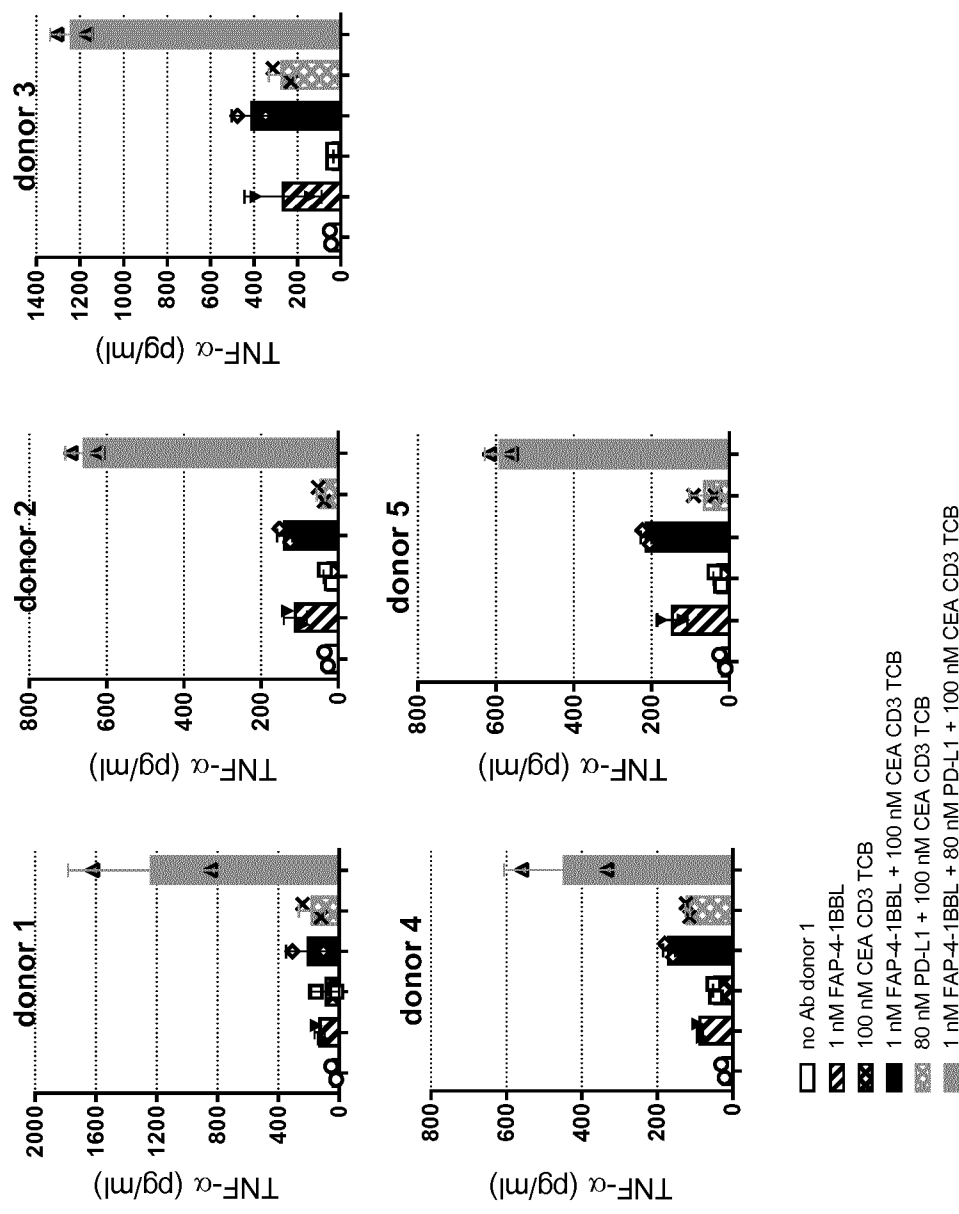
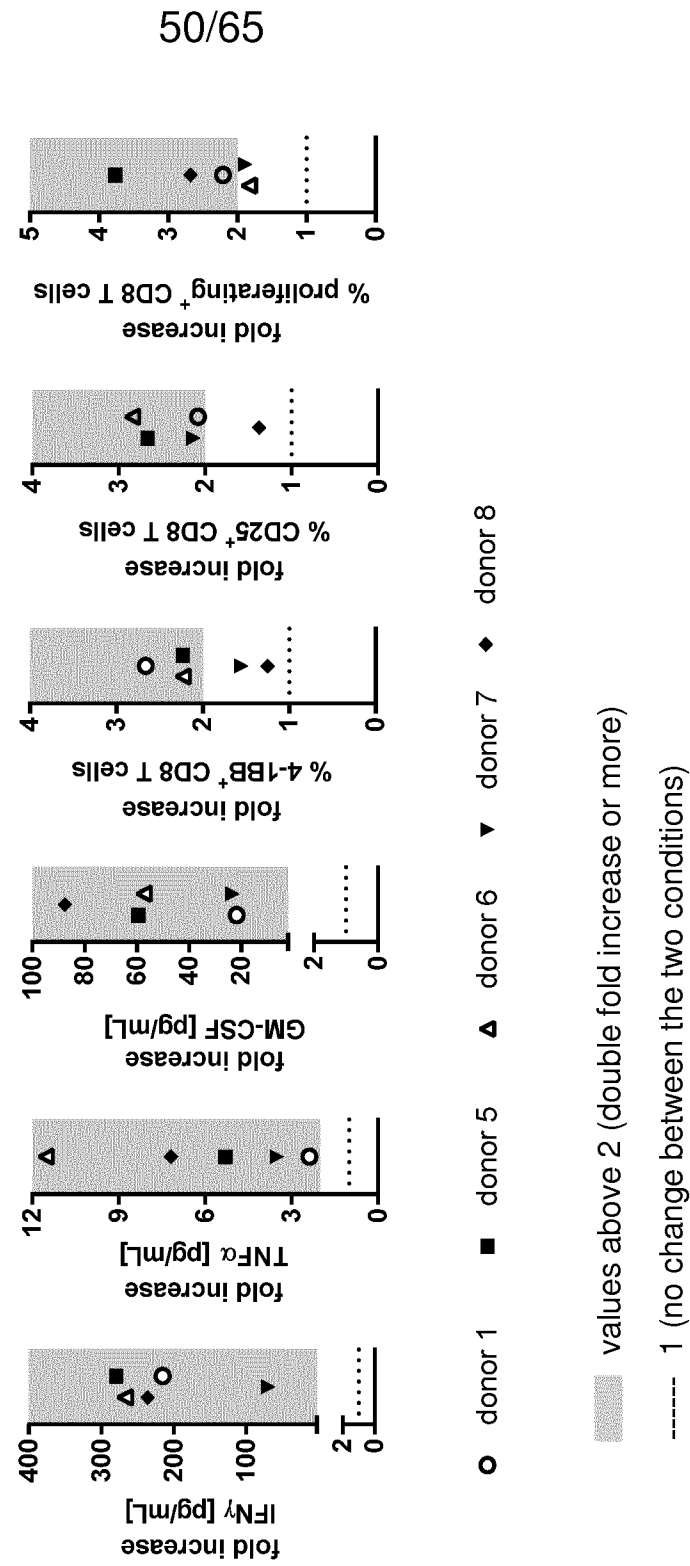


