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(54) Title: BISPECIFIC ANTIGEN BINDING MOLECULES TARGETING OX40 AND FAP

(57) Abstract: The invention relates to novel bispecific antigen binding molecules, comprising at least two antigen binding domains capable of specific binding to OX40 and a particular antigen binding domain capable of specific binding to Fibroblast Activation Protein (FAP), and to methods of producing these molecules and to methods of using the same.

Bispecific Antigen Binding Molecules targeting OX40 and FAP

FIELD OF THE INVENTION

The invention relates to new bispecific antigen binding molecules, comprising at least two antigen binding domains capable of specific binding to OX40 and an antigen binding domain capable of specific binding to Fibroblast Activation Protein (FAP), and a Fc domain, in particular a Fc domain comprising one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function. Further aspects of the invention are methods of producing these molecules and methods of using the same.

BACKGROUND

Cancer is one of the leading causes of death worldwide despite several new agents providing survival benefits to patients. Despite advances in treatment options, prognosis of patients with advanced cancer remains poor. Many cancer indications have a poor prognosis, and the management of most advanced solid tumors remains challenging because of the high rate of tumor recurrence or the development of distant metastases. Patients with advanced solid tumors have clearly benefitted from checkpoint inhibitor (CPI) therapy. Even after treatment discontinuation it can extend the overall survival in a subset of patients, likely via the generation of a memory immune response. Although this has changed the treatment landscape in many cancer types, unfortunately 60-80% of patients with metastatic disease do not derive long-term benefit from this type of cancer immunotherapy. Consequently, there is a persisting and urgent medical need to develop new and optimal therapies that can be added to existing treatments to increase survival of cancer patients without causing unacceptable toxicity.

The immune suppressive microenvironment in certain tumors is high in co-inhibitory signals, e.g. PD-L1, but lacks sufficient expression of OX40 ligand. OX40 (CD134; TNFRSF4) is a member of the tumor-necrosis factor (TNF) receptor superfamily that is transiently expressed by T cells upon engagement of the T-cell receptor (TCR). OX40 engagement modulates bi-directionally the interaction of T cells with $OX40L^+$ antigen presenting cells (e.g. B cells, dendritic cells (DCs), monocytes). In the context of TCR engagement, OX40 provides co-stimulatory signals predominantly to $CD4^+$, but also to $CD8^+$ effector T cells, resulting in enhanced proliferation, survival, and effector function

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

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

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

SUMMARY OF THE INVENTION

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

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

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

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

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

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

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

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

In one aspect, the antigen binding domains capable of specific binding to OX40 bind to a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:1.

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

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

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

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

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

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

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

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

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

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

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

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

In another aspect, provided is a bispecific antigen binding molecule as defined herein before, the antigen binding domains capable of specific binding to OX40 comprise

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

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

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

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

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

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

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

In a further aspect, provided is a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule comprises

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

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

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

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

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

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

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

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

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

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

In another aspect, provided is a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule consists of

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

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

(ac) a third Fab fragment capable of specific binding to OX40,

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

(c) the Fc region composed of a first and a second subunit capable of stable association, wherein the second Fab fragment (ab) is fused at the C-terminus of the VH-CH1 chain to the N-terminus of the VH-CH1 chain of the first Fab fragment (aa), which is in turn fused at its C-terminus to the N-terminus of the first subunit, and the third Fab fragment (ac) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit.

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

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

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

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

(ac) a third Fab fragment capable of specific binding to OX40,

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

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

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

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

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

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

(ac) a third Fab fragment capable of specific binding to OX40,

(ad) a fourth Fab fragment capable of specific binding to OX40,

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

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

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

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

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

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

Also encompassed by the invention is the bispecific antigen binding molecule or the antibody as described herein before, or the pharmaceutical composition comprising the bispecific antigen binding molecule, for use as a medicament.

In one aspect, provided is a bispecific antigen binding molecule as described herein before or the pharmaceutical composition of the invention, for use
(i) in inducing immune stimulation,
(ii) in stimulating tumor-specific T cell response,
(iii) in causing apoptosis of tumor cells,
(iv) in the treatment of cancer,
(v) in delaying progression of cancer,
(vi) in prolonging the survival of a patient suffering from cancer,
(vii) in the treatment of infections.

In a specific aspect, provided is the bispecific antigen binding molecule as described herein before or the pharmaceutical composition of the invention, for use in the treatment of cancer. In another specific aspect, the invention provides the bispecific antigen binding molecule as described herein before for use in the treatment of cancer, wherein the bispecific antigen binding molecule is for administration in combination with a chemotherapeutic agent, radiation and/ or other agents for use in cancer immunotherapy. In one aspect, provided is a bispecific agonistic OX40 antigen binding molecule or a pharmaceutical composition for use in the treatment of cancer, wherein the bispecific agonistic OX40 antigen binding molecule is for administration in combination with a T-cell activating anti-CD3 bispecific antibody, for example an anti-CEA/anti-CD3 bispecific antibody. In one further aspect, the bispecific antigen binding molecule as described herein is for use in the treatment of cancer, wherein the bispecific antigen binding molecule is for administration in combination with an agent blocking PD-L1/PD-1 interaction such as PD-L1 antibody, for example atezolizumab, or a PD-1 antibody, for example nivolumab or pembrolizumab. In another aspect, provided is the bispecific antigen binding molecule as described herein before or the pharmaceutical composition of the invention, for use in up-regulating or prolonging cytotoxic T cell activity.

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

Also provided is the use of the bispecific antigen binding molecule as described herein before for the manufacture of a medicament for the treatment of a disease in an individual in need thereof, in particular for the manufacture of a medicament for the

treatment of cancer, as well as a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the bispecific antigen binding molecule of the invention in a pharmaceutically acceptable form. In a specific aspect, the disease is cancer. In any of the above aspects the individual is a mammal, particularly a human.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A to E show schematic representations of bispecific antigen binding molecules which specifically bind to human OX40 and to FAP. **Fig. 1A** shows a schematic representation of a bispecific FAP-OX40 antibody in a 4+1 format consisting of four OX40 binding Fab fragments combined with one FAP (1G1a) binding moiety as crossFab fragment, wherein the VH-Ckappa chain is fused at the C-terminus of the Fc knob chain (tetravalent for OX40 and monovalent for FAP). **Fig. 1B** shows a schematic representation of a bispecific FAP-OX40 antibody in a 3+1 format consisting of three OX40 binding Fab fragments combined with one FAP (1G1a) binding moiety as crossFab fragment, wherein the VH-Ckappa chain is fused at the C-terminus of the Fc knob chain (trivalent for OX40 and monovalent for FAP). The arm comprising two OX40 binding Fab fragments fused to each other is on Fc knob chain. **Fig. 1C** shows a schematic representation of a bispecific FAP-OX40 antibody in a 3+1 format consisting of three OX40 binding Fab fragments combined with one FAP (1G1a) binding moiety as crossFab fragment, wherein the VH-Ckappa chain is fused at the C-terminus of the Fc knob chain (trivalent for OX40 and monovalent for FAP). The arm comprising two OX40 binding Fab fragments fused to each other is on Fc hole chain. **Fig. 1D** shows a schematic representation of a bispecific FAP-OX40 antibody in a 2+1 format consisting of two OX40 binding Fab fragments combined with one FAP (1G1a) binding moiety as crossFab fragment, wherein the VH-Ckappa chain is fused at the C-terminus of the Fc knob chain (bivalent for OX40 and monovalent for FAP). **Fig. 1E** shows a schematic representation of a bispecific FAP-OX40 antibody P1AD4524 in a 4+1 format consisting of four OX40 binding Fab fragments combined with one FAP (4B9) binding moiety as VH and VL domain, wherein the VL domain is fused at the C-terminus of the Fc knob chain and the VH domain is fused at the C-terminus of the Fc hole chain (tetravalent for OX40 and monovalent for FAP). The black point symbolizes knob-into-hole mutations.

Figures 2A to **2F** show the cellular binding of bispecific antigen binding molecules comprising OX40 clone 49B4 in different formats. The FAP antigen binding domain H212 is the humanized version of FAP clone 212 that is called FAP (1G1a) herein. Human FAP negative tumor cells (A549- NLR)(**Fig. 2F**), FAP positive fibroblasts (NIH/3T3-huFAP-clone 19) (**Fig. 2E**), OX40 positive activated PBMC (activated CD4 and CD8 T cells, **Fig.**

2A and **2C**, respectively) as well as OX40 negative resting PBMC (resting CD4 and CD8 T cells, **Fig. 2B** and **2D**, respectively) were incubated with indicated serial dilutions of test antibody detected then by fluorescently labeled 2nd antibody against human Fcγ. Living cells were gated and the mean fluorescence intensity of the secondary antibody, baseline corrected by the media-only sample, was plotted from duplicates. Error bars indicate the SEM.

Figures 3A to **3F** show the cellular binding of bispecific antigen binding molecules comprising OX40 clone 8H9 in different formats and in comparison with a bispecific antigen binding molecule comprising OX40 clone 49B4 in 4+1 format (P1AE6838). Human FAP negative tumor cells (A549- NLR)(**Fig. 3F**), FAP positive fibroblasts (NIH/3T3-huFAP-clone 19) (**Fig. 3E**), OX40 positive activated PBMC (activated CD4 and CD8 T cells, **Fig. 3A** and **3C**, respectively) as well as OX40 negative resting PBMC (resting CD4 and CD8 T cells, **Fig. 3B** and **3D**, respectively) were incubated with indicated serial dilutions of test antibody detected then by fluorescently labeled 2nd antibody against human Fcγ. Living cells were gated and the mean fluorescence intensity of the secondary antibody, baseline corrected by the media-only sample, was plotted from duplicates. Error bars indicate the SEM. The clone 8H9 bound with subnanomolar affinity to OX40 positive cells and with comparable strength as tri- and bivalent antibody.

Figures 4A to **4F** show the cellular binding of bispecific antigen binding molecules comprising OX40 clone MOXR0916 in different formats and in comparison with a bispecific antigen binding molecule comprising OX40 clone 49B4 in 4+1 format (P1AE6838). Human FAP negative tumor cells (A549- NLR)(**Fig. 4F**), FAP positive fibroblasts (NIH/3T3-huFAP-clone 19) (**Fig. 4E**), OX40 positive activated PBMC (activated CD4 and CD8 T cells, **Fig. 4A** and **4C**, respectively) as well as OX40 negative resting PBMC (resting CD4 and CD8 T cells, **Fig. 4B** and **4D**, respectively) were incubated with indicated serial dilutions of test antibody detected then by fluorescently labeled 2nd antibody against human Fcγ. Living cells were gated and the mean fluorescence intensity of the secondary antibody, baseline corrected by the media-only sample, was plotted from duplicates. Error bars indicate the SEM. The clone MOXR0916 bound with nanomolar affinity to OX40 positive cells, with comparable strength as tri- and bivalent antibody.

Figures 5A to **5F** show the cellular binding of bispecific antigen binding molecules comprising OX40 clone CLC563 in different formats and in comparison with a bispecific antigen binding molecule comprising OX40 clone 49B4 in 4+1 format (P1AE6838). Human FAP negative tumor cells (A549- NLR)(**Fig. 5F**), FAP positive fibroblasts (NIH/3T3-huFAP-clone 19) (**Fig. 5E**), OX40 positive activated PBMC (activated CD4 and CD8 T cells, **Fig. 5A** and **5C**, respectively) as well as OX40 negative resting PBMC (resting CD4 and CD8 T cells, **Fig. 5B** and **5D**, respectively) were incubated with

indicated serial dilutions of test antibody detected then by fluorescently labeled 2nd antibody against human Fcγ. Living cells were gated and the mean fluorescence intensity of the secondary antibody, baseline corrected by the media-only sample, was plotted from duplicates. Error bars indicate the SEM. The clone CLC-563 bound with nanomolar affinity to OX40 positive cells, with comparable strength as tri- and bivalent antibody.

Figures 6A to **6F** show the cellular binding of bispecific antigen binding molecules comprising different variants of OX40 clone 49B4 with amino acid mutations in the VH domain in different formats and in comparison with a bispecific antigen binding molecule comprising OX40 clone 49B4 in 4+1 format (P1AE6838). Human FAP negative tumor cells (A549- NLR)(**Fig. 6F**), FAP positive fibroblasts (NIH/3T3-huFAP-clone 19) (**Fig. 6E**), OX40 positive activated PBMC (activated CD4 and CD8 T cells, **Fig. 6A** and **6C**, respectively) as well as OX40 negative resting PBMC (resting CD4 and CD8 T cells, **Fig. 6B** and **6D**, respectively) were incubated with indicated serial dilutions of test antibody detected then by fluorescently labeled 2nd antibody against human Fcγ. Living cells were gated and the mean fluorescence intensity of the secondary antibody, baseline corrected by the media-only sample, was plotted from duplicates. Error bars indicate the SEM. All antigen binding molecules comprising OX40 (49B4) variants with amino acid mutations showed slightly improved binding to OX40 positive cells compared to antigen binding molecule including clone 49B4.

Figures 7A to **7C** show the NFκB-mediated luciferase expression activity in OX40 expressing reporter cell line HeLa_hOx40_NFκB_Luc1. The concentration of bispecific antigen binding molecules comprising OX40 clone 49B4 in different formats or its controls are blotted against the units of released light (URL) measured after incubation and addition of Luciferase detection solution. Shown is the NFκB induction of the 4+1, 3+1 or 2+1 formats, either crosslinked with human FAP expressing NIH/3T3 fibroblasts (**Fig. 7A**), a secondary antibody at a 2 to 1 ratio (**Fig. 7B**) or w/o further crosslinking (**Fig. 7C**). The isotype control antibody did not induce any NFκB activation. All OX40 containing constructs induced dose dependent NFκB activation. The tetravalent format comprising four OX40 Fab fragments induced a certain NFκB activation due to the assembly of the trimeric core OX40 receptor-signaling unit already in the absence of crosslinking. The same clone in a trivalent or bivalent format showed accordingly less bioactivity. The higher the valency of the OX40 antigen binding domains, the stronger was the extent of NFκB activation and the lower the required concentration. Shown is the mean of duplicates. Error bars represent the SEM.

Figures 8A to **8C** show the NFκB-mediated luciferase expression activity in OX40 expressing reporter cell line HeLa_hOx40_NFκB_Luc1 of different bispecific antigen binding molecules comprising OX40 clone 8H9. The concentration of the bispecific antigen binding molecules in different formats or its controls are blotted against the units

of released light (URL) measured after incubation and addition of Luciferase detection solution. Shown is the NFκB induction of the 3+1 or 2+1 formats, either crosslinked with human FAP expressing NIH/3T3 fibroblasts (**Fig. 8A**), a secondary antibody at a 2 to 1 ratio (**Fig. 8B**) or w/o further crosslinking (**Fig. 8C**). The isotype control antibody did not induce any NFκB activation. All OX40 containing constructs induced dose dependent NFκB activation. The tetravalent format comprising four OX40 Fab fragments induced a certain NFκB activation due to the assembly of the trimeric core OX40 receptor-signaling unit already in the absence of crosslinking and was most potent. The bispecific antigen binding molecules comprising OX40 clone 8H9 in a trivalent or bivalent format showed accordingly less bioactivity. Additional crosslinking by human FAP expressing fibroblasts via the FAP binding moiety, or by a secondary crosslinking antibody vie the Fc region of the OX40 antigen binding molecule further increased the NFκB activation. The clone OX40 (8H9) achieved the highest induction of NFkB activation already in the 2+1 format. Trivalency did not add further benefit. Shown is the mean of duplicates. Error bars represent the SEM.

Figures 9A to **9C** show the NFκB-mediated luciferase expression activity in OX40 expressing reporter cell line HeLa_hOx40_NFκB_Luc1 of different bispecific antigen binding molecules comprising OX40 clone MOXR0916. The concentration of the bispecific antigen binding molecules in different formats or its controls are blotted against the units of released light (URL) measured after incubation and addition of Luciferase detection solution. Shown is the NFκB induction of the 3+1 or 2+1 formats, either crosslinked with human FAP expressing NIH/3T3 fibroblasts (**Fig. 9A**), a secondary antibody at a 2 to 1 ratio (**Fig. 9B**) or w/o further crosslinking (**Fig. 9C**). The isotype control antibody did not induce any NFκB activation. All OX40 containing constructs induced dose dependent NFκB activation. The tetravalent format comprising four OX40 Fab fragments induced a certain NFκB activation due to the assembly of the trimeric core OX40 receptor-signaling unit already in the absence of crosslinking and was most potent. The bispecific antigen binding molecules comprising OX40 clone MOXR0916 in a trivalent or bivalent format showed accordingly less bioactivity. Additional crosslinking by human FAP expressing fibroblasts via the FAP binding moiety, or by a secondary crosslinking antibody vie the Fc region of the OX40 antigen binding molecule further increased the NFκB activation. The clone OX40 (MOXR0916) achieved the highest induction of NFkB activation already in the 2+1 format, no further benefit was obtained with the 3+1 format. Shown is the mean of duplicates. Error bars represent the SEM.

Figures 10A to **10C** show the NFκB-mediated luciferase expression activity in OX40 expressing reporter cell line HeLa_hOx40_NFκB_Luc1 of different bispecific antigen binding molecules comprising OX40 clone CLC563. The concentration of the bispecific antigen binding molecules in different formats or its controls are blotted against

the units of released light (URL) measured after incubation and addition of Luciferase detection solution. Shown is the NFκB induction of the 3+1 or 2+1 formats, either crosslinked with human FAP expressing NIH/3T3 fibroblasts (**Fig. 10A**), a secondary antibody at a 2 to 1 ratio (**Fig. 10B**) or w/o further crosslinking (**Fig. 10C**). The isotype control antibody did not induce any NFκB activation. All OX40 containing constructs induced dose dependent NFκB activation. The tetravalent format comprising four OX40 Fab fragments induced a certain NFκB activation due to the assembly of the trimeric core OX40 receptor-signaling unit already in the absence of crosslinking and was most potent. The bispecific antigen binding molecules comprising OX40 clone CLC563 in a trivalent or bivalent format showed less bioactivity. Additional crosslinking by human FAP expressing fibroblasts via the FAP binding moiety, or by a secondary crosslinking antibody vie the Fc region of the OX40 antigen binding molecule further increased the NFκB activation. The clone OX40 (CLC563) achieved the highest induction of NFκB activation in the 3+1 format, which was slightly more potent than the 2+1 format. Shown is the mean of duplicates. Error bars represent the SEM.

Figures 11A to **11C** show the NFκB-mediated luciferase expression activity in OX40 expressing reporter cell line HeLa_hOx40_NFκB_Luc1 of different variants of OX40 clone 49B4 with amino acid mutations in the VH domain in different formats and in comparison with a bispecific antigen binding molecule comprising OX40 clone 49B4 in 4+1 format (P1AE6838). The concentration of the bispecific antigen binding molecules in different formats or its controls are blotted against the units of released light (URL) measured after incubation and addition of Luciferase detection solution. Shown is the NFκB induction, either cross-linked with human FAP expressing NIH/3T3 fibroblasts (**Fig. 11A**), a secondary antibody at a 2 to 1 ratio (**Fig. 11B**) or w/o further crosslinking (**Fig. 11C**). The isotype control antibody did not induce any NFκB activation. All amino acid variants induced dose dependent NKκB activation to a similar extent than the OX40 (49B4) antibody in the 4+1 format. The tetravalent use of OX40 antigen binding domains induced a certain NFκB activation due to the assembly of the trimeric core OX40 receptor-signaling unit. Additional crosslinking by human FAP expressing fibroblasts via the FAP binding moiety, or by a secondary crosslinking antibody vie the Fc region of the OX40 antigen binding molecule further increases the NFκB activation, which was already evident at lower concentrations. Shown is the mean of duplicates. Error bars represent the SEM.

Figures 12A and **12B** show the primary T cell bioactivity of the bispecific antigen binding molecule P1AE6838 in comparison with different reference molecules (P1AD3690, an untargeted molecule comprising four OX40 (49B4) Fab fragments, P1AD4524, a molecule comprising four OX40 (49B4) Fab fragments and FAP antibody 4B9 as C-terminal VH/VL, P1AD4353, a molecule comprising two OX40 (49B4) Fab fragments and FAP antibody 4B9, and P1AD3691, a molecule comprising two OX40

(49B4) Fab fragments and two FAP (28H1) Fab fragments). **Fig. 12A** shows the number of $CD4^+$ T cells and **Fig. 12B** shows the CD25 activation marker expression on $CD4^+$ T cells at endpoint. Increased proliferation and CD25 activation marker expression were observed with FAP-targeted tetravalent OX40 antigen binding molecules in a dose-dependent manner. Bivalent molecules led to reduced bioactivity as compared to tetravalent in line with the fact that 49B4 is an avidity-driven OX40 antibody. The untargeted OX40 molecule showed minimal activity at the highest tested concentration whereas isotype control showed no activation after baseline-correction. Shown is the mean of triplicates. Error bars represent the SEM.