Fig. 39



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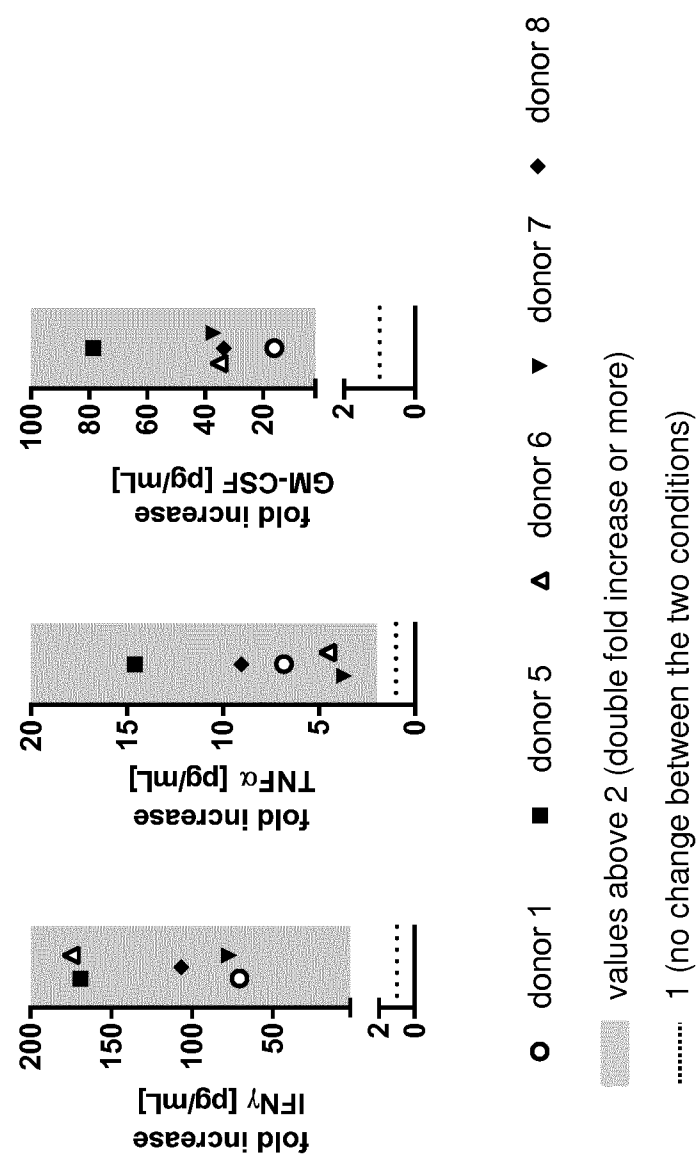


Fig. 40

Fig. 41A

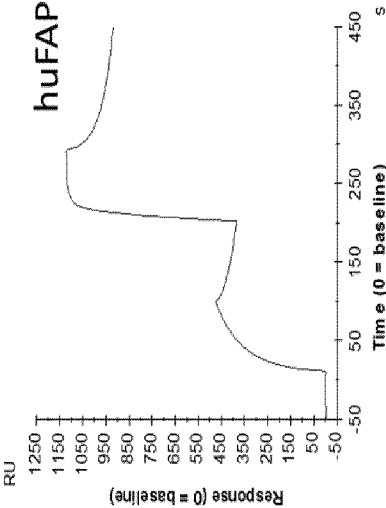


Fig. 41B

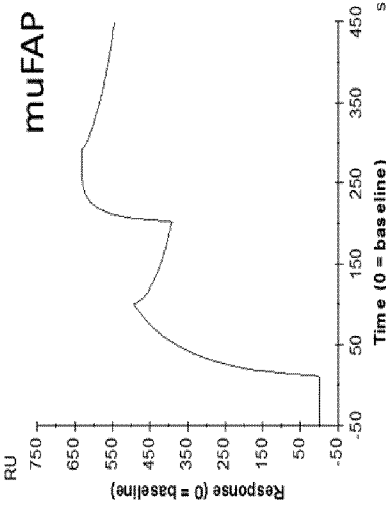
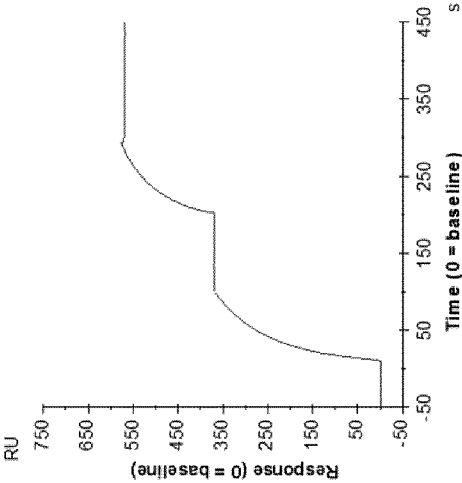