Figures 13A and **13B** show the primary T cell bioactivity of bispecific antigen binding molecules comprising OX40(49B4) and FAP (1G1a) in 4+1, 3+1 and 2+1 format in comparison to P1AD4524, a molecule comprising four OX40 (49B4) Fab fragments and FAP antibody 4B9 as C-terminal VH/VL. **Fig. 13A** shows the number of $CD4^+$ T cells and **Fig. 13B** shows the CD25 activation marker expression on $CD4^+$ T cells at endpoint. Increased proliferation and CD25 activation marker expression were observed with FAP-targeted tetravalent OX40 antigen binding molecules in a dose-dependent manner. Trivalent and to a bigger extent bivalent OX40 antigen binding molecules led to reduced bioactivity as compared to tetravalent, in line with the fact that 49B4 is an avidity driven antibody. Isotype control showed no activation after baseline-correction. Shown is the mean of triplicates. Error bars represent the SEM.

Figures 14A and **14B** show the primary T cell bioactivity of bispecific antigen binding molecules comprising clone OX40(8H9) in 3+1 and 2+1 format in comparison to P1AE6838, a molecule comprising four OX40 (49B4) Fab fragments. **Fig. 14A** shows the number of $CD4^+$ T cells and **Fig. 14B** shows the CD25 activation marker expression on $CD4^+$ T cells at endpoint. Increased proliferation and CD25 activation marker expression were observed with FAP-targeted tetravalent OX40 antigen binding molecules in a dose-dependent manner. Trivalent and bivalent antigen binding molecules comprising clone 8H9 led to slightly reduced maximal response as compared to tetravalent clone 49B4, with comparable sub-nanomolar EC_{50}. Isotype control showed no activation after baseline-correction. Shown is the mean of triplicates. Error bars represent the SEM.

Figures 15A and **15B** show the primary T cell bioactivity of bispecific antigen binding molecules comprising clone OX40(MOXR0916) in 3+1 and 2+1 format in comparison to P1AE6838, a molecule comprising four OX40 (49B4) Fab fragments. **Fig. 15A** shows the number of $CD4^+$ T cells and **Fig. 15B** shows the CD25 activation marker expression on $CD4^+$ T cells at endpoint. Increased proliferation and CD25 activation marker expression were observed with FAP-targeted tetravalent OX40 antigen binding molecules in a dose-dependent manner. Trivalent and bivalent antigen binding molecules comprising clone MOXR0916 led to similar numbers of $CD4^+$ T cells and reduced

maximal CD25 expression as compared to the tetravalent molecule comprising clone 49B4. The drop in $CD4^+$ T cells at the highest tested concentration of the MOXR0916 containing molecules is an indicator of activation induced cell death. Isotype control showed no activation after baseline-correction. Shown is the mean of triplicates. Error bars represent the SEM.

Figures 16A and **16B** show the primary T cell bioactivity of bispecific antigen binding molecules comprising clone OX40 (CLC563) in 3+1 and 2+1 format in comparison to P1AE6838, a molecule comprising four OX40 (49B4) Fab fragments. **Fig. 16A** shows the number of $CD4^+$ T cells and **Fig. 16B** shows the CD25 activation marker expression on $CD4^+$ T cells at endpoint. Increased proliferation and CD25 activation marker expression were observed with FAP-targeted tetravalent OX40 antigen binding molecules in a dose-dependent manner. Trivalent and bivalent antigen binding molecules comprising clone CLC563 led to similar numbers of $CD4^+$ T cells and reduced maximal CD25 expression as compared to the tetravalent molecule comprising clone 49B4. Isotype control showed no activation after baseline-correction. Shown is the mean of triplicates. Error bars represent the SEM.

Figures 17A and **17B** show the primary T cell bioactivity of different variants of OX40 clone 49B4 with amino acid mutations in the VH domain in different formats and in comparison with a bispecific antigen binding molecule comprising OX40 clone 49B4 in 4+1 format (P1AE6838). **Fig. 17A** shows the number of $CD8^+$ T cells and **Fig. 17B** shows the CD25 activation marker expression on $CD4^+$ T cells at endpoint. Increased proliferation and CD25 activation marker expression were observed with FAP-targeted tetravalent OX40 antigen binding molecules in a dose-dependent manner. The three tetravalent amino acid variants displayed comparable activity than that of the parental antibody in terms of $CD8^+$ T cells proliferation and CD25 upregulation on $CD4^+$ T cells. Isotype control showed no activation after baseline-correction. Shown is the mean of triplicates. Error bars represent the SEM.

A normalized overview of the areas under the curve (AUC) of the CD25 activation marker expression on $CD4^+$ T cells as measured at endpoint for all antigen binding molecules is provided in **Figure 18**.

Figure 19 shows the serum concentration-time profiles of the reference molecule P1AD4524 as measured in three different female HuFcRn mice (F350, F351 and F353) and the mean curve.

Figures 20A to **20C** show an isopotential surface area of the Fab region of FAP clone 4B9 as included in the reference compound P1AD4524. Black mesh depicts positively charged patches while white mesh depicts negatively charged patches. **Fig. 20A** allows a view from the back, **Fig. 20B** shows the view from the front and **Fig. 20C** illustrates the top of the antibody. **Figures 20D** to **20F** show the isopotential surface area

of the Fab region of FAP clone 1G1a as included in molecules of the present invention. Black mesh depicts positively charged patches while white mesh depicts negatively charged patches. **Fig. 20D** allows a view from the back, **Fig. 20E** shows the view from the front and **Fig. 20F** illustrates the top of the Fab.

Figure 21 shows the serum concentration-time profiles of bispecific antigen binding molecule P1AE6836 comprising a C-terminal cross-fab fragment in comparison to the reference molecule P1AD4524 comprising a VH and VL domain C-terminally fused to the the Fc region as measured in three different female HuFcRn mice (F350, F351 and F353) and the mean curve.

Figures 22A to **22C** show an isopotential surface area of the Fab region of OX40 clone 49B9 as included in the reference compound P1AD4524. Black mesh depicts positively charged patches while white mesh depicts negatively charged patches. **Fig. 22A** allows a view from the back, **Fig. 22B** shows the view from the front and **Fig. 22C** illustrates the top of the antibody.

Figures 23A to **23C** show an isopotential surface area of the Fab region of OX40 clone 8H9. Black mesh depicts positively charged patches while white mesh depicts negatively charged patches. **Fig. 23A** allows a view from the back, **Fig. 23B** shows the view from the front and **Fig. 23C** illustrates the top of the antibody.

Figures 24A to **24C** show an isopotential surface area of the Fab region of OX40 clone CLC563. Black mesh depicts positively charged patches while white mesh depicts negatively charged patches. **Fig. 24A** allows a view from the back, **Fig. 24B** shows the view from the front and **Fig. 24C** illustrates the top of the antibody.

Figures 25A to **25C** show an isopotential surface area of the Fab region of OX40 clone MOXR0916. Black mesh depicts positively charged patches while white mesh depicts negatively charged patches. **Fig. 25A** allows a view from the back, **Fig. 25B** shows the view from the front and **Fig. 25C** illustrates the top of the antibody.

Figure 26 is a schematic drawing of the transwell system used in the ARC assay. Madin-Darby Canine Kidney cells (MDCK) are seeded in the apical compartment of a transwell system. The MDCK cells have been transfected with the human FcRn.

Figures 27A to 27C show the preexisting Anti-Drug Antibody (ADA) reactivity in a panel of human individual plasma samples as measured with the assay described in Example 7.1. High incidence with high signals was observed for the bispecific antigen binding molecule OX40 (49B4) x FAP (4B9) (4+1) as described in WO 2017/060144 A1 wherein a VH and VL domain are c-terminally linked to each of the heavy chains (**Fig. 27A)**. Less incidence was detected for bispecific antigen binding molecules wherein the VH and VL domain fused to the C-termini of the Fc domain were replaced by a Fab fragment. However, there still seem to be preexisting anti-drug antibodies against the Fab fragment as can be seen for OX40 (49B4) x FAP (4B9) (4+1) in **Fig. 27B** and for OX40

(49B4) x FAP (1G1a) (4+1) in **Fig. 27C**. 1G1a is a humanized variant of FAP clone 212 (H212).

Figure 28 compares the preexisting Anti-Drug Antibody (ADA) reactivity of bispecific antigen binding molecules in 2+1 format comprising different anti-OX40 clones (49B4, 8H9, MOX0916 and CLC-563) in a panel of human individual plasma samples.

Fig. 29A shows that the control molecules, i.e. an untargeted tetravalent OX40 (49B4) antigen binding molecule (P1AD3690), the FAP (1G1a) antibody (P1AE1689) or a Germline control antibody (DP47) did not cause preexisting Anti-Drug Antibody (ADA) reactivity, whereas the bispecific antigen molecules comprising a Fab fragment fused at the C-terminus of the Fc domain all caused preexisting IgG interference as shown in **Fig. 29B**. Surprisingly, the smaller 2+1 molecule induced a slightly higher incidence than the molecules in 3+1 and 4+1 format.

Figures 30A to **30C** relate to the testing of the preexisting Anti-Drug Antibody (ADA) reactivity of the bispecific antigen binding molecule OX40 (49B4) x FAP (1G1a) (3+1). The molecule comprising a CH1 domain with a "free" C-terminus EPKSC induces preexisting ADA reactivity as can be seen in **Fig. 30A**. The individual background signal of the buffer as measured by performing the assay without the drug molecule is shown in **Fig. 30B** and **Fig. 30C** shows the preexisting ADA reactivity of the molecule with the background signal substracted.

Figures 31A to **31C**: The preexisting Anti-Drug Antibody (ADA) reactivity in a panel of human individual plasma samples of the bispecific antigen binding molecule OX40 (49B4) x FAP (1G1a) (3+1) as determined in Fig. 30C is also shown in **Fig. 31A** and compared with the preexisting IgG reactivity induced by the bispecific molecule OX40 (MOXR0916) x FAP (1G1a) (3+1) comprising a EPKSCD terminus (**Fig. 31B**) or by the bispecific molecule OX40 (MOXR0916) x FAP (1G1a) (3+1) comprising a EPKSCS terminus (**Fig. 31C**). A massive reduction was observed with the EPKSD variant whereas the EPKSCS variant led to complete elimination of preexisting ADA reactivity.

Figures 32A to 32C show a respective molecule set in 2+1 format, and confirm the previous results that a bispecific antigen binding molecule OX40 (MOXR0916) x FAP (1G1a) (2+1) with EPKSCD terminus, P1AF4852 (**Fig. 32B**) reduces, while a bispecific antigen binding molecule OX40 (MOXR0916) x FAP (1G1a) (2+1) with EPKSCS terminus, P1AF4858 (**Fig. 32C**) eliminates the reactivity with preexisting antibodies in plasma compared to a molecule OX40 (49B4) x FAP (1G1a) (2+1) with a free C-terminus EPKSC (P1AE6840, **Fig. 32A**).

Figures 33A to **33F** confirm that the same effect was observed with three other examples. The preexisting ADA reactivity in a panel of human individual plasma samples is shown for OX40 (CLC563) x FAP (1G1a) (3+1) with EPKSCD terminus (P1AF6454) in **Fig. 33A**, for OX40 (CLC563) x FAP (1G1a) (3+1) with EPKSCS terminus (P1AF6455)

in **Fig. 33B**, for OX40 (CLC563) x FAP (1G1a) (4+1) with EPKSCD terminus (P1AF7205) in **Fig. 33C**, for OX40 (CLC563) x FAP (1G1a) (4+1) with EPKSCS terminus (P1AF7217) in **Fig. 33D**, for OX40 (49B4_K23E_K73E) x FAP (1G1a) (3+1) with EPKSCD terminus (P1AF6456) in **Fig. 33E** and for OX40 (49B4_K23E_K73E) x FAP (1G1a) (3+1) with EPKSCS terminus (P1AF6457) in **Fig. 33F**.

Figures 34A to **34F** show the cellular binding of bispecific antigen-binding molecules comprising OX40 clones OX40(49B4_K23E_K73E) or OX40(CLC563) in 3+1 and 4+1 formats as D- and S-variant as indicated. OX40 positive activated PBMC gated on activated CD4 cells (**Fig. 34A, 34C, 34E**) and activated CD8 T cells (**Fig. 34C, 34D, 34F**), respectively, were incubated with indicated serial dilutions of test antibody detected then by fluorescently labeled 2nd antibody against human Fcγ. Living cells were gated and the mean fluorescence intensity of the secondary antibody, baseline corrected by the media-only sample, was plotted from duplicates. **Fig. 34A** shows the binding of the OX40(CLC563) 3+1 constructs as D- and S-variants to activated CD4 cells and the binding to activated CD8 T cells is shown in **Fig. 34B**. In **Fig. 34C** and **Fig. 34D** is shown the binding of the OX40(CLC563) 4+1 constructs as D- and S-variants on activated CD4 cells and on activated CD8 cells, respectively. The binding of OX40(49B4_K23E_K73E) 4+1 constructs as D- and S-variant to activated CD4 cells and to activated CD8 T cells is shown in **Fig. 34E** and **Fig. 34F**, respectively. As control molecules, the untargeted tetravalent OX40(49B4) 4+0 construct (P1AD3690), the tetravalent OX40(49B4)-FAP(4B9) 4+1 construct (P1AD4524) and isotype control were used.

Figures 35A to **35F** show the NFκB-mediated luciferase expression activity in OX40 expressing reporter cell line HeLa_hOx40_NFκB_Luc1. The concentration of bispecific antigen binding molecules comprising OX40 clones OX40(49B4_K23E_K73E) or OX40(CLC563) in 3+1 and 4+1 formats as D and S variant are blotted against the units of released light (URL) measured after incubation and addition of Luciferase detection solution. Shown is the NFκB induction, either crosslinked with human FAP expressing NIH/3T3 fibroblasts (**Fig. 35A** for the OX40(CLC563) 3+1 constructs as D- and S-variants, **Fig. 35C** for the OX40(CLC563) 4+1 constructs as D- and S-variants and **Fig. 35E** for the OX40(49B4_K23E_K73E) 4+1 constructs as D- and S-variant), or without further crosslinking (**Fig. 35B** for the OX40(CLC563) 3+1 constructs as D- and S-variants, **Fig. 35D** for the OX40(CLC563) 4+1 constructs as D- and S-variants and **Fig. 35F** for the OX40(49B4_K23E_K73E) 4+1 constructs as D- and S-variant). The isotype control antibody did not induce any NFκB activation. All OX40 containing constructs induced dose dependent NFκB activation. The tetravalent format comprising four OX40 Fab fragments induced a certain NFκB activation due to the assembly of the trimeric core OX40 receptor-signaling unit already in the absence of crosslinking. The S and D variant performed similar. Shown is the mean of duplicates. Error bars represent the SEM.

Figures 36A to **36F** show the primary T cell bioactivity of bispecific antigen-binding molecules comprising OX40 clones OX40(49B4_K23E_K73E) or OX40(CLC563) in 3+1 and 4+1 formats as D and S variant as indicated. The evaluated bioactivity marker was here the CD25 activation marker expression on $CD4^+$ T cells (**Fig. 36A** for OX40(CLC563) 3+1 constructs, **Fig. 36C** for OX40(CLC563) 4+1 constructs and **Fig. 36E** for OX40(49B4_K23E_K73E) 4+1 constructs) and $CD8^+$ T cells (**Fig. 36B** for OX40(CLC563) 3+1 constructs, **Fig. 36D** for OX40(CLC563) 4+1 constructs and **Fig. 36F** for OX40(49B4_K23E_K73E) 4+1 constructs) cells at endpoint. Increased proliferation and CD25 activation marker expression were observed with FAP-targeted OX40 antigen binding molecules in a dose-dependent manner. The untargeted OX40 molecule showed activity only at the highest tested concentrations whereas isotype control showed no activation after baseline-correction. No statistically significant difference could be detected between the S- and D- variants. Shown is the mean of duplicates. Error bars represent the SEM.

Figures 37A to **37F** show that co-stimulation with FAP targeted OX40 agonists enhances the cytokine secretion of PBMC induced by CEACAM5 TCB mediated lysis of tumor cells. PBMC were cocultured with MKN45 NLR target cells, FAP^+ NIH/3T3-huFAP clone 19, CECAM5 TCB [2 nM] and bispecific antigen binding molecules comprising OX40 clones OX40(49B4_K23E_K73E) or OX40(CLC563) in 3+1 and 4+1 formats as D and S variant as indicated for 48 hrs. The evaluated bioactivity marker was here the fold increase of GM-CSF (**Fig. 37A** for OX40(CLC563) 3+1 constructs, **Fig. 37C** for OX40(CLC563) 4+1 constructs and **Fig. 37E** for OX40(49B4_K23E_K73E) 4+1 constructs) and TNF-α (**Fig. 37B** for OX40(CLC563) 3+1 constructs, **Fig. 37D** for OX40(CLC563) 4+1 constructs and **Fig. 37F** for OX40(49B4_K23E_K73E) 4+1 constructs), over TCB only treated samples in the assay supernatant. Cytokine induction was only seen for FAP-crosslinked OX40 agonists in a dose-dependent manner. The untargeted OX40 control molecule (P1AD3690) and isotype control showed no activity here. The S- variants show a trend to reduced bioactivity compared to the D- variants. Shown is the mean of triplicates.

Figure 38A to **38F** also show that co-stimulation with FAP-targeted OX40 agonists enhances the cytokine secretion of PBMC induced by CEACAM5 TCB mediated lysis of tumor cells. PBMC were cocultured with MKN45 NLR target cells, FAP^+ NIH/3T3-huFAP clone 19, CECAM5 TCB [2 nM] and bispecific antigen binding molecules comprising OX40 clones OX40(49B4_K23E_K73E) or OX40(CLC563) in 3+1 and 4+1 formats as D and S variant as indicated for 48 hrs. The evaluated bioactivity marker was here the fold increase of IFNγ (**Fig. 38A** for OX40(CLC563) 3+1 constructs, **Fig. 38C** for OX40(CLC563) 4+1 constructs and **Fig. 38E** for OX40(49B4_K23E_K73E) 4+1 constructs) and IL-2 (**Fig. 38B** for OX40(CLC563) 3+1 constructs, **Fig. 38D** for

OX40(CLC563) 4+1 constructs and **Fig. 38F** for OX40(49B4_K23E_K73E) 4+1 constructs), over TCB only treated samples in the assay supernatant. Cytokine induction was only observed for FAP-crosslinked OX40 agonists in a dose-dependent manner. The untargeted OX40 control molecule (P1AD3690) and isotype control showed no activity here. The S- variants show a trend to reduced bioactivity compared to the D- variant. Shown is the mean of triplicates.

Figure 39 summarizes the data and shows that co-stimulation with all FAP targeted OX40 agonists enhances the cytokine secretion of PBMC induced by CEACAM5 TCB mediated lysis of tumor cells. The AUC of the dose response curves in Figures 37A to 37F and 38A to 38F were calculated and normalized against that of the OX40(CLC563) x FAP(1G1a_EPKSCD) 3+1 antigen binding molecule (P1AF6454, also called 3+1 CLC563/H212-D). Each symbol represents one cytokine in the Box-Whisker Blot.

Figures 40A to **40C** show that co-stimulation with FAP targeted OX40 agonists suppresses the induction of FoxP3 on Treg cells by TGFβ. Human PBMC preparations containing naive CD4 T cells were cultured in the presence of TGFβ during T cell activation with antibodies against CD28 and CD3. OX40 agonism was provided through serial dilution rows of bispecific antigen binding molecules comprising OX40 clones OX40(CLC563) or OX40(49B4_K23E_K73E) in 3+1 and 4+1 formats as D and S variant. Crosslinking was provided by FAP antigen coated to beads. OX40 agonism interfered with Treg induction visible by reduced FoxP3 expression. Alive $CD4^+CD25^+$Treg singlet cells were gated and the MFI of the αFoxP3 antibody reported. The FoxP3 MFI of each concentration was corrected by the MFI of the sample without OX40 antibody, thus only TGBβ, present. **Fig. 40A** shows the effect of OX40(CLC563) 3+1 constructs**, Fig. 40B** for OX40(CLC563) 4+1 constructs and **Fig. 40C** for OX40(49B4_K23E_K73E) 4+1 constructs. The D and S variant of each FAP targeted OX40 bispecific antigen binding molecule suppressed FoxP3 to a similar range. Shown is the mean of triplicates, error bars represent the SEM.

Figure 41 shows the single dose plasma concentration-time profiles in Hu FcRn mice for the OX40(CLC563) x FAP(1G1a_EPKSCD) 3+1 antigen binding molecule (P1AE6454) and for the OX40(49B4) x FAP(4B9) 4+1 antigen binding molecule (P1AD4524).