Fig. 41C



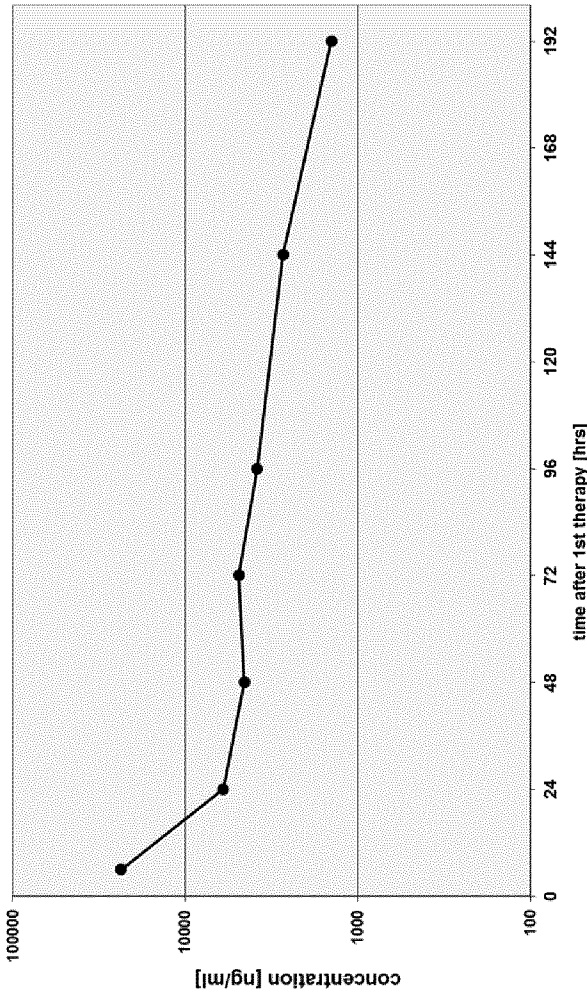


Fig. 42

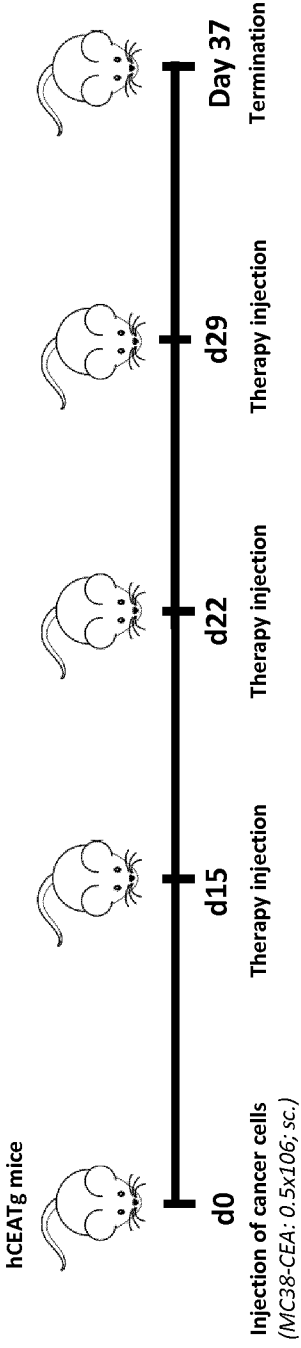


Fig. 43

Fig. 44A

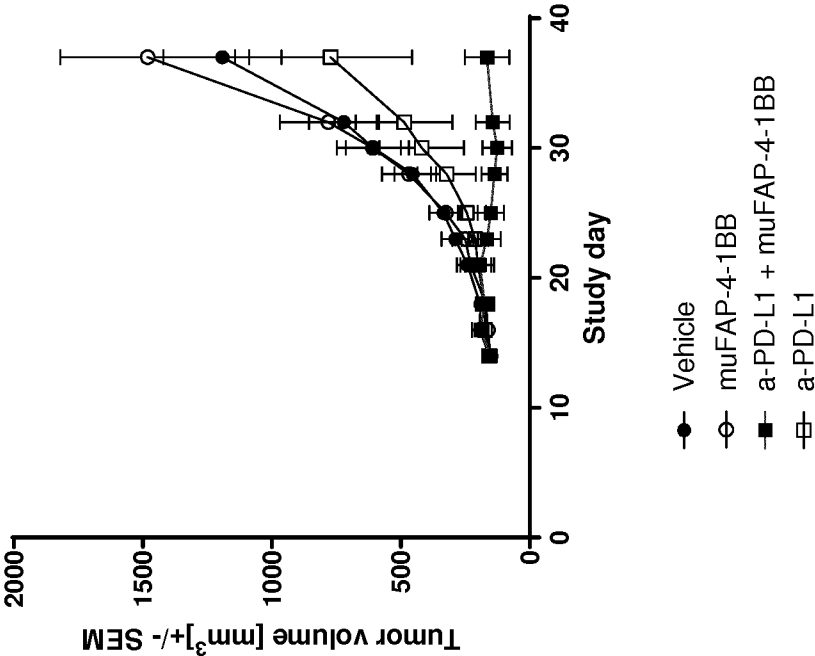
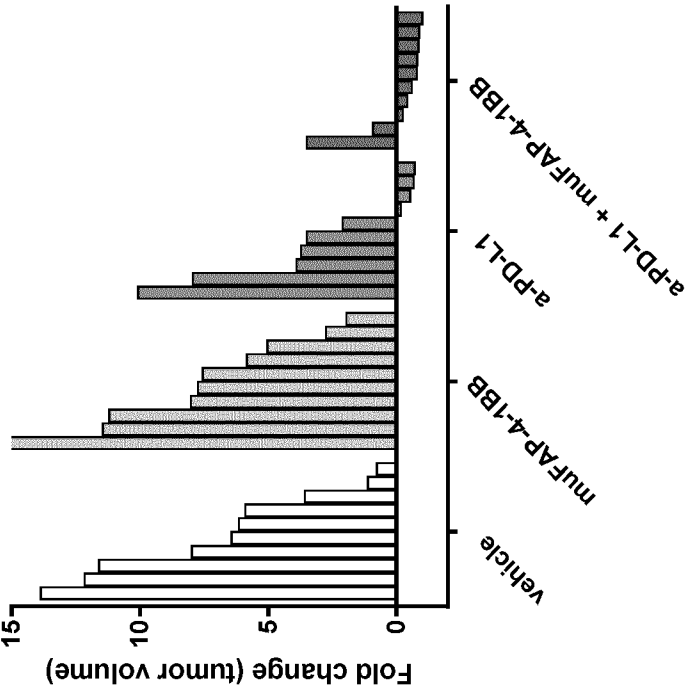


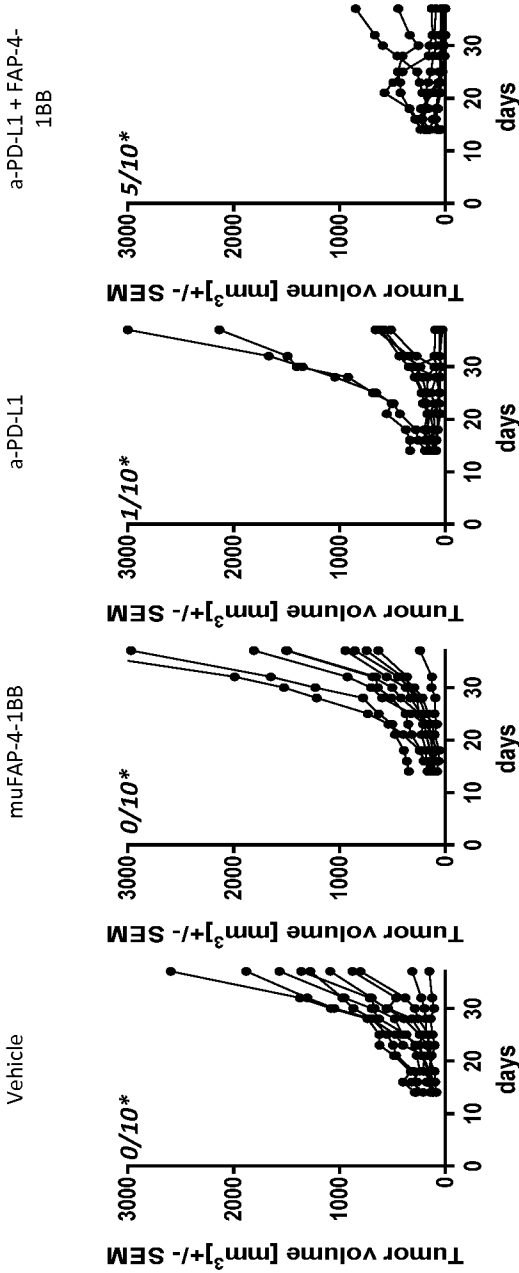
Fig. 44B



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Fig. 44C



*: Tumor free mice at study termination

Fig. 45A

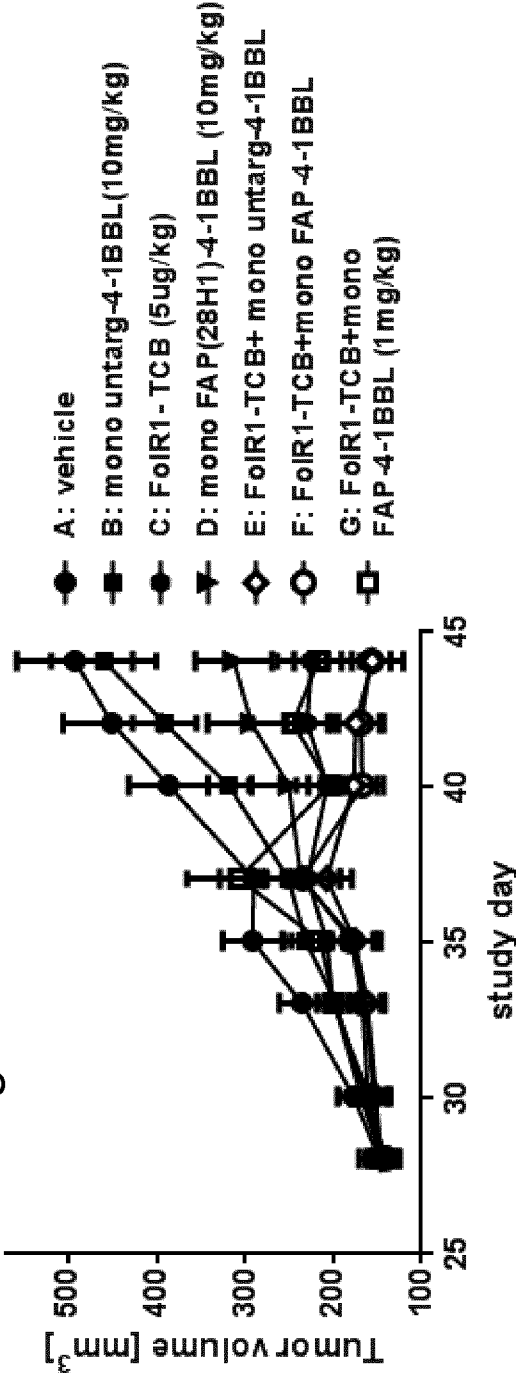
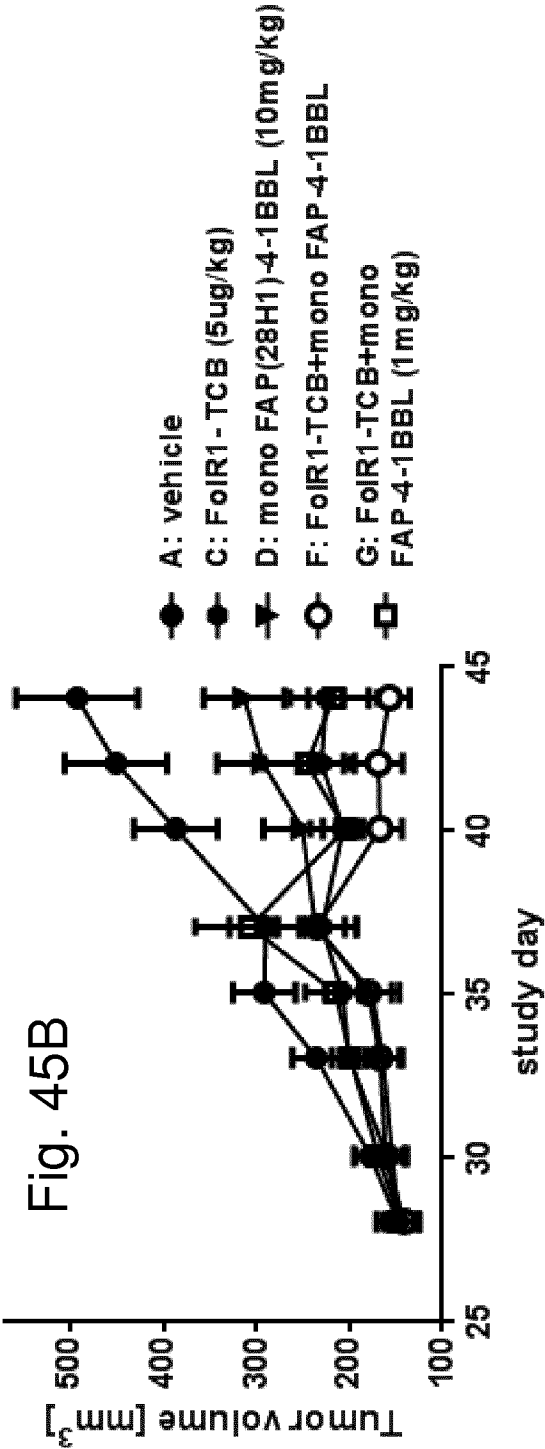