Figures 42A and **42B** show the release of the cytokine IL-6 in human whole blood samples when incubated with FAP x OX40 bispecific antigen binding molecules. Shown is the median signal for each donor and different concentrations in relation to the signal of Erbitux® as negative comparator. In **Fig. 42A** the median signals for the bispecific antibodies C1, C2 und C3 (see Example 8.1.1) are shown, whereas the median signals for the bispecific antibodies C4, C5, C6 and C7 are shown in **Fig. 42B**.

Figures 43A and **43B** show the release of the cytokine IL-8 in human whole blood samples when incubated with FAP x OX40 bispecific antigen binding molecules. Shown is the median signal for each donor and different concentrations in relation to the signal of Erbitux® as negative comparator. In **Fig. 43A** the median signals for the bispecific antibodies C1, C2 und C3 (see Example 8.1.1 and in **Fig. 43B** the median signals for the bispecific antibodies C4, C5, C6 and C7 are shown.

Figures 44A and **44B** show the results of the DC:$CD4^+$ T cell assay evaluating the sequence-related risk of immunogenicity of the bispecific FAP x OX40 antibodies P1AF6454 and P1AF6455 in comparison to Keyhole limpet haemocyanin (KLH) as positive control and Bevacizumab (Avastin®). The IFNγ stimulation plotted against a stimulation index is shown in **Fig. 44A** and Fig. 44B gives the response rate overview in % responders.

Figures 45A and **45B** relate to the results of the DC:$CD4^+$ T cell assay evaluating P1AD4524 against Keyhole limpet haemocyanin (KLH) as positive control and Bevacizumab (Avastin®) and adalimumab (Humira®). The IFNγ stimulation plotted against a stimulation index is shown in **Fig. 45A** and **Fig. 45B** gives the response rate overview in % responders.

Figures 46, 47 and **48** show the results of a 4-week immunogenicity study in C57BL/6 wild type mice and in transgenic C57BL/6-Tg (hIgG1,k,l) mice that are immunologically tolerant to human IgG1 antibodies. The immune responses for the two individual mouse groups treated with P1AF6455 are shown in **Fig. 46**, those for the two individual mouse groups treated with P1AF6454 are shown in **Fig. 47**, and the immune responses obtained for the two individual mouse groups treated with P1AD4524 are illustrated in **Fig. 48**.

Figure 49 shows the study design of an efficacy study with bispecific FAP x OX40 antibodies (comparison of different OX40 clones) in combination with CEACAM5 TCB in MKN45 Xenograft in humanized mice. Shown is the design and the different treatment groups. Compared were bispecific FAP x OX40 antibodies comprising the antibodies OX40 (49B4_K23E_K73E, here called 49B4 CPV), OX40 (CLC563), OX40 (8H9) and OX40 (49B4).

Figures 50A and 50B show the results of the efficacy study with the FAP x OX40 bispecific antibodies in combination with CEACAM5 TCB in MKN45 Xenograft in humanized mice. Shown is the average tumor volume (**Fig. 50A**) or the percent change of tumor volume in individual mice for the different treatment groups as plotted on the y-axis (**Fig. 50B**).

DETAILED DESCRIPTION OF THE INVENTION

Definitions

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

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

As used herein, the term "**antigen binding domain capable of specific binding to a target cell antigen**" or "moiety capable of specific binding to a target cell antigen" refers to a polypeptide molecule that specifically binds to an antigenic determinant. In one aspect, the antigen binding domain is able to activate signaling through its target cell antigen. In a particular aspect, the antigen binding domain is able to direct the entity to which it is attached (e.g. the OX40 agonistic antibody) to a target site, for example to a specific type of tumor cell or tumor stroma bearing the antigenic determinant. Antigen binding domains capable of specific binding to a target cell antigen include antibodies and fragments thereof as further defined herein. In addition, antigen binding domains capable of specific binding to a target cell antigen include scaffold antigen binding proteins as further defined herein, e.g. binding domains which are based on designed repeat proteins or designed repeat domains (see e.g. WO 2002/020565). In particular, the antigen binding domain capable of specific binding to a target cell antigen is an antigen binding domain capable of specific binding to Fibroblast Activation Protein (FAP).

In relation to an antibody or fragment thereof, the term "antigen binding domain capable of specific binding to a target cell antigen" refers to the part of the molecule that comprises the area which specifically binds to and is complementary to part or all of an antigen. An antigen binding domain capable of specific antigen binding may be provided, for example, by one or more antibody variable domains (also called antibody variable regions). Particularly, an antigen binding domain capable of specific antigen binding comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH). In one particular aspect, the "antigen binding domain capable of specific binding to a target cell antigen" is a Fab fragment or a cross-Fab fragment.

The term "**antibody**" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal

antibodies, monospecific and multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

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

The term "**monospecific**" antibody as used herein denotes an antibody that has one or more binding sites each of which bind to the same epitope of the same antigen. The term "**bispecific**" means that the antigen binding molecule is able to specifically bind to at least two distinct antigenic determinants. 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 simultaneously binding two antigenic determinants, particularly two antigenic determinants expressed on two distinct cells. A bispecific antigen binding molecule as described herein can also form part of a multispecific antibody.

The term "**valent**" as used within the current application denotes the presence of a specified number of binding sites specific for one distinct antigenic determinant in an antigen binding molecule that are specific for one distinct antigenic determinant. As such, the terms "bivalent", "trivalent", "tetravalent", and "hexavalent" denote the presence of two binding sites, three binding sites, four binding sites, and six binding sites specific for a certain antigenic determinant, respectively, in an antigen binding molecule. In particular aspects of the invention, the bispecific antigen binding molecules according to the invention can be monovalent for a certain antigenic determinant, meaning that they have only one binding site for said antigenic determinant or they can be bivalent or tetravalent for a certain antigenic determinant, meaning that they have two binding sites or four binding sites, respectively, for said antigenic determinant.

The terms "full length antibody", "intact antibody", and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure. "**Native antibodies**" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG-class

antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two light chains and two heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3), also called a heavy chain constant region. Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a light chain constant domain (CL), also called a light 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. γ1 (IgG1), γ2 (IgG2), γ3 (IgG3), γ4 (IgG4), α1 (IgA1) and α2 (IgA2). The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

An "**antibody fragment**" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies, triabodies, tetrabodies, cross-Fab fragments; linear antibodies; single-chain antibody molecules (e.g. scFv); and single domain antibodies. For a review of certain antibody fragments, 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 fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific, see, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat Med 9, 129-134 (2003); and Hollinger et al., Proc Natl Acad Sci USA 90, 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat Med 9, 129-134 (2003). Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see e.g. U.S. Patent No. 6,248,516 B1). Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. E. coli or phage), as described herein.

Papain digestion of intact antibodies produces two identical antigen-binding fragments, called "Fab" fragments containing each the heavy- and light-chain variable domains and also the constant domain of the light chain and the first constant domain

(CH1) of the heavy chain. As used herein, Thus, the term "**Fab fragment**" refers to an antibody fragment comprising a light chain fragment comprising a VL domain and a constant domain of a light chain (CL), 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 wherein the cysteine residue(s) of the constant domains bear a free thiol group. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites (two Fab fragments) and a part of the Fc region. According to the present invention, the term "Fab fragment" also includes "cross-Fab fragments" or "crossover Fab fragments" as defined below.

The term "**cross-Fab fragment**" or "xFab fragment" or "crossover Fab fragment" refers to a Fab fragment, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged. Two different chain compositions of a crossover Fab molecule are possible and comprised in the bispecific antibodies of the invention: On the one hand, the variable regions of the Fab heavy and light chain are exchanged, i.e. the crossover Fab molecule comprises a peptide chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1), and a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL). This crossover Fab molecule is also referred to as CrossFab $_{(VLVH)}$. On the other hand, when the constant regions of the Fab heavy and light chain are exchanged, the crossover Fab molecule comprises a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL), and a peptide chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1). This crossover Fab molecule is also referred to as CrossFab $_{(CLCH1)}$.

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

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

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

"**Scaffold antigen binding proteins**" are known in the art, for example, fibronectin and designed ankyrin repeat proteins (DARPins) have been used as alternative scaffolds for antigen-binding domains, see, e.g., Gebauer and Skerra, Engineered protein scaffolds as next-generation antibody therapeutics. Curr Opin Chem Biol 13:245-255 (2009) and Stumpp et al., Darpins: A new generation of protein therapeutics. Drug Discovery Today 13: 695-701 (2008). In one aspect of the invention, a scaffold antigen binding protein is selected from the group consisting of CTLA-4 (Evibody), Lipocalins (Anticalin), a Protein A-derived molecule such as Z-domain of Protein A (Affibody), an A-domain (Avimer/Maxibody), a serum transferrin (*trans*-body); a designed ankyrin repeat protein (DARPin), a variable domain of antibody light chain or heavy chain (single-domain antibody, sdAb), a variable domain of antibody heavy chain (nanobody, aVH), V_{NAR} fragments, a fibronectin (AdNectin), a C-type lectin domain (Tetranectin); a variable domain of a new antigen receptor beta-lactamase (V_{NAR} fragments), a human gamma-crystallin or ubiquitin (Affilin molecules); a kunitz type domain of human protease

inhibitors, microbodies such as the proteins from the knottin family, peptide aptamers and fibronectin (adnectin). CTLA-4 (Cytotoxic T Lymphocyte-associated Antigen 4) is a CD28-family receptor expressed on mainly $CD4^+$ T-cells. Its extracellular domain has a variable domain- like Ig fold. Loops corresponding to CDRs of antibodies can be substituted with heterologous sequence to confer different binding properties. CTLA-4 molecules engineered to have different binding specificities are also known as Evibodies (e.g. US7166697B1). Evibodies are around the same size as the isolated variable region of an antibody (e.g. a domain antibody). For further details see Journal of Immunological Methods 248 (1-2), 31-45 (2001). Lipocalins are a family of extracellular proteins which transport small hydrophobic molecules such as steroids, bilins, retinoids and lipids. They have a rigid beta-sheet secondary structure with a number of loops at the open end of the conical structure which can be engineered to bind to different target antigens. Anticalins are between 160-180 amino acids in size, and are derived from lipocalins. For further details see Biochim Biophys Acta 1482: 337-350 (2000), US7250297B1 and US20070224633. An affibody is a scaffold derived from Protein A of Staphylococcus aureus which can be engineered to bind to antigen. The domain consists of a three-helical bundle of approximately 58 amino acids. Libraries have been generated by randomization of surface residues. For further details see Protein Eng. Des. Sel. 2004, 17, 455-462 and EP 1641818A1. Avimers are multidomain proteins derived from the A-domain scaffold family. The native domains of approximately 35 amino acids adopt a defined disulfide bonded structure. Diversity is generated by shuffling of the natural variation exhibited by the family of A-domains. For further details see Nature Biotechnology 23(12), 1556 - 1561 (2005) and Expert Opinion on Investigational Drugs 16(6), 909-917 (June 2007). A transferrin is a monomeric serum transport glycoprotein. Transferrins can be engineered to bind different target antigens by insertion of peptide sequences in a permissive surface loop. Examples of engineered transferrin scaffolds include the Trans-body. For further details see J. Biol. Chem 274, 24066-24073 (1999). Designed Ankyrin Repeat Proteins (DARPins) are derived from Ankyrin which is a family of proteins that mediate attachment of integral membrane proteins to the cytoskeleton. A single ankyrin repeat is a 33 residue motif consisting of two alpha-helices and a beta-turn. They can be engineered to bind different target antigens by randomizing residues in the first alpha-helix and a beta-turn of each repeat. Their binding interface can be increased by increasing the number of modules (a method of affinity maturation). For further details see J. Mol. Biol. 332, 489-503 (2003), PNAS 100(4), 1700-1705 (2003) and J. Mol. Biol. 369, 1015-1028 (2007) and US20040132028A1. A single-domain antibody is an antibody fragment consisting of a single monomeric variable antibody domain. The first single domains were derived from the variable domain of the antibody heavy chain from camelids (nanobodies or V_HH fragments). Furthermore, the term single-domain antibody includes an autonomous human

heavy chain variable domain (aVH) or V_{NAR} fragments derived from sharks. Fibronectin is a scaffold which can be engineered to bind to antigen. Adnectins consists of a backbone of the natural amino acid sequence of the 10th domain of the 15 repeating units of human fibronectin type III (FN3). Three loops at one end of the .beta.-sandwich can be engineered to enable an Adnectin to specifically recognize a therapeutic target of interest. For further details see Protein Eng. Des. Sel. 18, 435- 444 (2005), US20080139791, WO2005056764 and US6818418B1. Peptide aptamers are combinatorial recognition molecules that consist of a constant scaffold protein, typically thioredoxin (TrxA) which contains a constrained variable peptide loop inserted at the active site. For further details see Expert Opin. Biol. Ther. 5, 783-797 (2005). Microbodies are derived from naturally occurring microproteins of 25-50 amino acids in length which contain 3-4 cysteine bridges - examples of microproteins include KalataBI and conotoxin and knottins. The microproteins have a loop which can beengineered to include upto 25 amino acids without affecting the overall fold of the microprotein. For further details of engineered knottin domains, see WO2008098796.

An "**antigen binding molecule that binds to the same epitope**" as a reference molecule refers to an antigen binding molecule that blocks binding of the reference molecule to its antigen in a competition assay by 50% or more, and conversely, the reference molecule blocks binding of the antigen binding molecule to its antigen in a competition assay by 50% or more. An "**antigen binding molecule that does not bind to the same epitope**" as a reference molecule refers to an antigen binding molecule that does not block binding of the reference molecule to its antigen in a competition assay by 50% or more, and conversely, the reference molecule does not block binding of the antigen binding molecule to its antigen in a competition assay by 50% or more.

The term "**antigen binding domain**" or "antigen-binding site" refers to the part of an antigen binding molecule 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) 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 (Kd) of $\leq$ 1 μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 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 (Kd), which is the ratio of dissociation and association rate constants (koff and kon, 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).

An "**affinity matured**" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does

not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

A "**target cell antigen**" as used herein refers to an antigenic determinant presented on the surface of a target cell, in particular a target cell in a tumor such as a cancer cell or a cell of the tumor stroma. Thus, the target cell antigen is a tumor-associated antigen. In particular, the tumor target cell antigen is Fibroblast Activation Protein (FAP).

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 that 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:2), 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: 62. The amino acid sequence of mouse FAP is shown in UniProt accession no. P97321 (version 126, SEQ ID NO:63), or NCBI RefSeq NP_032012.1. The extracellular domain (ECD) of mouse FAP extends from amino acid position 26 to 761. SEQ ID NO:64 shows the amino acid of a His-tagged mouse FAP ECD. SEQ ID NO:65 shows the amino acid of a His-tagged cynomolgus FAP ECD. Preferably, an anti-FAP binding molecule of the invention binds to the extracellular domain of FAP.

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 which determine antigen binding specificity, for example "**complementarity determining**

regions" ("CDRs"). Generally, antibodies comprise six CDRs: three in the VH (CDR-H1, CDR-H2, CDR-H3), and three in the VL (CDR-L1, CDR-L2, CDR-L3). Exemplary CDRs herein include:

(a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));

(b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991)); and

(c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996)).

Unless otherwise indicated, the CDRs are determined according to Kabat et al., *supra*. One of skill in the art will understand that the CDR designations can also be determined according to Chothia, *supra*, McCallum, *supra*, or any other scientifically accepted nomenclature system.

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

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. IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. 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 CDRs 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 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.

The term "**CH1 domain**" denotes the part of an antibody heavy chain polypeptide that extends approximately from EU position 118 to EU position 215 (EU numbering system according to Kabat). In one aspect, a CH1 domain has the amino acid sequence of ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKV (SEQ ID NO: 166). Usually, a segment having the amino acid sequence of EPKSC (SEQ ID NO:163) is following to link the CH1 domain to the hinge region. The inventors found that a CH1 domain that is not fused to a hinge region may lead to reactivity with pre-existing antibodies (ADAs) in the human body which are not present if a variant EPKSCD (SEQ ID NO:164) or EPKSCS (SEQ ID NO:165) is present. A CH1 domain with a free C-terminal end can be found for instance in a crossfab fragment.

The term "**hinge region**" denotes the part of an antibody heavy chain polypeptide that joins in a wild-type antibody heavy chain the CH1 domain and the CH2 domain, e. g. from about position 216 to about position 230 according to the EU number system of Kabat, or from about position 226 to about position 230 according to the EU number system of Kabat. The hinge regions of other IgG subclasses can be determined by aligning with the hinge-region cysteine residues of the IgG1 subclass sequence. The hinge region is normally a dimeric molecule consisting of two polypeptides with identical amino acid sequence. The hinge region generally comprises up to 25 amino acid residues and is flexible allowing the associated target binding sites to move independently. The hinge region can be subdivided into three domains: the upper, the middle, and the lower hinge domain (see e.g. Roux, et al., J. Immunol. 161 (1998) 4083). In one aspect, the hinge region has the amino acid sequence DKTHTCPXCP (SEQ ID NO: 160), wherein X is either S or P. In one aspect, the hinge region has the amino acid sequence HTCPXCP (SEQ ID NO: 161), wherein X is either S or P. In one aspect, the hinge region has the amino acid sequence CPXCP (SEQ ID NO: 162), wherein X is either S or P.

The term "Fc domain" or "**Fc region**" herein is used to define a C-terminal region of an 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. (EU numbering system according to Kabat). In one aspect, a CH2 domain has the amino acid sequence of APELLGGPSV FLFPPKPKDT LMISRTPEVT CVWDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQESTYRW SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAK (SEQ ID NO: 153). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native Fc-region. It has been speculated that the carbohydrate may provide a substitute for the domain-domain pairing and help stabilize the CH2 domain. Burton, Mol. Immunol. 22 (1985) 161-206. In one embodiment, a carbohydrate chain is attached to the CH2 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 according to EU numbering system according to Kabat of an IgG). In one aspect, the CH3 domain has the amino acid sequence of GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPG (SEQ ID NO: 154). 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 term "**wild-type Fc domain**" denotes an amino acid sequence identical to the amino acid sequence of an Fc domain found in nature. Wild-type human Fc domains include a native human IgG1 Fc-region (non-A and A allotypes), native human IgG2 Fc-region, native human IgG3 Fc-region, and native human IgG4 Fc-region as well as naturally occurring variants thereof. Wild-type Fc-regions are denoted in SEQ ID NO: 155 (IgG1, caucasian allotype), SEQ ID NO: 156 (IgG1, afroamerican allotype), SEQ ID NO: 157 (IgG2), SEQ ID NO:158 (IgG3) and SEQ ID NO:159 (IgG4). The term "**variant**

(human) Fc domain" denotes an amino acid sequence which differs from that of a "wild-type" (human) Fc domain amino acid sequence by virtue of at least one "amino acid mutation". In one aspect, the variant Fc-region has at least one amino acid mutation compared to a native Fc-region, e.g. from about one to about ten amino acid mutations, and in one aspect from about one to about five amino acid mutations in a native Fc-region. In one aspect, the (variant) Fc-region has at least about 95 % homology with a wild-type Fc-region.

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

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

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

- FcγRI (CD64) binds monomeric IgG with high affinity and is expressed on macrophages, monocytes, neutrophils and eosinophils. Modification in the Fc-region IgG at least at one of the amino acid residues E233-G236, P238, D265, N297, A327 and P329 (numbering according to EU index of Kabat) reduce binding to FcγRI. IgG2 residues at positions 233–236, substituted into IgG1 and IgG4, reduced binding to FcγRI by 10^3-fold and eliminated the human monocyte response to antibody-sensitized red blood cells (Armour, K.L., et al., Eur. J. Immunol. 29 (1999) 2613–2624).

-FcγRII (CD32) binds complexed IgG with medium to low affinity and is widely expressed. This receptor can be divided into two sub-types, FcγRIIA and FcγRIIB. FcγRIIA is found on many cells involved in killing (e.g. macrophages, monocytes, neutrophils) and seems able to activate the killing process. FcγRIIB seems to play a role in inhibitory processes and is found on B cells, macrophages and on mast cells and

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

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

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

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

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

The term "**OX40**", as used herein, refers to any native OX40 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed OX40 as well as any form of OX40 that results from processing in the cell. The term also encompasses naturally occurring variants of OX40, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human OX40 is shown in SEQ ID NO: 1

(Uniprot P43489, version 112) and the amino acid sequence of an exemplary murine OX40 is shown in SEQ ID NO: 66 (Uniprot P47741, version 101).

The term "**OX40 agonist**" as used herein includes any moiety that agonizes the OX40/OX40L interaction. OX40 as used in this context refers preferably to human OX40, thus the OX40 agonist is preferably an agonist of human OX40. Typically, the moiety will be an agonistic OX40 antibody or antibody fragment, in particular a Fab fragment.