Fig. 45B



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Fig. 46

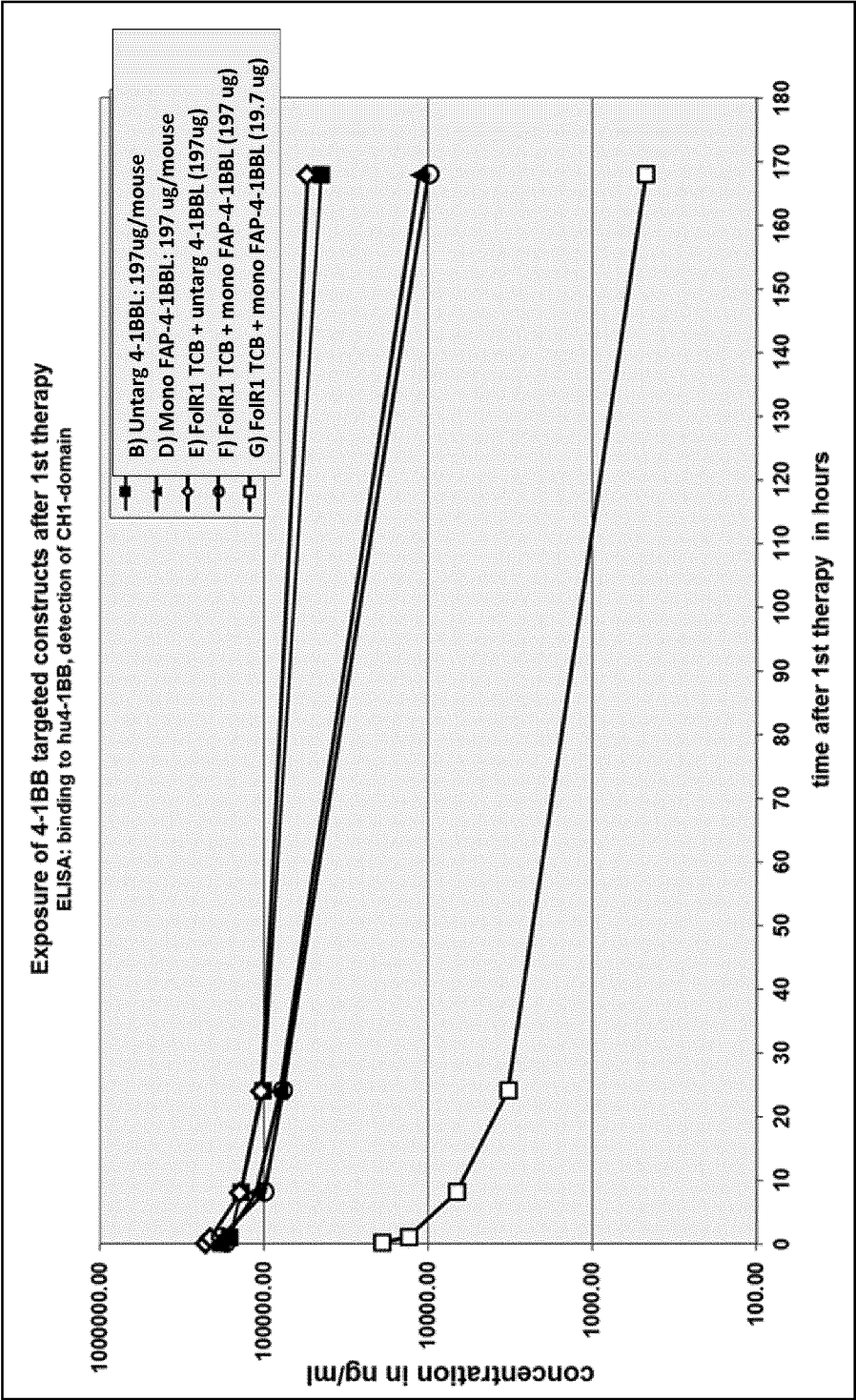


Fig. 47

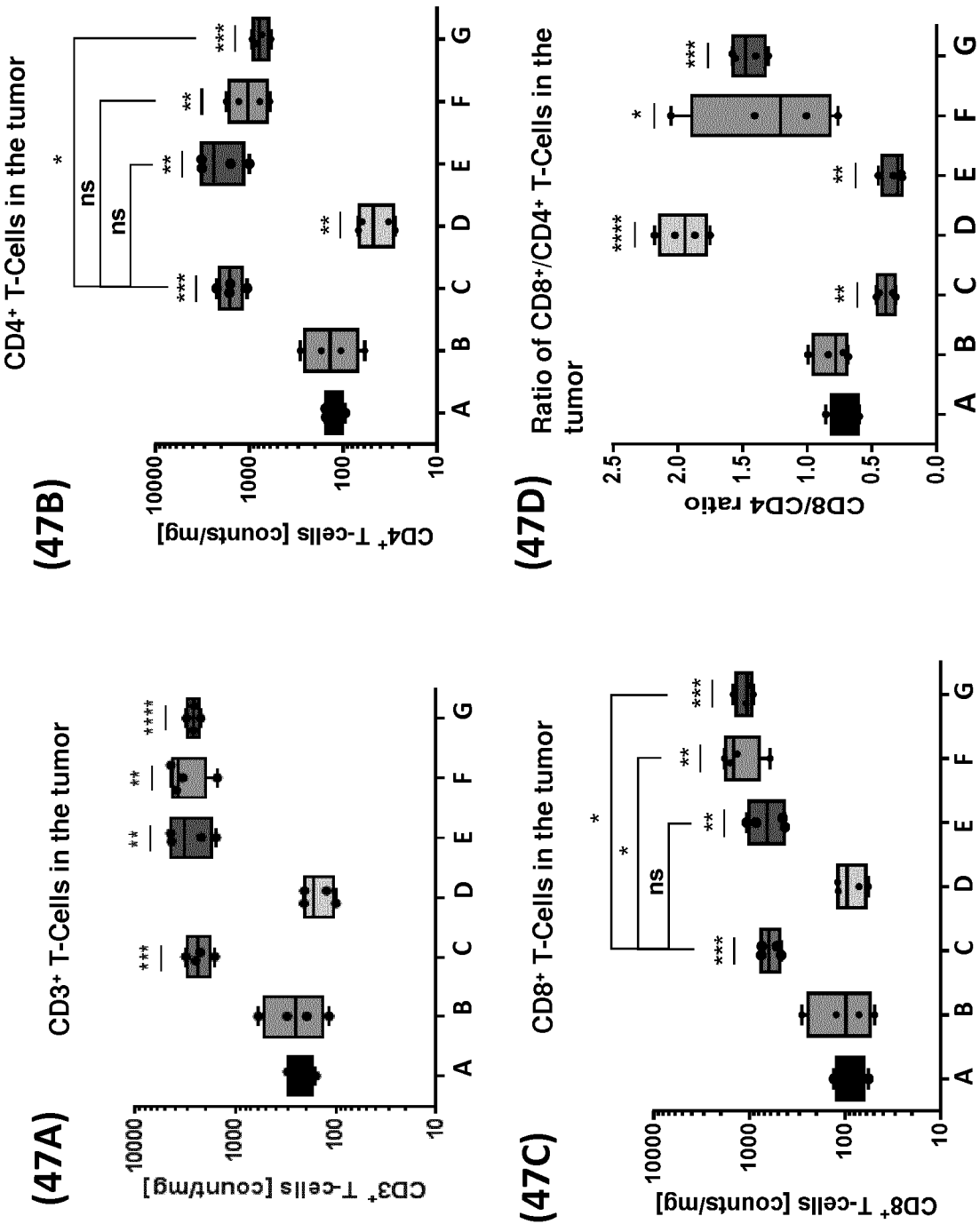
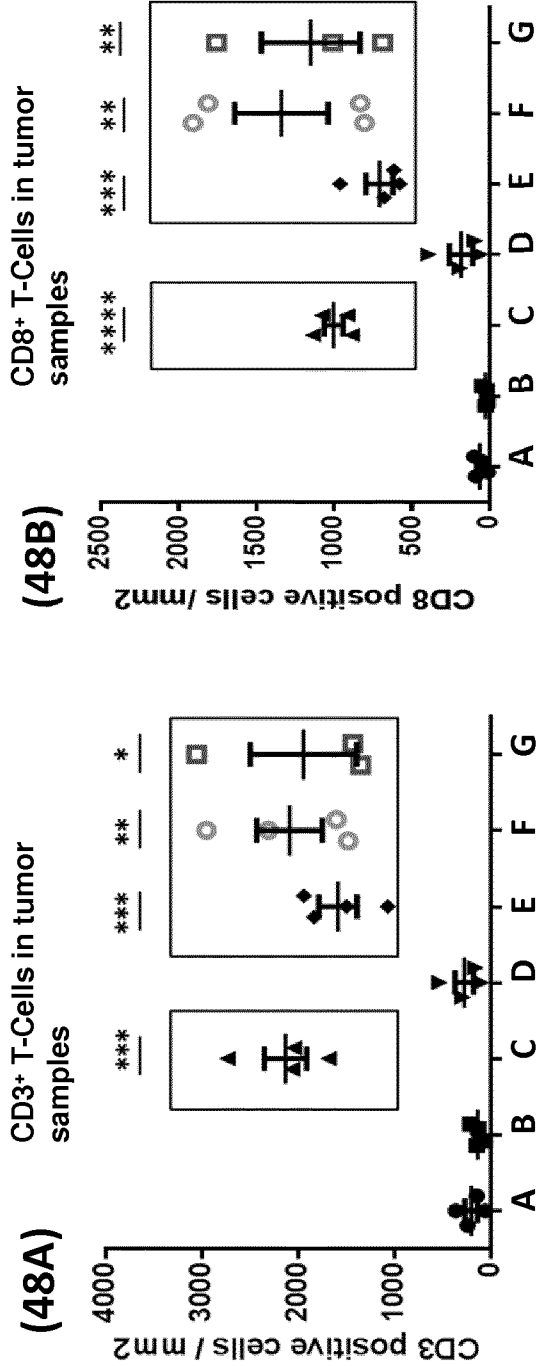


Fig. 48



(48C) Ratio of CD8⁺/CD3⁺ T-Cells in tumor samples

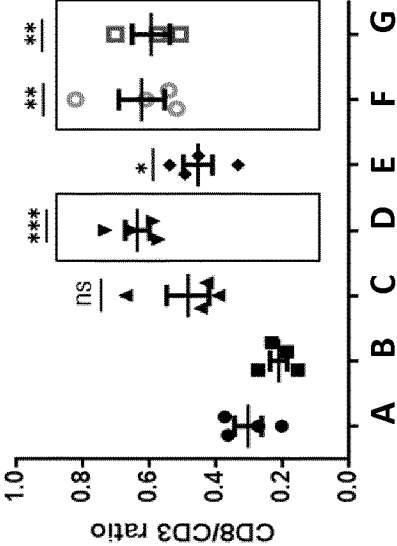


Fig. 49

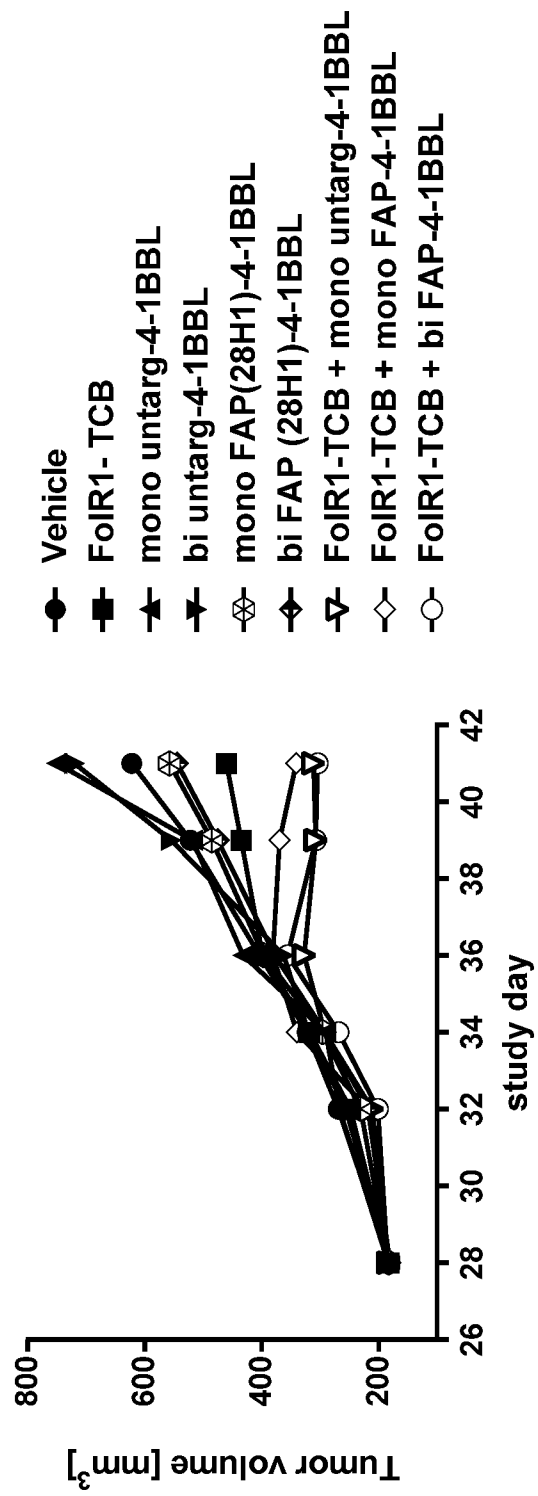


Fig. 50