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

The term "**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 GGGGS (SEQ ID NO:67), GGGGSGGGGS (SEQ ID NO:68), SGGGGSGGGG (SEQ ID NO:69) and GGGGSGGGGSGGGG (SEQ ID NO:70), but also include the sequences GSPGSSSSGS (SEQ ID NO:71), $(G4S)_3$ (SEQ ID NO:72), $(G4S)_4$ (SEQ ID NO:73), GSGSGSGS (SEQ ID NO:74), GSGSGNGS (SEQ ID NO:75), GGSGSGSG (SEQ ID NO:76), GGSGSG (SEQ ID NO:77), GGSG (SEQ ID NO:78), GGSGNGSG (SEQ ID NO:79), GGNGSGSG (SEQ ID NO:80) and GGNGSG (SEQ ID NO:81). Peptide linkers of particular interest are (G4S) (SEQ ID NO:67), $(G_4S)_2$ or GGGGSGGGGS (SEQ ID NO:68), $(G4S)_3$ (SEQ ID NO:72) and $(G4S)_4$ (SEQ ID NO:73).

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

By "**fused**" or "**connected**" is meant that the components (e.g. a heavy chain of an antibody and a Fab fragment) 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 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 bispecific 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 TNF ligand trimer-containing antigen binding molecules. Amino acid sequence variants of the TNF ligand trimer-containing antigen binding molecules may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the molecules, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding. Sites of interest for substitutional mutagenesis include the HVRs and Framework (FRs). Conservative substitutions are provided in Table B under the heading "Preferred Substitutions" and further described below in reference to amino acid side chain classes (1) to (6). Amino acid substitutions may be introduced into the molecule of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE A

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

Original Residue	Exemplary Substitutions	Preferred Substitutions
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) 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 HVR residues are mutated and the variant antigen binding molecules displayed on phage and screened for a particular biological activity (e.g. binding affinity). In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antigen binding molecule to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. A useful method for identification of residues or regions of an antibody

that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antigen binding molecule complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

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

In certain embodiments, the bispecific antigen binding molecules provided herein are altered to increase or decrease the extent to which the antibody is glycosylated. Glycosylation variants of the molecules may be conveniently obtained by altering the amino acid sequence such that one or more glycosylation sites is created or removed. Where the antigen binding molecule comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the “stem” of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in TNF family ligand trimer-containing antigen binding molecule may be made in order to create variants with certain improved properties. In one aspect, variants of bispecific antigen binding molecules or antibodies of the invention are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. Such fucosylation variants may have improved ADCC function, see e.g. US Patent Publication Nos. US 2003/0157108 (Presta, L.) or US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). In another aspect, variants of the bispecific antigen binding molecules or antibodies of the invention are provided with

bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region is bisected by GlcNAc. Such variants may have reduced fucosylation and/or improved ADCC function., see for example WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function and are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

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

The term "nucleic acid" or "**polynucleotide**" includes any compound and/or substance that comprises a polymer of nucleotides. Each nucleotide is composed of a base, specifically a purine- or pyrimidine base (i.e. cytosine (C), guanine (G), adenine (A), thymine (T) or uracil (U)), a sugar (i.e. deoxyribose or ribose), and a phosphate group. Often, the nucleic acid molecule is described by the sequence of bases, whereby said bases represent the primary structure (linear structure) of a nucleic acid molecule. The sequence of bases is typically represented from 5' to 3'. Herein, the term nucleic acid molecule encompasses deoxyribonucleic acid (DNA) including e.g., complementary DNA (cDNA) and genomic DNA, ribonucleic acid (RNA), in particular messenger RNA (mRNA), synthetic forms of DNA or RNA, and mixed polymers comprising two or more of these molecules. The nucleic acid molecule may be linear or circular. In addition, the term nucleic acid molecule includes both, sense and antisense strands, as well as single stranded and double stranded forms. Moreover, the herein described nucleic acid molecule can contain naturally occurring or non-naturally occurring nucleotides. Examples of non-naturally occurring nucleotides include modified nucleotide bases with derivatized sugars or phosphate backbone linkages or chemically modified residues. Nucleic acid molecules also encompass DNA and RNA molecules which are suitable as a vector for direct expression of an antibody of the invention in vitro and/or in vivo, e.g., in a host or patient.

Such DNA (e.g., cDNA) or RNA (e.g., mRNA) vectors, can be unmodified or modified. For example, mRNA can be chemically modified to enhance the stability of the RNA vector and/or expression of the encoded molecule so that mRNA can be injected into a subject to generate the antibody in vivo (see e.g., Stadler ert al, Nature Medicine 2017, published online 12 June 2017, doi:10.1038/nm.4356 or EP 2 101 823 B1).

An "**isolated**" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

"**Isolated nucleic acid encoding a bispecific antigen binding molecule or antibody**" refers to one or more nucleic acid molecules encoding the heavy and light chains (or fragments thereof) of the bispecific antigen binding molecule or antibody, including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

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

The term "**expression cassette**" refers to a polynucleotide generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be

incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among 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.

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.

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.

The term "**pharmaceutical composition**" or "pharmaceutical formulation" 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 pharmaceutical composition would be administered.

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

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

As used herein, "**treatment**" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, the molecules of the invention are used to delay development of a disease or to slow the progression of a disease.

The term "**cancer**" as used herein refers to proliferative diseases, such as lymphomas, lymphocytic leukemias, lung cancer, non-small cell lung (NSCL) cancer, bronchioloalviolar cell lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, gastric cancer, colon cancer, breast

cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, neoplasms of the central nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma multiforme, astrocytomas, schwanomas, ependymonas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenoma and Ewings sarcoma, including refractory versions of any of the above cancers, or a combination of one or more of the above cancers.

The term "**chemotherapeutic agent**" as used herein refers to a chemical compound useful in the treatment of cancer. In one aspect, the chemotherapeutic agent is an antimetabolite. In one aspect, the antimetabolite is selected from the group consisting of Aminopterin, Methotrexate, Pemetrexed, Raltitrexed, Cladribine, Clofarabine, Fludarabine, Mercaptopurine, Pentostatin, Thioguanine, Capecitabine, Cytarabine, Fluorouracil, Floxuridine, and Gemcitabine. In one particular aspect, the antimetabolite is capecitabine or gemcitabine. In another aspect, the antimetabolite is fluorouracil. In one aspect, the chemotherapeutic agent is an agent that affects microtubule formation. In one aspect, the agent that affects microtubule formation is selected from the group consisting of: paclitaxel, docetaxel, vincristine, vinblastine, vindesine, vinorelbin, taxotere, etoposide, and teniposide. In another aspect, the chemotherapeutic agent is an alkylating agent such as cyclophosphamide. In one aspect, the chemotherapeutic agent is a cytotoxic antibiotic such as a topoisomerase II inhibitor. In one aspect, the topoisomerase II inhibitor is doxorubicin.

Bispecific antibodies of the invention

The invention provides novel bispecific antigen binding molecules comprising a new anti-FAP antibody (clone 212). The bispecific antigen binding molecules comprising this new anti-FAP antibody possess particularly advantageous properties such as producibility, stability, binding affinity, biological activity, targeting efficiency, reduced internalization, superior pharmacokinetic (PK) properties such as improved clearance, reduced toxicity, an extended dosage range that can be given to a patient and thereby a possibly enhanced efficacy. In addition, the bispecific antigen binding molecules have been prepared in advantageous formats depending on the OX40 antibody included.

Exemplary bispecific antigen binding molecules

In one aspect, the invention provides bispecific antigen binding molecules that are characterized by targeted agonistic binding to OX40. In particular, the bispecific antigen binding molecule is an OX40 agonist that is targeted against FAP. In another particular aspect, the bispecific antigen binding molecules of the invention comprise a Fc region composed of a first and a second subunit capable of stable association which comprises mutations that reduce effector function. The use of an Fc region comprising mutations that reduce or abolish effector function will prevent unspecific agonism by crosslinking via Fc receptors and will prevent ADCC of $OX40^+$ cells. The bispecific antigen binding molecules as described herein possess the advantage over conventional antibodies capable of specific binding to OX40 in that they selectively induce immune response at the target cells, which are typically close to the tumor, i.e. in the tumor stroma.

The bispecific antigen binding molecules are thus characterized by FAP-targeted agonistic binding to OX40. In the presence of FAP-expressing cells the bispecific antigen binding molecules are able to induce NFκB activation in human OX40 positive NFκB reporter cells.

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

In one aspect, the antigen binding domain capable of specific binding to Fibroblast Activation Protein (FAP) comprises a heavy chain variable region (V_HFAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, (ii) CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:5, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence

selected from the group consisting of SEQ ID NO:6, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:7, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8.

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

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

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

In one aspect, the antigen binding domains capable of specific binding to OX40 bind to a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NO:1.

In a further aspect, provided is a bispecific antigen binding molecule, wherein the antigen binding domain capable of specific binding to OX40 comprises
(a) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:27, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:28, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:29, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:30, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:32, or
(b) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:35, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:36, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:37, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:38, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:39, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:40, or
(c) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:43, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:44, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:45, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:47, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:48, or
(d) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:51, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:52, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:53, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:54, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:55, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:56.

In one aspect, the antigen binding domains capable of specific binding to OX40 comprise a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:27, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:28, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:29, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:30, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:32. In a further aspect, the antigen binding domains capable of specific binding to OX40 comprise a heavy chain variable region (V_HOX40) comprising (i) CDR-H1

comprising the amino acid sequence of SEQ ID NO:35, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:36, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:37, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:38, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:39, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:40. In another aspect, the antigen binding domains capable of specific binding to OX40 comprise a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:43, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:44, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:45, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:47, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:48. In yet another aspect, the antigen binding domains capable of specific binding to OX40 comprise a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:51, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:52, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:53, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:54, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:55, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:56. In one particular aspect, the antigen binding domains capable of specific binding to OX40 comprise a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:35, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:36, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:37, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:38, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:39, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:40.

In one aspect, provided is a bispecific antigen binding molecule as defined herein before, wherein the antigen binding domains capable of specific binding to OX40 comprise

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

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

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

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

In one aspect, provided is a bispecific antigen binding molecule as defined herein before, wherein the antigen binding domain capable of specific binding to OX40 comprises a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:33 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34. In another aspect, provided is a bispecific antigen binding molecule as defined herein before, wherein the antigen binding domain capable of specific binding to OX40 comprises a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:41 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:42. In a further aspect, provided is a bispecific antigen binding molecule as defined herein before, wherein the antigen binding domain capable of specific binding to OX40 comprises a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:49 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:50. In yet as further aspect, provided is a bispecific antigen binding molecule as defined herein before, wherein the antigen binding domain capable of specific binding to OX40 comprises a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:57 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:58. In one particular aspect, the antigen binding domains capable of specific binding to OX40 (each) comprise a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:41 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:42.

In another aspect, provided is a bispecific antigen binding molecule as defined herein before, the antigen binding domains capable of specific binding to OX40 comprise

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

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

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

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

Bispecific antigen binding molecules binding to OX40 and FAP

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

(a) at least two antigen binding domains capable of specific binding to OX40, comprising a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:27, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:28, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:29, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:30, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:32,
(b) an antigen binding domain capable of specific binding to Fibroblast Activation Protein (FAP) comprising a heavy chain variable region (V_HFAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, (ii) CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:11 and SEQ ID NO:12, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:5, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:13 and SEQ ID NO:14, (v) CDR-L2 comprising the amino acid

sequence of SEQ ID NO:7, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and

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

In a further aspect, provided is a bispecific antigen binding molecule, comprising

(a) at least two antigen binding domains capable of specific binding to OX40, comprising a heavy chain variable region (V_HOX40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:33, SEQ ID NO:59, SEQ ID NO:60 and SEQ ID NO:61, and a light chain variable region (V_LOX40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:34,

(b) at least one antigen binding domain capable of specific binding to FAP, comprising a heavy chain variable region (V_HFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18. SEQ ID NO:19 and SEQ ID NO:20, and a light chain variable region (V_LFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 and SEQ ID NO:26, and

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

In a particular aspect, provided is a bispecific antigen binding molecule, comprising

(a) at least two antigen binding domains capable of specific binding to OX40, comprising a heavy chain variable region (V_HOX40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:59, SEQ ID NO:60 and SEQ ID NO:61, and a light chain variable region (V_LOX40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:34,

(b) at least one antigen binding domain capable of specific binding to FAP, comprising a heavy chain variable region (V_HFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:15, and a light chain variable region (V_LFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:21, and

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

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

(a) at least two antigen binding domains capable of specific binding to OX40, comprising a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:35, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:36, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:37, and a

light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:38, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:39, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:40,
(b) an antigen binding domain capable of specific binding to Fibroblast Activation Protein (FAP) comprising a heavy chain variable region (V_HFAP) comprising
(i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, (ii) CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:11 and SEQ ID NO:12, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:5, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:13 and SEQ ID NO:14, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:7, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and
(c) a Fc region composed of a first and a second subunit capable of stable association.

In a further aspect, provided is a bispecific antigen binding molecule, comprising

(a) at least two antigen binding domains capable of specific binding to OX40, comprising a heavy chain variable region (V_HOX40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:41, and a light chain variable region (V_LOX40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:42,

(b) at least one antigen binding domain capable of specific binding to FAP, comprising a heavy chain variable region (V_HFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18. SEQ ID NO:19 and SEQ ID NO:20, and a light chain variable region (V_LFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 and SEQ ID NO:26, and

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

In a particular aspect, provided is a bispecific antigen binding molecule, comprising

(a) at least two antigen binding domains capable of specific binding to OX40, comprising a heavy chain variable region (V_HOX40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:41 and a light chain variable region (V_LOX40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:42,

(b) at least one antigen binding domain capable of specific binding to FAP, comprising a heavy chain variable region (V_HFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:15, and a light chain variable region (V_LFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:21, and

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

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

(a) at least two antigen binding domains capable of specific binding to OX40, comprising a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:43, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:44, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:45, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:47, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:48,
(b) an antigen binding domain capable of specific binding to Fibroblast Activation Protein (FAP) comprising a heavy chain variable region (V_HFAP) comprising
(i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, (ii) CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:11 and SEQ ID NO:12, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:5, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:13 and SEQ ID NO:14, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:7, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and
(c) a Fc region composed of a first and a second subunit capable of stable association.

In a further aspect, provided is a bispecific antigen binding molecule, comprising

(a) at least two antigen binding domains capable of specific binding to OX40, comprising a heavy chain variable region (V_HOX40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:49, and a light chain variable region (V_LOX40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:50,

(b) at least one antigen binding domain capable of specific binding to FAP, comprising a heavy chain variable region (V_HFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18. SEQ ID NO:19 and SEQ ID NO:20, and a light chain variable region

(V_LFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 and SEQ ID NO:26, and

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

In a particular aspect, provided is a bispecific antigen binding molecule, comprising

(a) at least two antigen binding domains capable of specific binding to OX40, comprising a heavy chain variable region (V_HOX40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:49 and a light chain variable region (V_LOX40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:50,

(b) at least one antigen binding domain capable of specific binding to FAP, comprising a heavy chain variable region (V_HFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:15, and a light chain variable region (V_LFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:21, and

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

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

(a) at least two antigen binding domains capable of specific binding to OX40, comprising a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:51, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:52, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:53, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:54, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:55, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:56,

(b) an antigen binding domain capable of specific binding to Fibroblast Activation Protein (FAP) comprising a heavy chain variable region (V_HFAP) comprising
(i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, (ii) CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:11 and SEQ ID NO:12, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:5, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:13 and SEQ ID NO:14, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:7, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and

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

In a further aspect, provided is a bispecific antigen binding molecule, comprising

(a) at least two antigen binding domains capable of specific binding to OX40, comprising a heavy chain variable region (V_HOX40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:57, and a light chain variable region (V_LOX40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:58,

(b) at least one antigen binding domain capable of specific binding to FAP, comprising a heavy chain variable region (V_HFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18. SEQ ID NO:19 and SEQ ID NO:20, and a light chain variable region (V_LFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 and SEQ ID NO:26, and

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

In a particular aspect, provided is a bispecific antigen binding molecule, comprising

(a) at least two antigen binding domains capable of specific binding to OX40, comprising a heavy chain variable region (V_HOX40) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:57 and a light chain variable region (V_LOX40) comprising the amino acid sequence selected from the group consisting of SEQ ID NO:58,

(b) at least one antigen binding domain capable of specific binding to FAP, comprising a heavy chain variable region (V_HFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:15, and a light chain variable region (V_LFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:21, and

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

Bispecific antigen binding molecules bivalent for binding to OX40 and monovalent for binding to FAP (2+1 format)

In another aspect, the invention provides a bispecific antigen binding molecule comprising

(a) two Fab fragments capable of specific binding to OX40,

(b) one cross-Fab fragment capable of specific binding to FAP comprising a heavy chain variable region (V_HFAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, (ii) CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:11 and SEQ ID NO:12, and (iii) CDR-H3

comprising the amino acid sequence of SEQ ID NO:5, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:13 and SEQ ID NO:14, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:7, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and
(c) a Fc domain composed of a first and a second subunit capable of stable association.

Thus, provided is a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule binds bivalently to OX40 and monovalently to FAP.

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

(a) two heavy chains, each heavy chain comprising a VH and CH1 domain of a Fab fragment capable of specific binding to OX40 and a Fc region subunit,

(b) two light chains, each light chain comprising a VL and CL domain of a Fab fragment capable of specific binding to OX40, and

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

In one aspect, the VH-CL (VH-Ckappa) chain is connected to the C-terminus of the Fc knob heavy chain. In one aspect, the VH-Ckappa chain is connected to the C-terminus of an Fc knob heavy chain comprising the amino acid substitutions S354C and T366W (numbering according to Kabat EU index).

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

(a) two heavy chains, each heavy chain comprising a VH and CH1 domain of a Fab fragment capable of specific binding to OX40 and a Fc region subunit,

(b) two light chains, each light chain comprising a VL and CL domain of a Fab fragment capable of specific binding to OX40, and

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

In one aspect, the VL-CH1 chain is connected to the C-terminus of the Fc knob heavy chain. In one aspect, the VL-CH1 chain is connected to the C-terminus of an Fc knob heavy chain comprising the amino acid substitutions S354C and T366W (numbering according to Kabat EU index).

In one aspect, the invention provides a bispecific antigen binding molecule comprising
(a) two light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:88, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:91, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:90, or
(b) two light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:94, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:91, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:90, or
(c) two light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:96, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:91, and a second heavy chain an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:90. In one aspect, the invention provides a bispecific antigen binding molecule comprising (a) two light chains, each comprising the amino acid sequence of SEQ ID NO:87, one light chain comprising the amino acid sequence of SEQ ID NO:88, a first heavy chain comprising the amino acid sequence of SEQ ID NO:91, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:90, or (b) two light chains, each comprising the amino acid sequence of SEQ ID NO:87, one light chain comprising the amino acid sequence of SEQ ID NO:94, a first heavy chain comprising the amino acid sequence of SEQ ID NO:91, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:90, or (c) two light chains, each comprising the amino acid sequence of SEQ ID NO:87, one light chain comprising the amino acid sequence of SEQ ID NO:96, a first heavy chain comprising the amino acid sequence of SEQ ID NO:91, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:90.

In another aspect, the invention provides a bispecific antigen binding molecule comprising

(a) two light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:93, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:88, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:97, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:98, or

(b) two light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:93, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:94, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:97, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:98, or

(c) two light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:93, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:96, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:97, and a second heavy chain an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:98. In one aspect, the invention provides a bispecific antigen binding molecule comprising (a) two light chains, each comprising the amino acid sequence of SEQ ID NO:93, one light chain comprising the amino acid sequence of SEQ ID NO:88, a first heavy chain comprising the amino acid sequence of SEQ ID NO:97, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:98, or (b) two light chains, each comprising the amino acid sequence of SEQ ID NO:93, one light chain comprising the amino acid sequence of SEQ ID NO:94, a first heavy chain comprising the amino acid sequence of SEQ ID NO:97, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:98, or (c) two light chains, each comprising the amino acid sequence of SEQ ID NO:93, one light chain comprising the amino acid sequence of SEQ ID NO:96, a first heavy chain comprising the amino acid sequence of SEQ ID NO:97, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:98.

In another aspect, the invention provides a bispecific antigen binding molecule comprising

(a) two light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:100, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:88, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:99, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:102, or

(b) two light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:100, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:94, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:99, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:102, or

(c) two light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:100, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:96, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:99, and a second heavy chain an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:102. In one aspect, the invention provides a bispecific antigen binding molecule comprising (a) two light chains, each comprising the amino acid sequence of SEQ ID NO:100, one light chain comprising the amino acid sequence of SEQ ID NO:88, a first heavy chain comprising the amino acid sequence of SEQ ID NO:99, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:102, or (b) two light chains, each comprising the amino acid sequence of SEQ ID NO:100, one light chain comprising the amino acid sequence of SEQ ID NO:94, a first heavy chain comprising the amino acid sequence of SEQ ID NO:99, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:102, or (c) two light chains, each comprising the amino acid sequence of SEQ ID NO:100, one light chain comprising the amino acid sequence of SEQ ID NO:96, a first heavy chain comprising the amino acid sequence of SEQ ID NO:99, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:102.