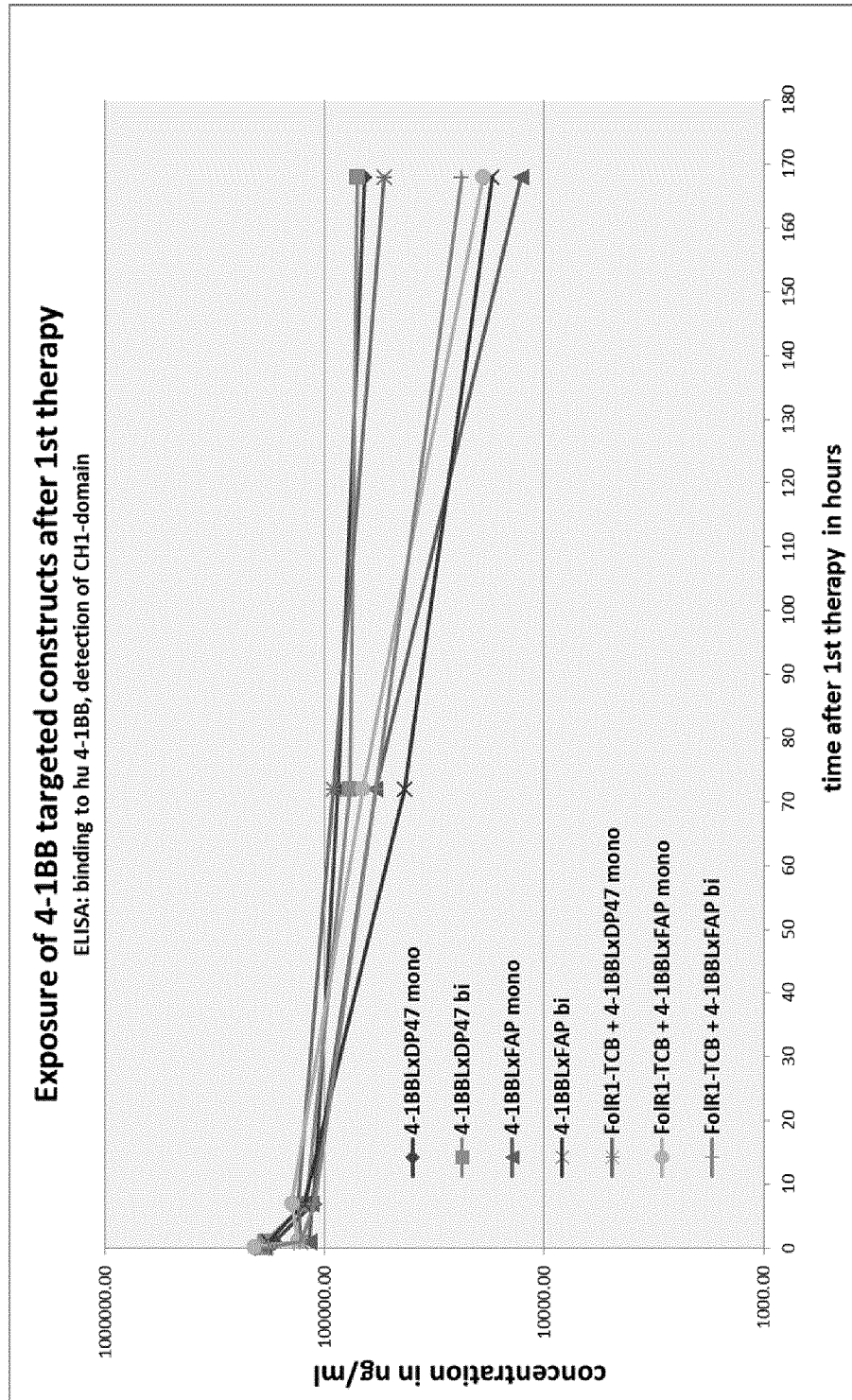
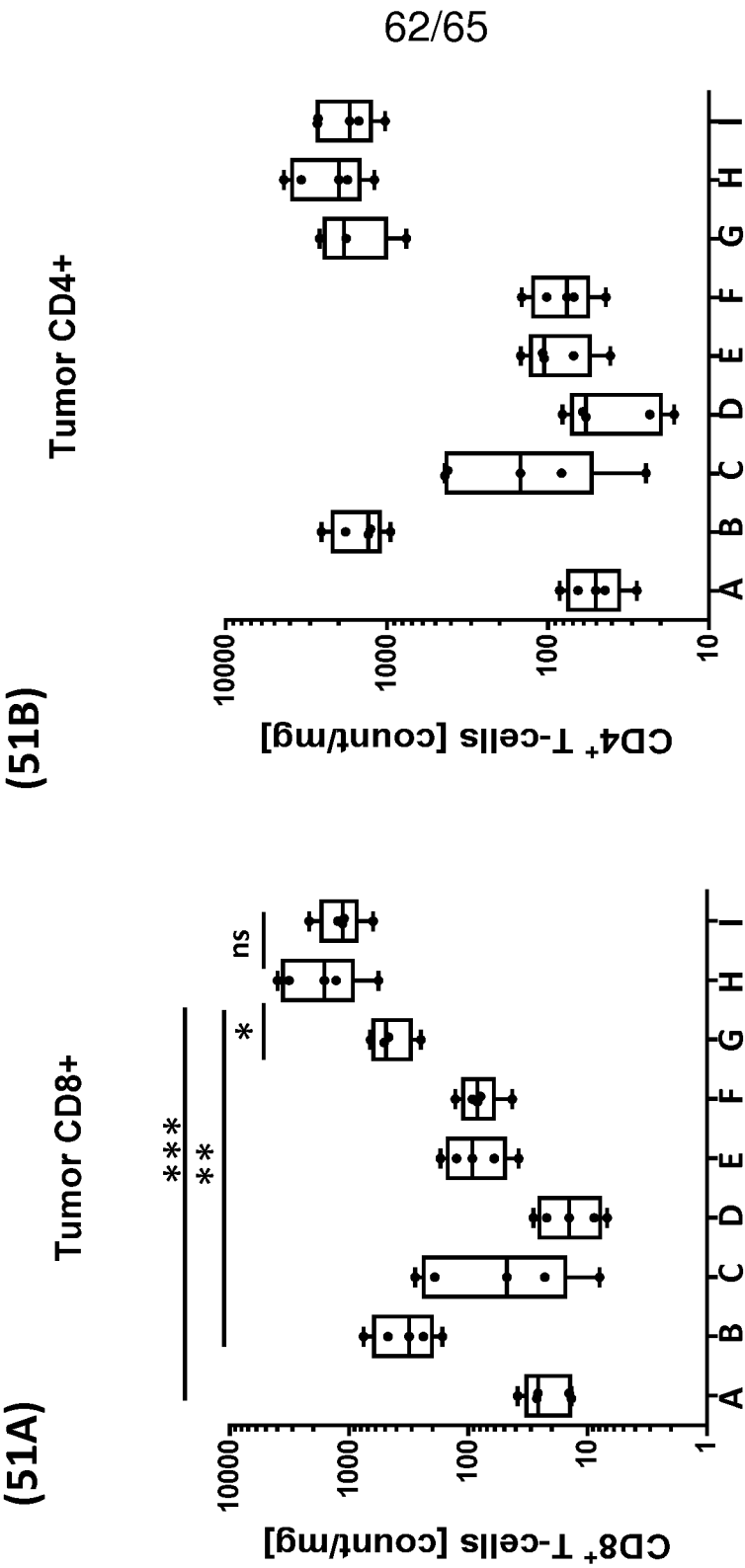
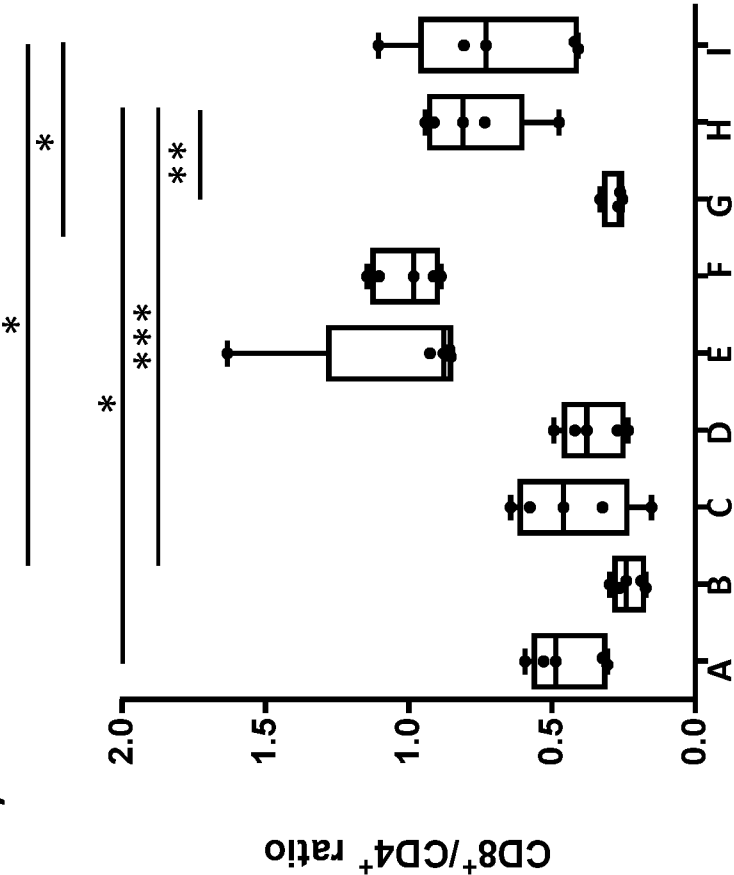


Fig. 51



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Fig. 51
(51C)