In another aspect, the invention provides a bispecific antigen binding molecule comprising

(a) two light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:104, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:88, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:103, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:106, or

(b) two light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:104, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:94, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:103, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:106, or

(c) two light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:104, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:96, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:103, and a second heavy chain an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:106. In one aspect, the invention provides a bispecific antigen binding molecule comprising (a) two light chains, each comprising the amino acid sequence of SEQ ID NO:104, one light chain comprising the amino acid sequence of SEQ ID NO:88, a first heavy chain comprising the amino acid sequence of SEQ ID NO:103, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:106, or (b) two light chains, each comprising the amino acid sequence of SEQ ID NO:104, one light chain comprising the amino acid sequence of SEQ ID NO:94, a first heavy chain comprising the amino acid sequence of SEQ ID NO:103, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:106, or (c) two light chains, each comprising the amino acid sequence of SEQ ID NO:104, one light chain comprising the amino acid sequence of SEQ ID NO:96, a first heavy chain comprising the amino acid sequence of SEQ ID NO:103, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:106.

Bispecific antigen binding molecules trivalent for binding to OX40 and monovalent for binding to FAP (3+1 format)

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

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

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

(ac) a third Fab fragment capable of specific binding to OX40,

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

(c) the Fc region composed of a first and a second subunit capable of stable association, wherein the second Fab fragment (ab) is fused at the C-terminus of the VH-CH1 chain to the N-terminus of the VH-CH1 chain of the first Fab fragment (aa), which is in turn fused at its C-terminus to the N-terminus of the first subunit, and the third Fab fragment (ac) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit.

In one aspect, the bispecific antigen binding molecule consists of

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

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

(ac) a third Fab fragment capable of specific binding to OX40,

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

(c) the Fc region composed of a first and a second subunit capable of stable association, wherein the second Fab fragment (ab) is fused at the C-terminus of the VH-CH1 chain to the N-terminus of the VH-CH1 chain of the first Fab fragment (aa), which is in turn fused at its C-terminus to the N-terminus of the first subunit, and the third Fab fragment (ac) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit.

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

(a) a heavy chain comprising a VH-CH1 chain of a first Fab fragment capable of specific binding to OX40 fused at its N-terminus to the VH-CH1 chain of a second Fab fragment capable of specific binding to OX40, optionally via a peptide linker, and a Fc region subunit,

(b) a heavy chain comprising a VH-CH1 domain of a Fab fragment capable of specific binding to OX40, a Fc region subunit, and a VH-CL chain of a Fab fragment

capable of specific binding to FAP fused to the C-terminus of the Fc region subunit, optionally via a peptide linker,

(c) three light chains, each light chain comprising a VL and CL domain of a Fab fragment capable of specific binding to OX40, and

(d) a light chain comprising a VL and CH1 domain of a Fab fragment capable of specific binding to FAP.

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

(a) a heavy chain comprising a VH-CH1 chain of a first Fab fragment capable of specific binding to OX40 fused at its N-terminus to the VH-CH1 chain of a second Fab fragment capable of specific binding to OX40, optionally via a peptide linker, a Fc region subunit, and a VH-CL chain of a Fab fragment capable of specific binding to FAP fused to the C-terminus of the Fc region subunit, optionally via a peptide linker,

(b) a heavy chain comprising a VH-CH1 domain of a Fab fragment capable of specific binding to OX40 and a Fc region subunit,

(c) three light chains, each light chain comprising a VL and CL domain of a Fab fragment capable of specific binding to OX40, and

(d) a light chain comprising a VL and CH1 domain of a Fab fragment capable of specific binding to FAP.

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

(a) a heavy chain comprising a VH-CH1 chain of a first Fab fragment capable of specific binding to OX40 fused at its N-terminus to the VH-CH1 chain of a second Fab fragment capable of specific binding to OX40, optionally via a peptide linker, and a Fc region subunit,

(b) a heavy chain comprising a VH-CH1 domain of a Fab fragment capable of specific binding to OX40, a Fc region subunit, and a VL-CH1 chain of a Fab fragment capable of specific binding to FAP fused to the C-terminus of the Fc region subunit, optionally via a peptide linker,

(c) three light chains, each light chain comprising a VL and CL domain of a Fab fragment capable of specific binding to OX40, and

(d) a light chain comprising a VH and CL domain of a Fab fragment capable of specific binding to FAP.

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

(a) a heavy chain comprising a VH-CH1 chain of a first Fab fragment capable of specific binding to OX40 fused at its N-terminus to the VH-CH1 chain of a second Fab fragment capable of specific binding to OX40, optionally via a peptide linker, a Fc region subunit, and a VL-CH1 chain of a Fab fragment capable of specific binding to FAP fused to the C-terminus of the Fc region subunit, optionally via a peptide linker,

(b) a heavy chain comprising a VH-CH1 domain of a Fab fragment capable of specific binding to OX40 and a Fc region subunit,

(c) three light chains, each light chain comprising a VL and CL domain of a Fab fragment capable of specific binding to OX40, and

(d) a light chain comprising a VH and CL domain of a Fab fragment capable of specific binding to FAP.

In one particular aspect, the peptide linker is selected from GGGGS (SEQ ID NO:67) GGGGSGGGGS (SEQ ID NO:68), SGGGGSGGGG (SEQ ID NO:69), GGGGSGGGGSGGGG (SEQ ID NO:70), GSPGSSSSGS (SEQ ID NO:71), $(G4S)_3$ (SEQ ID NO:72), $(G4S)_4$ (SEQ ID NO:73), GSGSGSGS (SEQ ID NO:74), GSGSGNGS (SEQ ID NO:75), GGSGSGSG (SEQ ID NO:76), GGSGSG (SEQ ID NO:77), GGSG (SEQ ID NO:78), GGSGNGSG (SEQ ID NO:79), GGNGSGSG (SEQ ID NO:80) and GGNGSG (SEQ ID NO:81). Peptide linkers of particular interest are (G4S) (SEQ ID NO:67), $(G_4S)_2$ or GGGGSGGGGS (SEQ ID NO:68), $(G4S)_3$ (SEQ ID NO:72) and $(G4S)_4$ (SEQ ID NO:73).

In one aspect, provided is a bispecific antigen binding molecule comprising
(a) a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:86, a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:90, three light chains each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87 and a light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:88, or
(b) a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:86, a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:90, three light chains each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87 and a light chain comprising an

amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:94, or
(c) a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:86, a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:90, three light chains each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87 and a light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:96. In one aspect, provided is a bispecific antigen binding molecule comprising (a) a first heavy chain comprising the amino acid sequence of SEQ ID NO:86, a second heavy chain comprising the amino acid sequence of SEQ ID NO:90, three light chains each comprising the amino acid sequence of SEQ ID NO:87 and a light chain comprising the amino acid sequence of SEQ ID NO:88, or (b) a first heavy chain comprising the amino acid sequence of SEQ ID NO:86, a second heavy chain comprising the amino acid sequence of SEQ ID NO:90, three light chains each comprising the amino acid sequence of SEQ ID NO:87 and a light chain comprising the amino acid sequence of SEQ ID NO:94, or (c) a first heavy chain comprising the amino acid sequence of SEQ ID NO:86, a second heavy chain comprising the amino acid sequence of SEQ ID NO:90, three light chains each comprising the amino acid sequence of SEQ ID NO:87 and a light chain comprising the amino acid sequence of SEQ ID NO:96.

In one aspect, provided is a bispecific antigen binding molecule comprising
(a) a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:97, a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:95, three light chains each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:93 and a light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:88, or
(b) a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:97, a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:95, three light chains each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:93 and a light chain comprising an

amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:94, or
(c) a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:97, a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:95, three light chains each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:93 and a light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:96. In one aspect, provided is a bispecific antigen binding molecule comprising (a) a first heavy chain comprising the amino acid sequence of SEQ ID NO:97, a second heavy chain comprising the amino acid sequence of SEQ ID NO:95, three light chains each comprising the amino acid sequence of SEQ ID NO:93 and a light chain comprising the amino acid sequence of SEQ ID NO:88, or (b) a first heavy chain comprising the amino acid sequence of SEQ ID NO:97, a second heavy chain comprising the amino acid sequence of SEQ ID NO:95, three light chains each comprising the amino acid sequence of SEQ ID NO:93 and a light chain comprising the amino acid sequence of SEQ ID NO:94, or (c) a first heavy chain comprising the amino acid sequence of SEQ ID NO:97, a second heavy chain comprising the amino acid sequence of SEQ ID NO:95, three light chains each comprising the amino acid sequence of SEQ ID NO:93 and a light chain comprising the amino acid sequence of SEQ ID NO:96.

In another aspect, provided is a bispecific antigen binding molecule comprising
(a) a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:99, a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:101, three light chains each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:100 and a light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:88, or
(b) a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:99, a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:101, three light chains each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:100 and a light chain comprising an

amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:94, or
(c) a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:99, a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:101, three light chains each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:100 and a light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:96. In one aspect, provided is a bispecific antigen binding molecule comprising (a) a first heavy chain comprising the amino acid sequence of SEQ ID NO:99, a second heavy chain comprising the amino acid sequence of SEQ ID NO:101, three light chains each comprising the amino acid sequence of SEQ ID NO:100 and a light chain comprising the amino acid sequence of SEQ ID NO:88, or (b) a first heavy chain comprising the amino acid sequence of SEQ ID NO:99, a second heavy chain comprising the amino acid sequence of SEQ ID NO:101, three light chains each comprising the amino acid sequence of SEQ ID NO:100 and a light chain comprising the amino acid sequence of SEQ ID NO:94, or (c) a first heavy chain comprising the amino acid sequence of SEQ ID NO:99, a second heavy chain comprising the amino acid sequence of SEQ ID NO:101, three light chains each comprising the amino acid sequence of SEQ ID NO:100 and a light chain comprising the amino acid sequence of SEQ ID NO:96.

In another aspect, provided is a bispecific antigen binding molecule comprising (a) a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:103, a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:105, three light chains each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:104 and a light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:88, or
(b) a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:103, a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:105, three light chains each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:104 and a light chain comprising an

amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:94, or
(c) a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:103, a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:105, three light chains each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:104 and a light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:96. In one aspect, provided is a bispecific antigen binding molecule comprising (a) a first heavy chain comprising the amino acid sequence of SEQ ID NO:103, a second heavy chain comprising the amino acid sequence of SEQ ID NO:105, three light chains each comprising the amino acid sequence of SEQ ID NO:104 and a light chain comprising the amino acid sequence of SEQ ID NO:88, or (b) a first heavy chain comprising the amino acid sequence of SEQ ID NO:103, a second heavy chain comprising the amino acid sequence of SEQ ID NO:105, three light chains each comprising the amino acid sequence of SEQ ID NO:104 and a light chain comprising the amino acid sequence of SEQ ID NO:94, or (c) a first heavy chain comprising the amino acid sequence of SEQ ID NO:103, a second heavy chain comprising the amino acid sequence of SEQ ID NO:105, three light chains each comprising the amino acid sequence of SEQ ID NO:104 and a light chain comprising the amino acid sequence of SEQ ID NO:96.

Bispecific antigen binding molecules tetravalent for binding to OX40 and monovalent for binding to the target cell antigen (4+1 format)

In another aspect, the invention provides a bispecific antigen binding molecule comprising
(a) four antigen binding domains capable of specific binding to OX40,
(b) one antigen binding domain capable of specific binding to FAP comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, (ii) CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:11 and SEQ ID NO:12, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:5, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:13 and SEQ ID NO:14, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:7, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and
(c) a Fc domain composed of a first and a second subunit capable of stable association.

Thus, provided is a bispecific antigen binding molecule, wherein the bispecific antigen binding molecule binds tetravalently to OX40 and monovalently to FAP.

In one aspect, provided is a bispecific antigen binding molecule, wherein the four antigen binding domains capable of specific binding to OX40 are Fab fragments and each two thereof are fused to each other at the heavy chain, optionally via a peptide linker. In a particular aspect, the antigen binding molecule comprises two heavy chains comprising each a VHCH1-peptide linker-VHCH1 fragment. In a particular aspect, the peptide linker has the amino acid sequence of SEQ ID NO:68.

In one aspect, the bispecific antigen binding molecule consists of

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

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

(ac) a third Fab fragment capable of specific binding to OX40,

(ad) a fourth Fab fragment capable of specific binding to OX40,

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

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

In one aspect, provided is antigen binding molecule consisting of

(a) four light chains, each light chain comprising a VL and CL domain of a Fab fragment capable of specific binding to OX40,

(b) two heavy chains, wherein each of the heavy chain comprises a VH-CH1 domain of a Fab fragment capable of specific binding to OX40 fused to the N-terminus of a VH-CH1 domain of a second Fab fragment capable of specific binding to OX40, and a Fc region subunit, and

(c) a cross-fab fragment capable of specific binding to FAP, wherein the VH-CL domain is connected via a peptide linker to the C-terminus of one of the heavy chains.

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

(a) four light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%,

99% or 100% identical to the sequence of SEQ ID NO:88, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:86, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:89,

(b) four light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:94, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:86, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:89,

(c) four light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:96, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:86, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:89. In one aspect, provided is a bispecific antigen binding molecule comprising (a) four light chains, each comprising the amino acid sequence of SEQ ID NO:87, one light chain comprising the amino acid sequence of SEQ ID NO:88, a first heavy chain comprising the amino acid sequence of SEQ ID NO:86, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:89, (b) four light chains, each comprising the amino acid sequence of SEQ ID NO:87, one light chain comprising the amino acid sequence of SEQ ID NO:94, a first heavy chain comprising the amino acid sequence of SEQ ID NO:86, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:89, or (c) four light chains, each comprising the amino acid sequence of SEQ ID NO:87, one light chain comprising the amino acid sequence of SEQ ID NO:96, a first heavy chain comprising the amino acid sequence of SEQ ID NO:86, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:89.

In another aspect, provided is a bispecific antigen binding molecule comprising (a) four light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:93, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:88, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100%

identical to the sequence of SEQ ID NO:92, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:95,

(b) four light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:93, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:94, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:92, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:95, or

(c) four light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:93, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:96, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:92, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:95. In one aspect, provided is a bispecific antigen binding molecule comprising (a) four light chains, each comprising the amino acid sequence of SEQ ID NO:93, one light chain comprising the amino acid sequence of SEQ ID NO:88, a first heavy chain comprising the amino acid sequence of SEQ ID NO:92, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:95, (b) four light chains, each comprising the amino acid sequence of SEQ ID NO:93, one light chain comprising the amino acid sequence of SEQ ID NO:94, a first heavy chain comprising the amino acid sequence of SEQ ID NO:92, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:95, or (c) four light chains, each comprising the amino acid sequence of SEQ ID NO:93, one light chain comprising the amino acid sequence of SEQ ID NO:96, a first heavy chain comprising the amino acid sequence of SEQ ID NO:92, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:95.

In a further aspect, provided is a bispecific antigen binding molecule comprising (a) four light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:88, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:107, and a second heavy chain comprising an

amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:108,
(b) four light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:94, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:107, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:108, or
(c) four light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:96, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:107, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:108. In a further aspect, provided is a bispecific antigen binding molecule comprising (a) four light chains, each comprising the amino acid sequence of SEQ ID NO:87, one light chain comprising the amino acid sequence of SEQ ID NO:88, a first heavy chain comprising the amino acid sequence of SEQ ID NO:107, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:108, or (b) four light chains, each comprising the amino acid sequence of SEQ ID NO:87, one light chain comprising the amino acid sequence of SEQ ID NO:94, a first heavy chain comprising the amino acid sequence of SEQ ID NO:107, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:108, or (c) four light chains, each comprising the amino acid sequence of SEQ ID NO:87, one light chain comprising the amino acid sequence of SEQ ID NO:96, a first heavy chain comprising the amino acid sequence of SEQ ID NO:107, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:108.

In a further aspect, provided is a bispecific antigen binding molecule comprising (a) four light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:88, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:109, and a second heavy chain comprising an

amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:110, or

(b) four light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:94, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:109, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:110,

(c) four light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:96, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:109, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:110. In one aspect, provided is a bispecific antigen binding molecule comprising (a) four light chains, each comprising the amino acid sequence of SEQ ID NO:87, one light chain comprising the amino acid sequence of SEQ ID NO:88, a first heavy chain comprising the amino acid sequence of SEQ ID NO:109, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:110, or (b) four light chains, each comprising the amino acid sequence of SEQ ID NO:87, one light chain comprising the amino acid sequence of SEQ ID NO:94, a first heavy chain comprising the amino acid sequence of SEQ ID NO:109, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:110, or (c) four light chains, each comprising the amino acid sequence of SEQ ID NO:87, one light chain comprising the amino acid sequence of SEQ ID NO:96, a first heavy chain comprising the amino acid sequence of SEQ ID NO:109, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:110.

In a further aspect, provided is a bispecific antigen binding molecule comprising (a) four light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:88, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:111, and a second heavy chain comprising an

amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:112,
(b) four light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:94, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:111, and a second heavy chain an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:112,
(c) four light chains, each comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:87, one light chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:96, a first heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:111, and a second heavy chain comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:112. In a further aspect, provided is a bispecific antigen binding molecule comprising (a) four light chains, each comprising the amino acid sequence of SEQ ID NO:87, one light chain comprising the amino acid sequence of SEQ ID NO:88, a first heavy chain comprising the amino acid sequence of SEQ ID NO:111, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:112, or (b) four light chains, each comprising the amino acid sequence of SEQ ID NO:87, one light chain comprising the amino acid sequence of SEQ ID NO:94, a first heavy chain comprising the amino acid sequence of SEQ ID NO:111, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:112, or (c) four light chains, each comprising the amino acid sequence of SEQ ID NO:87, one light chain comprising the amino acid sequence of SEQ ID NO:96, a first heavy chain comprising the amino acid sequence of SEQ ID NO:111, and a second heavy chain comprising the amino acid sequence of SEQ ID NO:112.

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

The bispecific antigen binding molecules of the invention further comprise a Fc domain composed of a first and a second subunit capable of stable association.

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 acid positions.

The Fc domain confers favorable pharmacokinetic properties to the bispecific antibodies of the invention, including a long serum half-life which contributes to good accumulation in the target tissue and a favorable tissue-blood distribution ratio. At the same time it may, however, lead to undesirable targeting of the bispecific antibodies of the invention to cells expressing Fc receptors rather than to the preferred antigen-bearing cells. Accordingly, in particular embodiments the Fc domain of the bispecific antibodies of the invention exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG Fc domain, in particular an IgG1 Fc domain or an IgG4 Fc domain. More particularly, the Fc domain is an IgG1 Fc domain.

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

In a particular aspect, the Fc domain is engineered to have reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a non-engineered Fc domain. In a particular aspect, the Fc domain of the bispecific antigen binding molecule of the invention comprises one or more amino acid mutation that reduces the binding affinity of the Fc domain to an Fc receptor and/or effector function. Typically, the same one or more amino acid mutation is present in each of the two subunits of the Fc domain. In one aspect, the amino acid mutation reduces the binding affinity of the Fc domain to an Fc receptor. In another aspect, the amino acid mutation reduces the binding affinity of the Fc domain to an Fc receptor by at least 2-fold, at least 5-fold, or at least 10-fold. In one aspect, the bispecific antigen binding molecule of the invention comprising an engineered Fc domain exhibits less than 20%, particularly less than 10%, more particularly less than 5% of the binding affinity to an Fc receptor as compared to bispecific antibodies of the invention comprising a non-engineered Fc domain. In a particular aspect, the Fc receptor is an Fcγ receptor. In other aspects, the Fc receptor is a human Fc receptor. In one aspect, the Fc receptor is an inhibitory Fc receptor. In a specific aspect, the Fc receptor is an inhibitory human Fcγ receptor, more specifically human FcγRIIB. In some aspects the Fc receptor is an activating 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. Preferably, binding to each of these receptors is reduced. In some aspects, binding affinity to a complement component, specifically binding affinity to C1q, is also reduced. In one aspect, binding affinity to neonatal Fc receptor (FcRn) is not reduced. Substantially similar binding to FcRn, i.e. preservation of the binding affinity of the Fc domain to said receptor, is achieved when the Fc domain (or the bispecific antigen binding molecule of the invention comprising said Fc domain) exhibits greater than about 70% of the binding affinity of a non-engineered form of the Fc domain (or the bispecific antigen binding molecule of the invention comprising said non-engineered form of the Fc domain) to FcRn. The Fc domain, or the the bispecific antigen binding molecule of the invention comprising said Fc domain, may exhibit greater than about 80% and even greater than about 90% of such affinity. In certain embodiments the Fc domain of the bispecific antigen binding molecule of the invention is engineered to have reduced effector function, as compared to a non-engineered Fc domain. The reduced effector function can include, but is not limited to, one or more of the following: reduced complement dependent cytotoxicity (CDC), reduced antibody-dependent cell-mediated cytotoxicity (ADCC), reduced antibody-dependent cellular phagocytosis (ADCP), reduced cytokine secretion, reduced immune complex-mediated antigen uptake by antigen-presenting cells, reduced binding to NK cells, reduced binding to macrophages, reduced binding to monocytes, reduced binding to polymorphonuclear cells, reduced direct signaling inducing apoptosis, reduced dendritic cell maturation, or reduced T cell priming.

Antibodies with reduced effector function include those with substitution of one or more of Fc region 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). Certain antibody variants with improved or diminished binding to FcRs are described. (e.g. U.S. Patent No. 6,737,056; WO 2004/056312, and Shields, R.L. et al., J. Biol. Chem. 276 (2001) 6591-6604).

In one aspect, the Fc domain comprises an amino acid substitution at a position of E233, L234, L235, N297, P331 and P329. In some aspects, the Fc domain comprises the amino acid substitutions L234A and L235A ("LALA"). In one such embodiment, the Fc domain is an IgG1 Fc domain, particularly a human IgG1 Fc domain. In one aspect, the Fc domain comprises an amino acid substitution at position P329. In a more specific aspect, the amino acid substitution is P329A or P329G, particularly P329G. In one embodiment the Fc domain comprises an amino acid substitution at position P329 and a further amino acid substitution selected from the group consisting of E233P, L234A, L235A, L235E, N297A, N297D or P331S. In more particular embodiments the Fc domain comprises the amino acid mutations L234A, L235A and P329G ("P329G LALA"). The "P329G LALA" combination of amino acid substitutions almost completely abolishes Fcγ receptor binding of a human IgG1 Fc domain, as described in PCT Patent Application No. WO 2012/130831 A1. Said document also describes methods of preparing such mutant Fc domains and methods for determining its properties such as Fc receptor binding or effector functions. Such antibody is an IgG1 with mutations L234A and L235A or with mutations L234A, L235A and P329G (numbering according to EU index of Kabat et al , Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991).

In one aspect, the Fc domain is an IgG4 Fc domain. In a more specific embodiment, 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 embodiment, the Fc domain is an IgG4 Fc domain comprising amino acid substitutions L235E and S228P and P329G. This amino acid substitution reduces in vivo Fab arm exchange of IgG4 antibodies (see Stubenrauch et al., Drug Metabolism and Disposition 38, 84-91 (2010)).

Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer, R.L. et al., J. Immunol. 117 (1976) 587-593, and Kim, J.K. et al., J. Immunol. 24 (1994)

2429-2434), are described in US 2005/0014934. Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826). See also Duncan, A.R. and Winter, G., Nature 322 (1988) 738-740; US 5,648,260; US 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

Binding to Fc receptors can be easily determined e.g. by ELISA, or by Surface Plasmon Resonance (SPR) using standard instrumentation such as a BIAcore instrument (GE Healthcare), and Fc receptors such as may be obtained by recombinant expression. A suitable such binding assay is described herein. Alternatively, binding affinity of Fc domains or cell activating bispecific antigen binding molecules comprising an Fc domain for Fc receptors may be evaluated using cell lines known to express particular Fc receptors, such as human NK cells expressing FcγIIIa receptor. Effector function of an Fc domain, or bispecific antigen binding molecules 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, ACTI™ 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 an animal model such as that disclosed in Clynes et al., Proc Natl Acad Sci USA 95, 652-656 (1998).

The following section describes preferred aspects of the bispecific antigen binding molecules of the invention comprising Fc domain modifications reducing Fc receptor binding and/or effector function. In one aspect, the invention relates to the bispecific antigen binding molecule (a) at least two antigen binding domains capable of specific binding to OX40, (b) an antigen binding domain capable of specific binding to FAP, and (c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the Fc domain comprises one or more amino acid substitution that reduces the binding affinity of the antibody to an Fc receptor, in particular towards Fcγ receptor. In another aspect, the invention relates to the bispecific antigen binding molecule comprising (a) at least two antigen binding domain capable of specific binding to OX40, (b) an antigen

binding domain capable of specific binding to FAP, and (c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the Fc domain comprises one or more amino acid substitution that reduces effector function. In particular aspect, the Fc domain is of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

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 antigen binding molecules 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 two antigen binding domains capable of specific binding to OX40, (b) an antigen binding domain capable of specific binding to FAP, and (c) a Fc domain composed of a 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.

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 the bispecific antigen binding molecule comprising (a) at least two antigen binding domains capable of specific binding to OX40, (b) an antigen binding domain capable of specific binding to FAP, and (c) a Fc domain composed of a first and a second subunit capable 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 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 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).

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 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 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 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 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).

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 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 aspect 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 bispecific antigen binding molecule comprising (a) at least two Fab fragments capable of specific binding to OX40, (b) a Fab fragment capable of specific binding to FAP, 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 (CrossMab VH-VL or CrossMab CH-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) at least two Fab fragments capable of specific binding to OX40, (b) a Fab fragment capable of specific binding to FAP, and (c) a Fc domain composed of a first and a second subunit capable of stable association, wherein in one of the Fab fragments the constant domains CL and CH1 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. More particularly, in the

second Fab fragment capable of specific binding to a target cell antigen the constant domains CL and CH1 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 a particular aspect, the invention relates a bispecific antigen binding molecule comprising (a) at least two Fab fragments capable of specific binding to OX40, (b) a Fab fragment capable of specific binding to FAP, wherein in the Fab fragment capable of specific binding to FAP the constant domains CL and CH1 are replaced by each other so that the CH1 domain is part of the light chain and the CL (Ckappa) domain is part of the heavy chain.

Thus, in one aspect, the invention comprises a bispecific antigen binding molecule, comprising (a) two light chains and two heavy chains of an antibody comprising two Fab fragments capable of specific binding to OX40 and the Fc region, and (b) a crossFab fragment capable of specific binding to FAP fused to the C-terminus of one of subunits of the Fc region.

In another aspect, and to further improve correct pairing, the bispecific antigen binding molecule comprising (a) at least two Fab fragments capable of specific binding to OX40, (b) a crossFab fragment capable of specific binding to FAP, 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 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/or wherein 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 numbering) and/or at position 213 (EU numbering) have been substituted by glutamic acid (E).

Exemplary antibodies of the invention

In one aspect, the invention provides new antibodies and antibody fragments that specifically bind to FAP. These antibodies bind to a different epitope than the known FAP antibodies 4B9 or 28H1 that make them especially suitable for the incorporation into bispecific antigen binding molecules that can be used in combination with other FAP-targeted molecules. The new antibodies are further characterized in that they are producable in high amounts and with high titers, that they show high thermal stability (as measured by the aggregation temperature T_{agg}), that they are supposed to possess excellent

PK properties and that they bind with high affinity to human FAP as measured by Biacore assay.

In one aspect, provided is an antibody that specifically binds to FAP (clone 212), wherein said antibody comprises a heavy chain variable region (V_HFAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, (ii) CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:11 and SEQ ID NO:12, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:5, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:13 and SEQ ID NO:14, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:7, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8.

In one aspect, provided is a humanized antibody that specifically binds to FAP, wherein said antibody comprises a heavy chain variable region (V_HFAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, (ii) CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:11 and SEQ ID NO:12, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:5, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:13 and SEQ ID NO:14, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:7, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8.

In another aspect, provided is an antibody that competes for binding with an antibody that specifically binds to FAP, wherein said antibody comprises any of the heavy chain variable regions (V_HFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18. SEQ ID NO:19 and SEQ ID NO:20, and any of the light chain variable regions (V_LFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 and SEQ ID NO:26.

In one aspect, provided is an antibody that competes for binding with an antibody that specifically binds to FAP, wherein said antibody comprises a heavy chain variable region VH comprising an amino acid sequence of SEQ ID NO:15 and a light chain variable region VL comprising an amino acid sequence of SEQ ID NO:21.

In a further aspect, provided is an antibody that specifically binds to FAP, wherein said antibody comprises

(a) a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:15 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:21,

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

In a further aspect, provided is an antibody that specifically binds to FAP comprising a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:15 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:21.

In another aspect, the invention provides new antibodies and antibody fragments that specifically bind to OX40. These antibodies are variants of OX40 antibody 49B4 and have less positive charge patches compared to 49B4. These new antibodies are are supposed to possess improved PK properties compared to 49B4 and that they bind with high affinity to human OX40 as measured by Biacore assay.

Thus, provided is a humanized antibody that specifically binds to OX40, wherein said antibody comprises
(a) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:59 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34,
(b) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:60 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34,
(c) a heavy chain variable region (V_HOX40) comprising the amino acid sequence of SEQ ID NO:61 and a light chain variable region (V_LOX40) comprising the amino acid sequence of SEQ ID NO:34.

Polynucleotides

The invention further provides isolated nucleic acid encoding a bispecific antigen binding molecule as described herein or a fragment thereof or isolated nucleic acid encoding an antibody as described herein.

The isolated polynucleotides encoding bispecific antigen binding molecules 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 a polypeptide comprised in the bispecific molecule according to the invention as described herein.

In one aspect, the present invention is directed to an isolated polynucleotide encoding a bispecific antigen binding molecule, comprising (a) at least two antigen binding domains capable of specific binding to OX40, (b) an antigen binding domain capable of specific binding to a FAP comprising a heavy chain variable region (V_HFAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:3, (ii) CDR-H2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:11 and SEQ ID NO:12, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:5, and a light chain variable region (V_LFAP) comprising (iv) CDR-L1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:13 and SEQ ID NO:14, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:7, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:8, and (c) a Fc domain composed of a first and a second subunit capable of stable association.

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

promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription.

Suitable promoters and other transcription control regions are disclosed herein. A variety of transcription control regions are known to those skilled in the art. These include, without 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 â-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 bispecific antigen binding molecule or polypeptide fragments thereof is desired, DNA encoding a signal sequence may be placed upstream of the nucleic acid encoding the bispecific antigen binding molecule of the invention or polypeptide fragments thereof. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells generally have a 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 a bispecific antigen binding molecule 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 aspects, 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) a bispecific antigen binding molecule 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

PLANTIBODIES™ 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 a bispecific antigen binding molecule of the invention or polypeptide fragments thereof is provided, wherein the method comprises culturing a host cell comprising polynucleotides encoding the bispecific antigen binding molecule of the invention or polypeptide fragments thereof, as provided herein, under conditions suitable for expression of the bispecific antigen binding molecule of the invention or polypeptide fragments thereof, and recovering the bispecific antigen binding molecule of the invention or polypeptide fragments thereof from the host cell (or host cell culture medium).

Bispecific molecules of the invention prepared as described herein may be purified by art-known techniques such as high performance liquid chromatography, ion exchange chromatography, gel electrophoresis, affinity chromatography, size exclusion

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

Assays

The antigen binding molecules provided herein may be characterized for their binding properties and/or biological activity by various assays known in the art. In particular, they are characterized by the assays described in more detail in the examples.

1. Binding assay

Binding of the bispecific antigen binding molecule provided herein to the corresponding target expressing cells may be evaluated for example by using a murine fibroblast cell line expressing human Fibroblast Activation Protein (FAP) and flow cytometry (FACS) analysis. Binding of the bispecific antigen binding molecules provided herein to OX40 may be determined by using activated human PBMCs as described in Example 3.1.

2. Activity assays

Bispecific antigen binding molecules of the invention are tested for biological activity. Biological activity may include efficacy and specificity of the bispecific antigen binding molecules. Efficacy and specificity are demonstrated by assays showing agonistic signaling through the OX40 receptor upon binding of the target antigen. Furthermore, the stimulation of OX40 signaling is measures through induced NFκB activation in human OX40 positive NFκB reporter cells as described in Example 4.1.

Pharmaceutical Compositions, Formulations and Routes of Administration

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

Pharmaceutical compositions of the present invention comprise a therapeutically effective amount of one or more bispecific antigen binding molecules dissolved or dispersed in a pharmaceutically acceptable carrier. 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 preparation of a pharmaceutical composition that contains at least one bispecific antigen binding molecule according to the invention 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 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, intra-lesional, intravenous, intra-arterial, intramuscular, intrathecal or intraperitoneal injection. For injection, the bispecific 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 formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the bispecific antigen binding molecules 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 antigen binding molecules 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 cleats 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-(methylmethacylate) 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 insterstitial 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 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 bispecific 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 bispecific antigen binding molecules 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.

Therapeutic methods and compositions

Any of the bispecific antigen binding molecules provided herein may be used in therapeutic methods. For use in therapeutic methods, bispecific antigen binding molecules of the invention can 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.

In one aspect, bispecific antigen binding molecules of the invention for use as a medicament are provided.

In further aspects, bispecific antigen binding molecules of the invention for use (i) in inducing immune stimulation, (ii) in stimulating tumor-specific T cell response, (iii) in causing apoptosis of tumor cells, (iv) in the treatment of cancer, (v) in delaying progression of cancer, (vi) in prolonging the survival of a patient suffering from cancer, (vii) in the treatment of infections are provided. In a particular aspect, bispecific antigen binding molecules of the invention for use in treating a disease, in particular for use in the treatment of cancer, are provided.

In certain aspects, bispecific antigen binding molecules of the invention for use in a method of treatment are provided. In one aspect, the invention provides a bispecific

antigen binding molecule as described herein for use in the treatment of a disease in an individual in need thereof. In certain aspects, the invention provides a bispecific antigen binding molecule for use in a method of treating an individual having a disease comprising administering to the individual a therapeutically effective amount of the bispecific antigen binding molecule. In certain aspects the disease to be treated is cancer. The subject, patient, or "individual" in need of treatment is typically a mammal, more specifically a human.

In one aspect, provided is a method for i) inducing immune stimulation, (ii) stimulating tumor-specific T cell response, (iii) causing apoptosis of tumor cells, (iv) treating of cancer, (v) delaying progression of cancer, (vi) prolonging the survival of a patient suffering from cancer, or (vii) treating of infections, wherein the method comprises administering a therapeutically effective amount of the bispecific antigen binding molecule of the invention to an individual in need thereof.

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

physiological change is considered an "effective amount" or a "therapeutically effective amount".

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

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

receives from about two to about twenty, or e.g. about six doses of the fusion protein). In a particular aspect, the bispecific antigen binding molecule will be administered every three weeks. An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

The bispecific antigen binding molecule of the invention will generally be used in an amount effective to achieve the intended purpose. For use to treat or prevent a disease condition, the bispecific antigen binding molecule of the invention, or pharmaceutical compositions thereof, are administered or applied in a therapeutically effective amount. Determination of a therapeutically effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure provided herein. For systemic administration, a therapeutically effective dose can be estimated initially from in vitro assays, such as cell culture assays. A dose can then be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Initial dosages can also be estimated from in vivo data, e.g., animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

Dosage amount and interval may be adjusted individually to provide plasma levels of the bispecific antigen binding molecule of the invention which are sufficient to maintain therapeutic effect. Usual patient dosages for administration by injection range from about 0.1 to 50 mg/kg/day, typically from about 0.1 to 1 mg/kg/day. Therapeutically effective plasma levels may be achieved by administering multiple doses each day. Levels in plasma may be measured, for example, by HPLC. In cases of local administration or selective uptake, the effective local concentration of the bispecific antigen binding molecule or antibody of the invention may not be related to plasma concentration. One skilled in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

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

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

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

Other agents and treatments

The bispecific antigen binding molecule of the invention may be administered in combination with one or more other agents in therapy. For instance, the bispecific antigen binding molecule of the invention may be co-administered with at least one additional therapeutic agent. The term “therapeutic agent” encompasses any agent that can be administered for treating a symptom or disease in an individual in need of such treatment. Such additional therapeutic agent may comprise any active ingredients suitable for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. In certain aspects, an additional therapeutic agent is another anti-cancer agent, for example a microtubule disruptor, an antimetabolite, a topoisomerase inhibitor, a DNA intercalator, an alkylating agent, a hormonal therapy, a kinase inhibitor, a receptor antagonist, an activator of tumor cell apoptosis, or an antiangiogenic agent. In certain aspects, an additional therapeutic agent is an immunomodulatory agent, a cytostatic agent, an inhibitor of cell adhesion, a cytotoxic or cytostatic agent, an activator of cell apoptosis, or an agent that increases the sensitivity of cells to apoptotic inducers.

Thus, provided are bispecific antigen binding molecules of the invention or pharmaceutical compositions comprising them for use in the treatment of cancer, wherein the bispecific antigen binding molecule is administered in combination with a chemotherapeutic agent, radiation and/ or other agents for use in cancer immunotherapy.

Such other agents are suitably present in combination in amounts that are effective for the purpose intended. The effective amount of such other agents depends on the amount of fusion protein used, the type of disorder or treatment, and other factors discussed above. The bispecific antigen binding molecule or antibody of the invention 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. Other agents for use in cancer immunotherapy may also include vaccines, toll-like receptor (TLR) agents and oncolytic viruses.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate compositions), and separate administration, in which case, administration of the bispecific antigen binding molecule or antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant.

In a further aspect, provided is the bispecific antigen binding molecule as described herein before for use in the treatment of cancer, wherein the bispecific antigen binding molecule is administered in combination with another immunomodulator.

The term “immunomodulator” refers to any substance including a monoclonal antibody that effects the immune system. The molecules of the inventions can be considered immunomodulators. Immunomodulators can be used as anti-neoplastic agents for the treatment of cancer. In one aspect, immunomodulators include, but are not limited to anti-CTLA4 antibodies (e.g. ipilimumab), anti-PD1 antibodies (e.g. nivolumab or pembrolizumab), PD-L1 antibodies (e.g. atezolizumab, avelumab or durvalumab), ICOS antibodies, 4-1BB antibodies and GITR antibodies. In a further aspect, provided is the bispecific antigen binding molecule as described herein before for use in the treatment of cancer, wherein the bispecific antigen binding molecule is administered in combination with an agent blocking PD-L1/PD-1 interaction. In one aspect, 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 an anti-PD-L1 antibody, in particular an anti-PD-L1 antibody selected from the group consisting of atezolizumab, durvalumab, pembrolizumab and nivolumab. In one specific aspect, the agent blocking PD-L1/PD-1

interaction is atezolizumab (MPDL3280A, RG7446). 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:149 and a light chain variable domain VL(PDL-1) of SEQ ID NO:150. 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:151 and a light chain variable domain VL(PDL-1) of SEQ ID NO:152. In another aspect, the agent blocking PD-L1/PD-1 interaction is an anti-PD1 antibody, in particular an anti-PD1 antibody selected from pembrolizumab or nivolumab. Such other agents are suitably present in combination in amounts that are effective for the purpose intended. The effective amount of such other agents depends on the amount of bispecific antigen binding molecule used, the type of disorder or treatment, and other factors discussed above. The bispecific antigen binding molecules as described herein before 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.

In one aspect, provided is a bispecific agonistic OX40 antigen binding molecule or a pharmaceutical composition for use in the treatment of cancer, wherein the bispecific agonistic OX40 antigen binding molecule is for administration in combination with a T-cell activating anti-CD3 bispecific antibody. 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.

In a particular aspect, the anti-CD3 bispecific antibody for use in the combination comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) comprising CDR-H1 sequence of SEQ ID NO:117, CDR-H2 sequence of SEQ ID NO:118, and CDR-H3 sequence of SEQ ID NO:119; and/or a light chain variable region (V_LCD3) comprising CDR-L1 sequence of SEQ ID NO:120, CDR-L2 sequence of SEQ ID NO:121, and CDR-L3 sequence of SEQ ID NO:122. More particularly, the anti-CD3 bispecific comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:123 and/or a light chain variable region (V_LCD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:124. In a further aspect, the anti-CD3 bispecific antibody comprises a heavy chain variable region (V_HCD3) comprising the amino acid sequence of SEQ ID NO:123 and/or a light chain variable region (V_LCD3) comprising the amino acid sequence of SEQ ID NO:124.

In another aspect, the anti-CD3 bispecific antibody for use in the combination comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) comprising CDR-H1 sequence of SEQ ID NO:125, CDR-H2 sequence of SEQ ID NO:126, and CDR-H3 sequence of SEQ ID NO:127; and/or a light chain variable region (V_LCD3) comprising CDR-L1 sequence of SEQ ID NO:128, CDR-L2 sequence of SEQ ID NO:129, and CDR-L3 sequence of SEQ ID NO:130. More particularly, the anti-CD3 bispecific comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:131 and/or a light chain variable region (V_LCD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:132. In a further aspect, the anti-CD20/anti-CD3 bispecific antibody comprises a heavy chain variable region (V_HCD3) comprising the amino acid sequence of SEQ ID NO:131 and/or a light chain variable region (V_LCD3) comprising the amino acid sequence of SEQ ID NO:132.