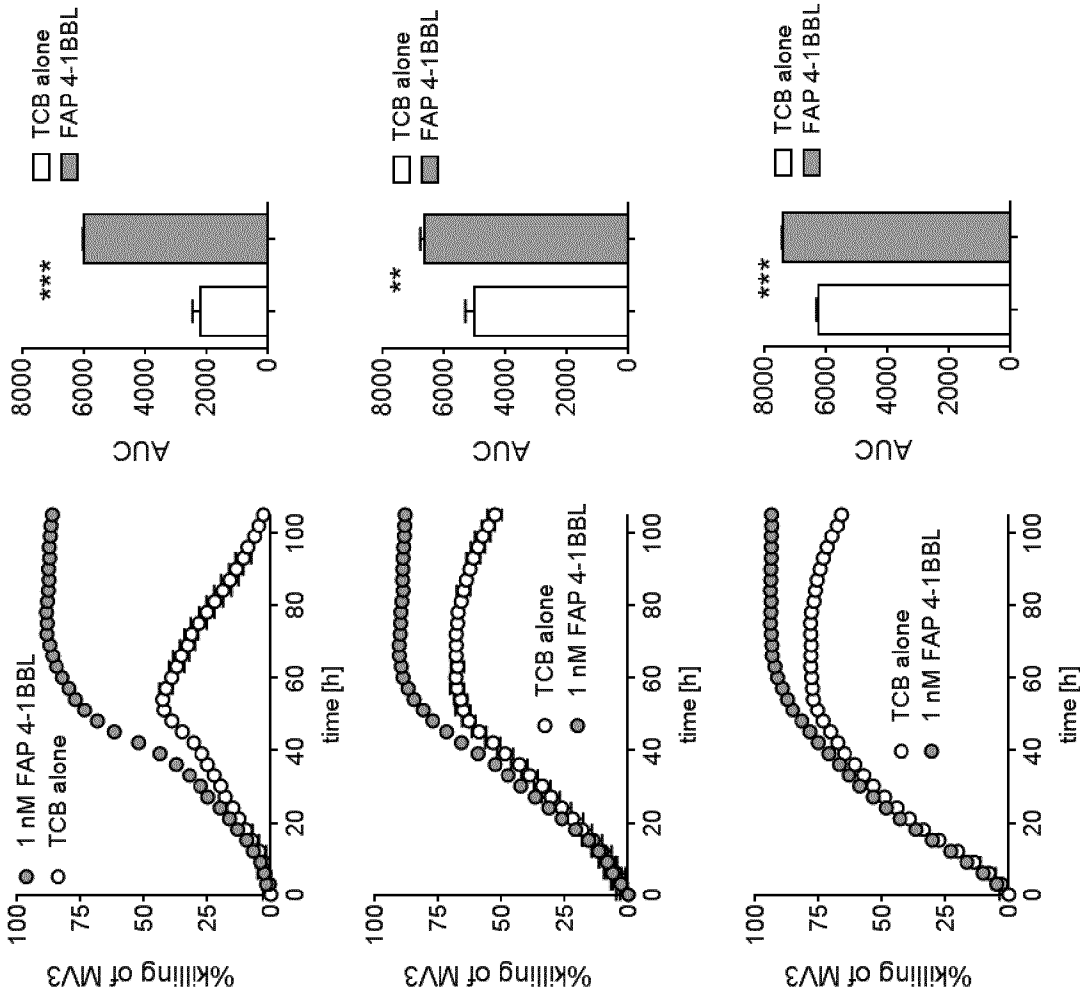
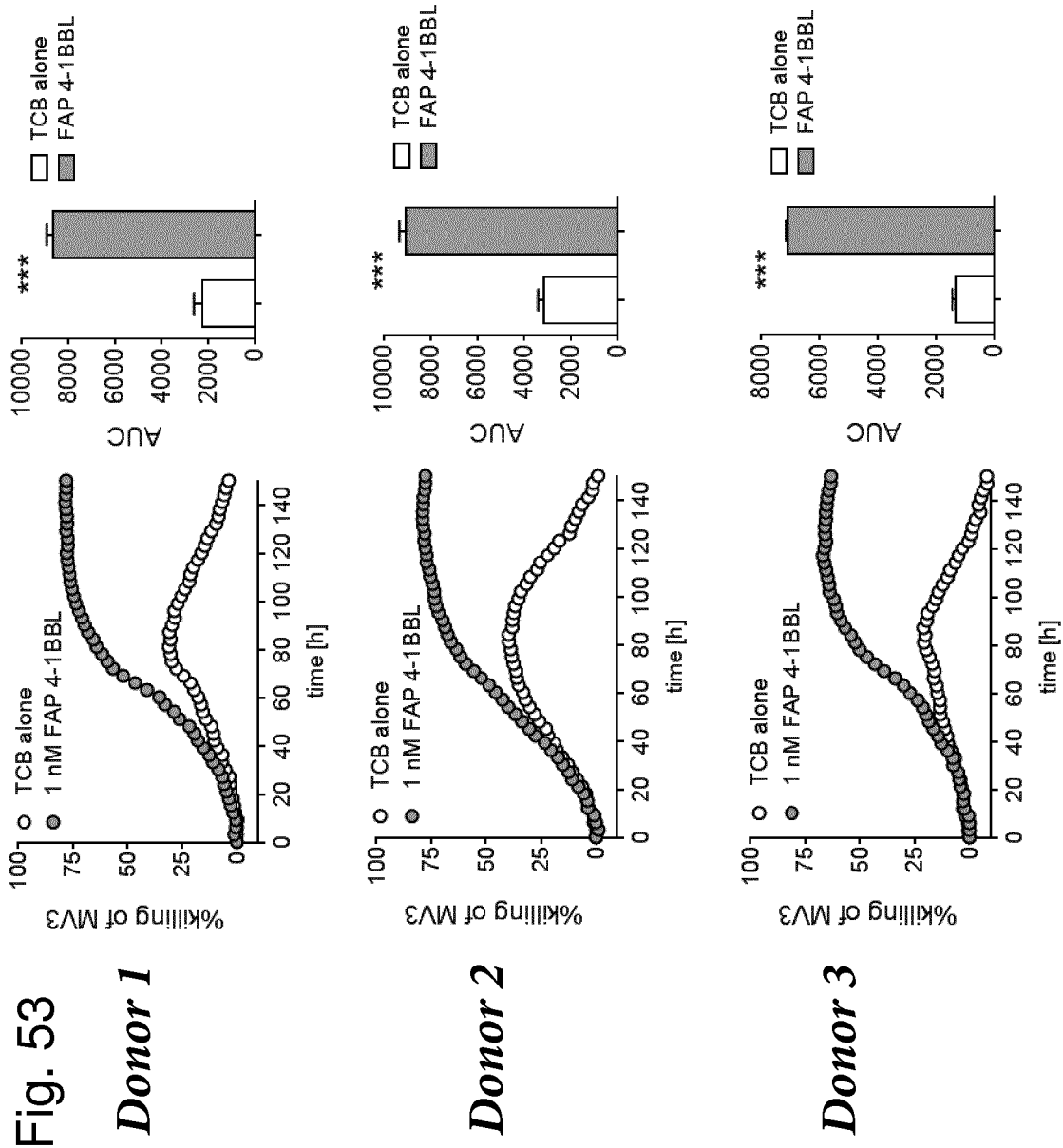


Fig. 52
Donor 1

Donor 2

Donor 3



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/083235

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 C07K14/525 C07K16/28 C07K16/30
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/156291 A1 (F HOFFMANN-LA ROCHE AG [CH]; HOFFMANN-LA ROCHE INC [US]) 6 October 2016 (2016-10-06) cited in the application	1-5, 7-11,14, 17,18, 21,23, 26, 28-32, 35-49
A	See examples, claims, figure 1B, Table 18 ----- -/--	6,12,13, 15,16, 19,20, 22,24, 25,27, 33,34,50



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

6 April 2018

Date of mailing of the international search report

23/04/2018

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Fax: (+31-70) 340-3016

Authorized officer

Nauche, Stéphane

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2017/083235

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/075278 A1 (HOFFMANN LA ROCHE [CH]; HOFFMANN LA ROCHE [US]) 19 May 2016 (2016-05-19) cited in the application	1-5, 7-11,14, 17,18, 21,23, 26, 28-32, 35-49
A	See claims	6,12,13, 15,16, 19,20, 22,24, 25,27, 33,34,50

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/083235

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2016156291 A1	06-10-2016	CN 107207579 A EP 3277305 A1 US 2016340399 A1 WO 2016156291 A1	26-09-2017 07-02-2018 24-11-2016 06-10-2016
WO 2016075278 A1	19-05-2016	AU 2015345024 A1 CA 2963718 A1 CL 2017001000 A1 CR 20170194 A EA 201791057 A1 EP 3224275 A1 JP 2018503356 A KR 20170085552 A PE 08962017 A1 PH 12017500892 A1 SG 11201703597T A TW 201629094 A US 2016200833 A1 WO 2016075278 A1	20-04-2017 19-05-2016 12-01-2018 10-07-2017 31-10-2017 04-10-2017 08-02-2018 24-07-2017 12-07-2017 06-11-2017 29-06-2017 16-08-2016 14-07-2016 19-05-2016