In another aspect, the anti-CD3 bispecific antibody for use in the combination comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) comprising CDR-H1 sequence of SEQ ID NO:133, CDR-H2 sequence of SEQ ID NO:134, and CDR-H3 sequence of SEQ ID NO:135; and/or a light chain variable region (V_LCD3) comprising CDR-L1 sequence of SEQ ID NO:136, CDR-L2 sequence of SEQ ID NO:137, and CDR-L3 sequence of SEQ ID NO:138. More particularly, the anti-CD3 bispecific comprises a first antigen binding domain comprising a heavy chain variable region (V_HCD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:139 and/or a light chain variable region (V_LCD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:140. In a further aspect, the anti-CD20/anti-CD3 bispecific antibody comprises a heavy chain variable region (V_HCD3) comprising the amino acid sequence of SEQ ID NO:139 and/or a light chain variable region (V_LCD3) comprising the amino acid sequence of SEQ ID NO:140.

In one 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: 141, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 142, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 143, and a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 144. In a further particular embodiment, the bispecific antibody comprises a polypeptide sequence of SEQ ID NO: 141, a polypeptide sequence of SEQ ID NO: 142, a polypeptide sequence of SEQ ID NO: 143 and a polypeptide sequence of SEQ ID NO: 144 (CEA TCB).

In another 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:145, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO:146, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO:147, and a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO:148. In a further particular embodiment, the bispecific antibody comprises a polypeptide sequence of SEQ ID NO:145, a polypeptide sequence of SEQ ID NO:146, a polypeptide sequence of SEQ ID NO:147 and a polypeptide sequence of SEQ ID NO:148 (CEACAM5 TCB).

Particular bispecific antibodies are further 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.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate compositions), and separate administration, in which case, administration of the bispecific antigen binding molecule can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant.

Articles of Manufacture

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

The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises a bispecific antigen binding molecule of the invention; and (b) a second container with a

composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition.

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

Table B (Sequences):

SEQ ID NO:	Name	Sequence
1	hu OX40	Uniprot No. P43489, aa 29-214 LH CVGDTYPSND RCCHECRPGN GMVSRCSRSQ NTVCRPCGPGFYNDVVSSKPCKPCTWCNLRSGSERKQLC TATQDTVCRCRAGTQPLDSYKPGVDCAPCPPGHFSPGDN QACKPWTNCTLAGKHTLQPASNSSDAICEDRDPPATQPQ ETQGPPARPITVQPTEAWPRTSQGPSTRPVEVPGGRAVA AILGLGLVLGLLGPLAILLALYLLRRDQRLPPDAHKPPG GGSFRTPIQEEQADAHSTLAKI
2	hu FAP	UniProt no. Q12884, version 168 MKTWVKIVFGVATSAVLALLVMCIVLRPSRVHNSEENTM RALTLKDILNGTFSYKTFFPNWISGQEYLHQSADNNIVL YNIETGQSYTILSNRTMKSVNASNYGLSPDRQFVYLESD YSKLWRYSYTATYYIYDLSNGEFVRGNELPRPIQYLCWS PVGSKLAYVYQNNIYLKQRPGDPPFQITFNGRENKIFNG IPDWVYEEEMLATKYALWWSPNGKFLAYAEFNDTDIPVI AYSYYGDEQYPRTINIPYPKAGAKNPVVRIFIIDTTYPA YVGPQEVPVPAMIASSDYYFSWLTWVTDERVCLQWLKRV QNVSVLSICDFREDWQTWDCPKTQEHIEESRTGWAGGFF VSTPVFSYDAISYYKIFSDKDGYKHIHYIKDTVENAIQI TSGKWEAINIFRVTQDSLFYSSNEFEEYPGRRNIYRISI GSYPPSKKCVTCHLRKERCQYYTASFSDYAKYYALVCYG PGIPISTLHDGRTDQEIKILEENKELENALKNIQLPKEE IKKLEVDEITLWYKMILPPQFDRSKKYPLLIQVYGGPCS QSVRSVFAVNWISYLASKEGMVIALVDGRGTAFQGDKLL YAVYRKLGVYEVEDQITAVRKFIEMGFIDEKRIAIWGWS YGGYVSSLALASGTGLFKCGIAVAPVSSWEYYASVYTER FMGLPTKDDNLEHYKNSTVMARAEYFRNVDYLLIHGTAD DNVHFQNSAQIAKALVNAQVDFQAMWYSDQNHGLSGLST NHLYTHMTHFLKQCFSLSD
3	FAP (212) CDR-H1	DYNMD
4	FAP (212) CDR-H2	DIYPNTGGTIYNQKFKG
5	FAP (212) CDR-H3	FRGIHYAMDY
6	FAP (212) CDR-L1	RASESVDNYGLSFIN
7	FAP (212) CDR-L2	GTSNRGS
8	FAP (212) CDR-L3	QQSNEVPYT
9	FAP (212) VH	EVLLQQSGPELVKPGASVKIACKASGYTLTDYNMDWVRQ

SEQ ID NO:	Name	Sequence
		SHGKSLEWIGDIYPNTGGTIYNQKFKGKATLTIDKSSST AYMDLRSLTSEDTAVYYCTRFRGIHYAMDYWGQGTSVTV SS
10	FAP (212) VL	DIVLTQSPVSLAVSLGQRATISCRASESVDNYGLSFINW FQQKPGQPPKLLIYGTSNRGSGVPARFSGSGSGTDFSLN IHPMEEDDTAMYFCQQSNEVPYTFGGGTNLEIK
11	FAP (VH1G3a) CDR-H2	DIYPNTGGTIYAQKFQG
12	FAP (VH2G3a) CDR-H2	DIYPNTGGTIYADSVKG
13	FAP (VL1G3a) CDR-L1	RASESVDNYGLSFLA
14	FAP (VL2G3a) CDR-L1	RASESIDNYGLSFLN
15	FAP (VH1G1a)	See Table 10
16	FAP (VH1G2a)	See Table 10
17	FAP (VH1G3a)	See Table 10
18	FAP (VH2G1a)	See Table 10
19	FAP (VH2G2a)	See Table 10
20	FAP (VH2G3a)	See Table 10
21	FAP (VL1G1a)	See Table 10
22	FAP (VL1G2a)	See Table 10
23	FAP (VL1G3a)	See Table 10
24	FAP (VL2G1a)	See Table 10
25	FAP (VL2G2a)	See Table 10
26	FAP (VL2G3a)	See Table 10
27	OX40 (49B4) CDR-H1	SYAIS
28	OX40 (49B4) CDR-H2	GIIPIFGTANYAQKFQG
29	OX40 (49B4) CDR-H3	EYYRGPYDY
30	OX40 (49B4) CDR-L1	RASQSISSWLA
31	OX40 (49B4) CDR-L2	DASSLES
32	OX40 (49B4) CDR-L3	QQYSSQPYT
33	OX40 (49B4) VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQ APGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTST AYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTVTVS S
34	OX40 (49B4) VL	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQK PGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISSL QPDDFATYYCQQYSSQPYTFGQGTKVEIK
35	OX40 (CLC563) CDR-H1	SYAMS
36	OX40 (CLC563) CDR-H2	AISGSGGSTYYADSVKG
37	OX40 (CLC563) CDR-H3	DVGAFDY
38	OX40 (CLC563) CDR-L1	RASQSVSSSYLA
39	OX40 (CLC563) CDR-L2	GASSRAT
40	OX40 (CLC563) CDR-L3	QQYGSSPLT
41	OX40 (CLC563) VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQ APGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVTVSS
42	OX40 (CLC563) VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQ KPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISR LEPEDFAVYYCQQYGSSPLTFGQGTKVEIK
43	OX40 (MOXR0916) CDR-H1	DSYMS
44	OX40 (MOXR0916) CDR-	DMYPDNGDSSYNQKFRE

SEQ ID NO:	Name	Sequence
	H2	
45	OX40 (MOXR0916) CDR-H3	APRWYFSV
46	OX40 (MOXR0916) CDR-L1	RASQDISNYLN
47	OX40 (MOXR0916) CDR-L2	YTSRLRS
48	OX40 (MOXR0916) CDR-L3	QQGHTLPPT
49	OX40 (MOXR0916) VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSWVRQ APGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDTSTST AYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTLVTVSS
50	OX40 (MOXR0916) VL	DIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQK PGKAPKLLIYYTSRLRSGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQGHTLPPTFGQGTKVEIK
51	OX40 (8H9) CDR-H1	SYAIS
52	OX40 (8H9) CDR-H2	GIIPIFGTANYAQKFQG
53	OX40 (8H9) CDR-H3	EYGWMDY
54	OX40 (8H9) CDR-L1	RASQSISSWLA
55	OX40 (8H9) CDR-L2	DASSLES
56	OX40 (8H9) CDR-L3	QQYLTYSRFT
57	OX40 (8H9) VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQ APGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTST AYMELSSLRSEDTAVYYCAREYGWMDYWGQGTTVTVSS
58	OX40 (8H9) VL	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQK PGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISSL QPDDFATYYCQQYLTYSRFTFGQGTKVEIK
59	OX40 (49B4_K73E) VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQ APGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD**E**STST AYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTVTVS S
60	OX40 (49B4_K23T_K73E) VH	QVQLVQSGAEVKKPGSSVKVSC**T**ASGGTFSSYAISWVRQ APGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD**E**STST AYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTVTVS S
61	OX40 (49B4_K23E_K73E) VH	QVQLVQSGAEVKKPGSSVKVSC**E**ASGGTFSSYAISWVRQ APGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD**E**STST AYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTVTVS S
62	hu FAP ectodomain+poly-lys-tag+his$_6$-tag	RPSRVHNSEENTMRALTLKDILNGTFSYKTFFPNWISGQ EYLHQSADNNIVLYNIETGQSYTILSNRTMKSVNASNYG LSPDRQFVYLESDYSKLWRYSYTATYYIYDLSNGEFVRG NELPRPIQYLCWSPVGSKLAYVYQNNIYLKQRPGDPPFQ ITFNGRENKIFNGIPDWVYEEEMLATKYALWWSPNGKFL AYAEFNDTDIPVIAYSYYGDEQYPRTINIPYPKAGAKNP VVRIFIIDTTYPAYVGPQEVPVPAMIASSDYYFSWLTWV TDERVCLQWLKRVQNVSVLSICDFREDWQTWDCPKTQEH IEESRTGWAGGFFVSTPVFSYDAISYYKIFSDKDGYKHI HYIKDTVENAIQITSGKWEAINIFRVTQDSLFYSSNEFE EYPGRRNIYRISIGSYPPSKKCVTCHLRKERCQYYTASF SDYAKYYALVCYGPGIPISTLHDGRTDQEIKILEENKEL ENALKNIQLPKEEIKKLEVDEITLWYKMILPPQFDRSKK YPLLIQVYGGPCSQSVRSVFAVNWISYLASKEGMVIALV DGRGTAFQGDKLLYAVYRKLGVYEVEDQITAVRKFIEMG

SEQ ID NO:	Name	Sequence
		FIDEKRIAIWGWSYGGYVSSLALASGTGLFKCGIAVAPV SSWEYYASVYTERFMGLPTKDDNLEHYKNSTVMARAEYF RNVDYLLIHGTADDNVHFQNSAQIAKALVNAQVDFQAMW YSDQNHGLSGLSTNHLYTHMTHFLKQCFSLSDGKKKKK GHHHHHH
63	Murine FAP	UniProt no. P97321
64	Murine FAP ectodomain+poly-lys-tag+his_6-tag	RPSRVYKPEGNTKRALTLKDILNGTFSYKTYFPNWISEQ EYLHQSEDDNIVFYNIETRESYIILSNSTMKSVNATDYG LSPDRQFVYLESDYSKLWRYSYTATYYIYDLQNGEFVRG YELPRPIQYLCWSPVGSKLAYVYQNNIYLKQRPGDPPFQ ITYTGRENRIFNGIPDWVYEEEMLATKYALWWSPDGKFL AYVEFNDSDIPIIAYSYYGDGQYPRTINIPYPKAGAKNP VVRVFIVDTTYPHHVGPMEVPVPEMIASSDYYFSWLTWV SSERVCLQWLKRVQNVSVLSICDFREDWHAWECPKNQEH VEESRTGWAGGFFVSTPAFSQDATSYYKIFSDKDGYKHI HYIKDTVENAIQITSGKWEAIYIFRVTQDSLFYSSNEFE GYPGRRNIYRISIGNSPPSKKCVTCHLRKERCQYYTASF SYKAKYYALVCYGPGLPISTLHDGRTDQEIQVLEENKEL ENSLRNIQLPKVEIKKLKDGGLTFWYKMILPPQFDRSKK YPLLIQVYGGPCSQSVKSVFAVNWITYLASKEGIVIALV DGRGTAFQGDKFLHAVYRKLGVYEVEDQLTAVRKFIEMG FIDEERIAIWGWSYGGYVSSLALASGTGLFKCGIAVAPV SSWEYYASIYSERFMGLPTKDDNLEHYKNSTVMARAEYF RNVDYLLIHGTADDNVHFQNSAQIAKALVNAQVDFQAMW YSDQNHGILSGRSQNHLYTHMTHFLKQCFSLSDGKKKKK KGHHHHHH
65	Cynomolgus FAP ectodomain+poly-lys-tag+his_6-tag	RPPRVHNSEENTMRALTLKDILNGTFSYKTFFPNWISGQ EYLHQSADNNIVLYNIETGQSYTILSNRTMKSVNASNYG LSPDRQFVYLESDYSKLWRYSYTATYYIYDLSNGEFVRG NELPRPIQYLCWSPVGSKLAYVYQNNIYLKQRPGDPPFQ ITFNGRENKIFNGIPDWVYEEEMLATKYALWWSPNGKFL AYAEFNDTDIPVIAYSYYGDEQYPRTINIPYPKAGAKNP FVRIFIIDTTYPAYVGPQEVPVPAMIASSDYYFSWLTWV TDERVCLQWLKRVQNVSVLSICDFREDWQTWDCPKTQEH IEESRTGWAGGFFVSTPVFSYDAISYYKIFSDKDGYKHI HYIKDTVENAIQITSGKWEAINIFRVTQDSLFYSSNEFE DYPGRRNIYRISIGSYPPSKKCVTCHLRKERCQYYTASF SDYAKYYALVCYGPGIPISTLHDGRTDQEIKILEENKEL ENALKNIQLPKEEIKKLEVDEITLWYKMILPPQFDRSKK YPLLIQVYGGPCSQSVRSVFAVNWISYLASKEGMVIALV DGRGTAFQGDKLLYAVYRKLGVYEVEDQITAVRKFIEMG FIDEKRIAIWGWSYGGYVSSLALASGTGLFKCGIAVAPV SSWEYYASVYTERFMGLPTKDDNLEHYKNSTVMARAEYF RNVDYLLIHGTADDNVHFQNSAQIAKALVNAQVDFQAMW YSDQNHGLSGLSTNHLYTHMTHFLKQCFSLSDGKKKKK GHHHHHH
66	Murine OX40	UniProt no. P47741, version 143 MYVWVQQPTALLLLALTLGVTARRLNCVKHTYPSGHKCC RECQPGHGMVSRCDHTRDTLCHPCETGFYNEAVNYDTCK QCTQCNHRSGSELKQNCTPTQDTVCRCRPGTQPRQDSGY KLGVDCVPCPPGHFSPGNNQACKPWTNCTLSGKQTRHPA SDSLDAVCEDRSLLATLLWETQRPTFRPTTVQSTTVWPR TSELPSPPTLVTPEGPAFAVLLGLGLGLLAPLTVLLALY LLRKAWRLPNTPKPCWGNSFRTPIQEEHTDAHFTLAKI
67	Peptide linker (G4S)	GGGGS
68	Peptide linker $(G4S)_2$	GGGGSGGGGS
69	Peptide linker $(SG4)_2$	SGGGGSGGGG

SEQ ID NO:	Name	Sequence
70	Peptide linker G4(SG4)$_2$	GGGGSGGGGSGGGG
71	peptide linker	GSPGSSSSGS
72	(G4S)$_3$ peptide linker	GGGGSGGGGSGGGGS
73	(G4S)$_4$ peptide linker	GGGGSGGGGSGGGGSGGGGS
74	peptide linker	GSGSGSGS
75	peptide linker	GSGSGNGS
76	peptide linker	GGSGSGSG
77	peptide linker	GGSGSG
78	peptide linker	GGSG
79	peptide linker	GGSGNGSG
80	peptide linker	GGNGSGSG
81	peptide linker	GGNGSG
82	Fc knob chain	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
83	Fc hole chain	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
84	Acceptor framework IGHJ6*01/02	YYYYYGMDVWGQGTTVTVSS
85	Acceptor framework IGKJ4*01/02	LTFGGGTKVEIK
86	OX40(49B4) VHCH1-OX40(49B4) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	See Table 13
87	OX40(49B4) light chain	See Table 13
88	FAP(1G1a) VLCH1-light chain	See Table 13
89	OX40(49B4) VHCH1-OX40(49B4) VHCH1-Fc hole_PGLALA	See Table 13
90	OX40(49B4) VHCH1- Fc hole_PGLALA	See Table 13
91	OX40(49B4) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	See Table 13
92	OX40(CLC563) VHCH1-OX40(CLC563) VHCH1-Fc knob_PGLALA-FAP(1G1a) VHCL	See Table 13
93	OX40(CLC563) light chain	See Table 13
94	FAP(1G1a) VLCH1-light	See Table 13

SEQ ID NO:	Name	Sequence
	chain (EPKSCD)	
95	OX40(CLC563) VHCH1-OX40(CLC563) VHCH1-Fc hole_PGLALA	See Table 13
96	FAP(1G1a) VLCH1-light chain (EPKSCS)	See Table 13
97	OX40(CLC563) VHCH1-Fc knob_PGLALA-FAP(1G1a) VHCL	See Table 13
98	OX40(CLC563) VHCH1 Fc hole_PGLALA	See Table 13
99	OX40(MOXR0916) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	See Table 13
100	OX40(MOXR0916) light chain	See Table 13
101	OX40(MOXR0916) VHCH1-OX40(MOXR0916) VHCH1- Fc hole_PGLALA-	See Table 13
102	OX40(MOXR0916) VHCH1- Fc hole_PGLALA	See Table 13
103	OX40(8H9) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	See Table 13
104	OX40(8H9) light chain	See Table 13
105	OX40(8H9) VHCH1-OX40(8H9) VHCH1- Fc hole_PGLALA	See Table 13
106	OX40(8H9) VHCH1- Fc hole_PGLALA	See Table 13
107	OX40(49B4_K73E) VHCH1-OX40(49B4_K73E) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	See Table 15
108	OX40(49B4_K73E) VHCH1-OX40(49B4_K73E) VHCH1-Fc hole_PGLALA	See Table 15
109	OX40(49B4_K23T_K73E) VHCH1-OX40(49B4_K23T_K73E) VHCH1- Fc	See Table 15

SEQ ID NO:	Name	Sequence
	knob_PGLALA-FAP(1G1a) VHCL	
110	OX40(49B4_K23T_K73E) VHCH1-OX40(49B4_K23T_K73E) VHCH1-Fc hole_PGLALA	See Table 15
111	OX40(49B4_K23E_K73E) VHCH1-OX40(49B4_K23E_K73E) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	See Table 15
112	OX40(49B4_K23E_K73E) VHCH1-OX40(49B4_K23E_K73E) VHCH1-Fc hole_PGLALA	See Table 15
113	OX40(49B4) VHCH1-OX40(49B4) VHCH1- Fc hole_PGLALA – FAP(4B9) VL	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQ APGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTST AYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSQV QLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAA GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCTLP PSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGSEIV LTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEP EDFAVYYCQQGIMLPPTFGQGTKVEIK
114	OX40(49B4) VHCH1-OX40(49B4) VHCH1- Fc knob_PGLALA – FAP(4B9) VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQ APGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTST AYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSQV QLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAA GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLP PCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGSEVQ

SEQ ID NO:	Name	Sequence
		LLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAIIGSGASTYYADSVKGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSS
115	OX40 (49B4) VLCkappa	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQK PGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISSL QPDDFATYYCQQYSSQPYTFGQGTKVEIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS GNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC
116	OX40(49B4) VHCH1-OX40(49B4) VHCH1- IgG1 Fc _PGLALA	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQ APGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTST AYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSQV QLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAA GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSP
117	CD3 CDR-H1	TYAMN
118	CD3 CDR-H2	RIRSKYNNYATYYADSVKG
119	CD3 CDR-H2	HGNFGNSYVSWFAY
120	CD3 CDR-L1	GSSTGAVTTSNYAN
121	CD3 CDR-L2	GTNKRAP
122	CD3 CDR-L3	ALWYSNLWV
123	CD3 VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQ APGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSK NTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQ GTLVTVSS
124	CD3 VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQ EKPGQAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLS GAQPEDEAEYYCALWYSNLWVFGGGTKLTVL
125	CD3 (Cl22) CDR-H1	SYAMN
126	CD3 (Cl22) CDR-H2	RIRSKYNNYATYYADSVKG
127	CD3 (Cl22) CDR-H3	HTTFPSSYVSYYGY
128	CD3 (Cl22) CDR-L1	GSSTGAVTTSNYAN
129	CD3 (Cl22) CDR-L2	GTNKRAP
130	CD3 (Cl22) CDR-L3	ALWYSNLWV
131	CD3 (Cl22) VH	EVQLLESGGGLVQPGGSLRLSCAASGFQFSSYAMNWVRQ APGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSK NTLYLQMNSLRAEDTAVYYCVRHTTFPSSYVSYYGYWGQ GTLVTVSS
132	CD3 (Cl22) VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQ EKPGQAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLS GAQPEDEAEYYCALWYSNLWVFGGGTKLTVL
133	CD3 (V9) CDR-H1	GYSFTGYTMN

SEQ ID NO:	Name	Sequence
134	CD3 (V9) CDR-H2	LINPYKGVSTYNQKFKD
135	CD3 (V9) CDR-H3	SGYYGDSDWYFDV
136	CD3 (V9) CDR-L1	RASQDIRNYLN
137	CD3 (V9) CDR-L2	YTSRLES
138	CD3 (V9) CDR-L3	QQGNTLPWT
139	CD3 (V9) VH	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQ APGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNT AYLQMNSLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTL VTVSS
140	CD3 (V9) VL	DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQK PGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSL QPEDFATYYCQQGNTLPWTFGQGTKVEIK
141	Light chain „CEA $_{2F1}$" (CEA TCB)	DIQMTQSPSSLSASVGDRVTITCKASAAVGTYVAWYQQK PGKAPKLLIYSASYRKRGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCHQYYTYPLFTFGQGTKLEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC EVTHQGLSSPVTKSFNRGEC
142	Light Chain humanized CD3 $_{CH2527}$ (Crossfab, VL-CH1) (CEA TCB)	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQ EKPGQAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLS GAQPEDEAEYYCALWYSNLWVFGGGTKLTVLSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSC
143	CEA $_{CH1A1A\ 98/99}$ — humanized CD3 $_{CH2527}$ (Crossfab VH-Ck)—Fc(knob) P329GLALA (CEA TCB)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFGMNWVRQ APGQGLEWMGWINTKTGEATYVEEFKGRVTFTTDTSTST AYMELRSLRSDDTAVYYCARWDFAYYVEAMDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGG SEVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVR QAPGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDS KNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWG QGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECD KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP
144	CEA $_{CH1A1A\ 98/99}$ (VH-CH1)—Fc(hole) P329GLALA (CEA TCB)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFGMNWVRQ APGQGLEWMGWINTKTGEATYVEEFKGRVTFTTDTSTST AYMELRSLRSDDTAVYYCARWDFAYYVEAMDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQ VCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSP
145	CD3 VH-CL (CEACAM5 TCB)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQ APGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSK NTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQ

SEQ ID NO:	Name	Sequence
		GTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
146	humanized CEA VH-CH1(EE)-Fc (hole, P329G LALA) (CEACAM5 TCB)	QVQLVQSGAEVKKPGSSVKVSCKASGFNIKDTYMHWVRQ APGQGLEWMGRIDPANGNSKYVPKFQGRVTITADTSTST AYMELSSLRSEDTAVYYCAPFGYYVSDYAMAYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQ VCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSP
147	humanized CEA VH-CH1(EE)-CD3 VL-CH1-Fc (knob, P329G LALA) (CEACAM5 TCB)	QVQLVQSGAEVKKPGSSVKVSCKASGFNIKDTYMHWVRQ APGQGLEWMGRIDPANGNSKYVPKFQGRVTITADTSTST AYMELSSLRSEDTAVYYCAPFGYYVSDYAMAYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCDGGGGSGGGG SQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWV QEKPGQAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTL SGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRD ELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSP
148	humanized CEA VL-CL(RK) (CEACAM5 TCB)	EIVLTQSPATLSLSPGERATLSCRAGESVDIFGVGFLHW YQQKPGQAPRLLIYRASNRATGIPARFSGSGSGTDFTLT ISSLEPEDFAVYYCQQTNEDPYTFGQGTKLEIKRTVAAP SVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC
149	VH (PD-L1)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQ APGKGLEWVAWISPYGGSTYYADSVKGRFTISADTSKNT AYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVS S
150	VL (PD-L1)	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQK PGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQYLYHPATFGQGTKVEIK
151	VH (PD-L1)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQ APGKGLEWVANIKQDGSEKYYVDSVKGRFTISRDNAKNS LYLQMNSLRAEDTAVYYCAREGGWFGELAFDYWGQGTLV TVSS
152	VL (PD-L1)	EIVLTQSPGTLSLSPGERATLSCRASQRVSSSYLAWYQQ KPGQAPRLLIYDASSRATGIPDRFSGSGSGTDFTLTISR LEPEDFAVYYCQQYGSLPWTFGQGTKVEIK
153	CH2 domain	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVWDVSHED PEVKFNWYVDGVEVHNAKTKPREEQESTYRWSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAK
154	CH3 domain	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ

SEQ ID NO:	Name	Sequence
		QGNVFSCSVMHEALHNHYTQKSLSLSP
155	Fc IgG1, caucasian allotype	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK
156	Fc IgG1, afroamerican allotype	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK
157	Fc IgG2	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGT QTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNG KEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTT PPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
158	Fc IgG3	ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRCPEP KSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPC PRCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGN IFSCSVMHEALHNRFTQKSLSLSPGK
159	Fc IgG4	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT KTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFN WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
160	Hinge full	DKTHTCPXCP with X being S or P
161	Hinge middle	HTCPXCP with X being S or P
162	Hinge short	CPXCP with X being S or P
163	CH1 connector C-terminal end	EPKSC
164	CH1 connector C-terminal end D-variant	EPKSCD
165	CH1 connector C-terminal end S-variant	EPKSCS

SEQ ID NO:	Name	Sequence
166	CH1 domain	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDKKV

The following numbered paragraphs (paras) describe aspects of the present invention:

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

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

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

4. The bispecific antigen binding molecule of any one of paras 1 to 3, wherein the antigen binding domain capable of specific binding to FAP comprises
a heavy chain variable region (V_HFAP) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18. SEQ ID NO:19 and SEQ ID NO:20, and
a light chain variable region (V_LFAP) comprising an amino acid sequence selected from

the group consisting of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 and SEQ ID NO:26.

5. The bispecific antigen binding molecule of any one of paras 1 to 4, wherein the antigen binding domain capable of specific binding to FAP comprises
(a) a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:15 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:21, or
(b) a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:16 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:21, or
(c) a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:16 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:22, or
(d) a heavy chain variable region (V_HFAP) comprising the amino acid sequence of SEQ ID NO:19 and a light chain variable region (V_LFAP) comprising the amino acid sequence of SEQ ID NO:25.

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

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

(iii) a heavy chain variable region (V_HOX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:43, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:44, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:45, and a light chain variable region (V_LOX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:47, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:48, or

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

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

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

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

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

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

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

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

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

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

10. The bispecific antigen binding molecule of any one of paras 1 to 9, wherein the Fc region is an IgG, particularly an IgG1 Fc region or an IgG4 Fc region.

11. The bispecific antigen binding molecule of any one of paras 1 to 10, wherein the Fc region is of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

12. The bispecific antigen binding molecule of any one of paras 1 to 15, wherein the bispecific antigen binding molecule comprises

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

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

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

13. The bispecific antigen binding molecule of para 12, wherein the VH-Ckappa chain of the cross-fab fragment capable of specific binding to FAP is fused to the C-terminus of one of subunits of the Fc region.

14. The bispecific antigen binding molecule of any one of paras 1 to 13, consisting of

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

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

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

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

15. The bispecific antigen binding molecule of any one of paras 1 to 13, consisting of

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

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

(ac) a third Fab fragment capable of specific binding to OX40,

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

(c) the Fc region composed of a first and a second subunit capable of stable association, wherein the second Fab fragment (ab) is fused at the C-terminus of the VH-CH1 chain to the N-terminus of the VH-CH1 chain of the first Fab fragment (aa), which is in turn fused at its C-terminus to the N-terminus of the first subunit, and the third Fab fragment (ac) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit.

16. The bispecific antigen binding molecule of any one of paras 1 to 13, consisting of

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

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

(ac) a third Fab fragment capable of specific binding to OX40,

(ad) a fourth Fab fragment capable of specific binding to OX40,

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

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

17. Isolated nucleic acid encoding the bispecific antigen binding molecule of any one of paras 1 to 16.

18. An expression vector comprising the isolated nucleic acid of para 17.

19. A host cell comprising isolated nucleic acid of para 17 or the expression vector of para 18.

20. A method of producing a bispecific antigen binding molecule, comprising culturing the host cell of para 19 under conditions suitable for the expression of the bispecific antigen binding molecule, and isolating the bispecific antigen binding molecule.

21. A pharmaceutical composition comprising the bispecific antigen binding molecule of any one of paras 1 to 16 and a pharmaceutically acceptable carrier.

22. The pharmaceutical composition of para 21, further comprising an additional therapeutic agent.

23. The bispecific antigen binding molecule of any one of paras 1 to 16, or the pharmaceutical composition of para 21, for use as a medicament.

24. The bispecific antigen binding molecule of any one of claims 1 to 16, or the pharmaceutical composition of para 21, for use

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

25. The bispecific antigen binding molecule of any one of paras 1 to 16, or the pharmaceutical composition of para 21, for use in the treatment of cancer.

26. The bispecific antigen binding molecule according to any one of paras 1 to 16 or the pharmaceutical composition according to para 21 for use in the treatment of cancer, wherein the bispecific antigen binding molecule or pharmaceutical composition is for administration in combination with a chemotherapeutic agent, radiation and/ or other agents for use in cancer immunotherapy.

27. Use of the bispecific antigen binding molecule of any one of paras 1 to 16, or the pharmaceutical composition of para 21, in the manufacture of a medicament for the treatment of cancer.

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

EXAMPLES

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

Recombinant DNA techniques

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

DNA sequencing

DNA sequences were determined by double strand sequencing.

Gene synthesis

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 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 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.

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 3.0 followed by immediate neutralization of the sample. Aggregated protein was separated from monomeric antibodies by ion exchange chromatography (Poros XS) with equilibration

buffer 20 mM His, pH 5.5, 1,47 mS/cm and elution buffer 20 mM His, 500 mM NaCl, pH 5.5, 49.1 mS/cm, (gradient: to 100% elution buffer in 60 CV). In some cases a size exclusion chromatography (Superdex 200, GE Healthcare) in PBS or in 20 mM Histidine, 140 mM NaCl pH 6.0 was subsequently performed. 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 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-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.

CE-SDS

Purity, antibody integrity and molecular weight of bispecific and control antibodies were analyzed by CE-SDS using microfluidic Labchip technology (Caliper Life Science, USA). 5 µl of protein solution was prepared for CE-SDS analysis using the HT Protein Express Reagent Kit according manufacturer's instructions and analysed on LabChip GXII system using a HT Protein Express Chip. Data were analyzed using LabChip GX Software version 3.0.618.0.

Analytical size exclusion chromatography

The aggregate content of the molecule was analyzed using a BioSuite 250 5µm.7.8x300 analytical size-exclusion column (Tosoh) in 200 mM K-Phosophat 250 mM KCl pH 6.2 running buffer at 25° C.

Mass spectrometry

This section describes the characterization of the multispecific antibodies with VH/VL or CH/CL exchange (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/FabALACTICA or alternatively deglycosylated/GingisKHAN digested CrossMabs.

The 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 FabALACTICA or

GingisKHAN (Genovis AB; Sweden) digestions were performed in the buffers supplied by the vendor with 100 µg deglycosylated CrossMabs. 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 (Advion).

Example 1

Generation of new antibodies against fibroblast activation protein (FAP)

1.1 Immunization of mice

Balb/c and NMRI mice were used for immunization. The animals were housed according to the Appendix A "Guidelines for accommodation and care of animals" in an AAALACi accredited animal facility. All animal immunization protocols and experiments were approved by the Government of Upper Bavaria (permit number 55.2-1-54-2531-19-10) and performed according to the German Animal Welfare Act and the Directive 2010/63 of the European Parliament and Council. Balb/c and NMRI mice (n=5), 6-8 week old, received four rounds of immunization with recombinant produced extracellular domain of human fibroblast activation protein alpha (amino acid 27-759; accession number NP_004451) covalently attached to a His tag (SEQ ID NO:62). Before each immunization, mice were anesthetized with a gas mixture of oxygen and isoflurane. For the first immunization, 30 µg protein dissolved in PBS, pH 7.4, were mixed with an equal volume of CFA (BD Difco, #263810) and administered intraperitoneal (i.p.) Another 10 µg of protein emulsified in Abisco adjuvant was administered subcutaneously (s.c.) at week 6. A third dose of 5 µg protein without adjuvant was administered i.p. at week 10. Finally, three days prior to the preparation of splenocytes for antibody development using hybridoma technology, the mice were subjected to intravenous (i.v.) booster immunizations with 50 µg of protein. Serum was tested for antigen-specific total IgG antibody production by ELISA. Three days after the final immunization, mice were euthanized and the spleen was isolated aseptically and prepared for hybridoma generation. The mouse lymphocytes were isolated and fused with a mouse myeloma cell line using PEG based standard protocols to generate hybridomas. The resulting hybridoma cells were plated at approximately 10^4 in flat bottom 96 well micro titer plate, followed by about two weeks of incubation in selective medium and then screened for the production of antigen-specific antibodies. Once extensive hybridoma growth occurs, the antibody secreting hybridomas are replated. Hybridoma supernatants were screened for specific binding to recombinant human fibroblast activation protein alpha (huFAP) by ELISA, followed by evaluation of kinetic binding parameters to recombinant huFAP using Biacore measurement.

Culture of hybridomas: Generated muMAb hybridomas were cultured in RPMI 1640 (PAN – Catalogue No. (Cat. No.) PO4-17500) supplemented with 2 mM L-glutamine (GIBCO - Cat. No.35050-038), 1 mM Na-Pyruvat (GIBCO - Cat. No.11360-039), 1x NEAA (GIBCO - Cat. No.11140-035), 10% FCS (PAA - Cat. No. A15-649), 1x Pen Strep (Roche - Cat. No.1074440), 1x Nutridoma CS (Roche - Cat. No.1363743), 50 µM Mercaptoethanol (GIBCO - Cat. No.31350-010) and 50 U/ml IL 6 mouse (Roche - Cat. No.1 444 581) at 37°C and 5% CO_2.

1.2 Format-depending binding of anti-huFAP clones

In order to determine if the binding properties of the anti-FAP clones are not lost when they are C-terminally fused to an Fc domain, constructs comprising a Fc knob chain and a Fc hole chain wherein the VH domain is fused to the C-terminus of the Fc knob chain and the VL domain is fused to the C-terminus of the Fc hole chain (C-terminal VH/VL fusion) and constructs comprising a Fc knob chain and a Fc hole chain wherein the whole Fab is fused with its VH domain to the C-terminus of the Fc knob chain (C-terminal Fab fusion) were prepared. The Fc knob chain has the amino acid sequence of SEQ ID NO:82 and the Fc hole chain has the amino acid sequences of SEQ ID NO:83.

The affinity of the constructs towards biotinylated recombinant human FAP and biotinylated recombinant cynomolgus FAP as compared to the antibodies is shown in Table 3 below.

Table 3: Affinity to human FAP and cynomolgus FAP as measured by Biacore

	Affinity to human FAP KD [nM]			Affinity to cynomolgus FAP KD [nM]		
clone	free Fab	C-terminal Fab fusion	C-terminal VH/VL fusion	IgG	C-terminal Fab fusion	C-terminal VH/VL fusion
209	0.31	1.52	42.40	0.33	1.60	50.00
210	0.07	0.17	3.95	0.12	0.20	3.44
211	0.28	1.20	10.90	0.32	1.30	11.40
212	0.12	0.62	5.72	0.14	0.64	6.19
214	0.06	0.19	2.49	0.09	0.21	2.77

Cellular binding of the constructs to FAP-transfected HEK cells has also been determined as described herein before. The EC_{50} values are shown in Table 4. The C-terminal fusion constructs of all anti-FAP antibodies were able to bind to human and cynomolgus FAP, however the constructs wherein the whole Fab is fused with its VH domain to the C-terminus of the Fc knob chain were superior to those wherein the VH domain is fused to the C-terminus of the Fc knob chain and the VL domain is fused to the C-terminus of the Fc hole chain.

Table 4: Cellular binding to huFAP expressing cells

	Cellular binding to human FAP EC_{50} [µg/ml]			Cellular binding to cynomolgus FAP EC_{50} [µg/ml]		
clone	IgG	C-terminal Fab fusion	C-terminal VH/VL fusion	IgG	C-terminal Fab fusion	C-terminal VH/VL fusion
209	0,15	1.2	5.7	0.4	1.1	7.9
210	0,13	1.8	9.0	0.4	1.3	7.1
211	0,20	3.7	9.3	0.3	2.9	6.7
212	0,12	2.8	8.8	0.3	2.3	11.1
214	0,09	1.7	9.4	0.3	1.3	3.6

1.3 Competitive cellular binding of anti-huFAP antibodies to FAP clone 4B9 and 28H1

The resulting clones were tested for their binding behavior in comparison to FAP clone 4B9. The generation and preparation of FAP clones 4B9 and 28H1 is described in WO 2012/020006 A2, which is incorporated herein by reference. To determine whether the murine FAP clones recognize different epitopes as clones 4B9 and 28H1 a competition binding to human FAP expressed on transfected HEK cells was performed.

Briefly, the target cells were harvested with Cell Dissociation buffer, washed with FACS Buffer (PBS + 2 % FCS + 5 mM EDTA + 0.25 % sodium acide) and seeded into 96-U bottom plates (1x105 cells/well). Unlabeled primary anti-human FAP antibodies (mu IgG1) were added to the cells (final concentrations 60 µg/ml to 0.2 µg/ml; 1:3 dilutions) and incubated for 20 min at 4°C before addition of AlexaFluor647-labeled anti FAP

antibody 4B9 or 28H1 (end concentration 20 µg/ml). After 30 min incubation at 4°C, cells were washed, fixed and the fluorescent signal intensities of the AF647-labeled clones 4B9 and 28H1 were measured using a Miltenyi MACSQuant.

10 hybridoma-derived murine antibodies were identified (named clones 209, 210, 211, 212, 213, 214, 215, 216, 217 and 218) that did not compete for binding with anti FAP antibodies 4B9 or 28H1.

1.4 Target binding specificity of anti-huFAP murine antibodies

Fibroblast activation protein (FAP, FAP-α, seprase) is a type II transmembrane serine protease, belonging to the prolyl oligopeptidase family. This family comprises serine proteases that cleave peptides preferentially after proline residues. Other important members of this family that are expressed in the human proteome are prolyl oligopeptidase (PREP) and the dipeptidyl peptidases (DPPs). DPP-IV is the closest homolog of FAP. In contrast to FAP, DPP-IV is ubiquitously expressed and plays a role in various biological processes such as T cell co-stimulation, chemokine biology, glucose metabolism, and tumorigenesis and therefore the desired anti-human FAP antibodies should not bind to human DPP-IV.

Binding to human FAP and human DPP-IV was determined by flow cytometry using human FAP or human DPPIV-transfected HEK cells. Briefly, the target cells were harvested with Cell Dissociation buffer, washed with FACS Buffer (PBS + 2 % FCS + 5 mM EDTA + 0.25 % sodium acide) and seeded into 96-U bottom plates ($1x10^5$ cells/well). Unlabeled primary antibodies were added to the cells (final concentration 10 µg/ml) and incubated for 30 min at 4°C. After washing, cells were incubated with a goat anti-mouse IgG-PE F(ab')2 (Serotec) for 30 min at 4°C in the dark. Afterwards, cells were washed, fixed and measured using a BD FACS Canto™ II. No unspecific binding to human DPP-IV was detected for any of the 10 hybridoma derived anti-human FAP antibodies.

1.5 Generation of anti-huFAP antibodies in huIgG1_LALA_PG format

The DNA sequences of the new anti-huFAP antibodies were determined with standard sequencing methods. Based on the VH and VL domains new anti-FAP antibodies were expressed as huIgG1 antibodies with an effector silent Fc (P329G; L234, L235A) to abrogate binding to Fcγ receptors according to the method described in WO 2012/130831 A1. In detail, antibodies were expressed by transient transfection of HEK293-F cells grown in suspension with expression vectors encoding the different peptide chains. Transfection into HEK293-F cells (Invitrogen, USA) was performed according to the cell supplier's instructions using Maxiprep (Qiagen, Germany) preparations of the antibody vectors, F17

based medium (Invitrogen, USA), PEIpro (Polyscience Europe GmbH) and an initial cell density of 1–2 million viable cells/ml in serum free FreeStyle 293 expression medium (Invitrogen). Cell culture supernatants were harvested after 7 days of cultivation in shake flasks or stirred fermenters by centrifugation at 14000 g for 30 minutes and filtered through a 0.22 µm filter.

The antibodies were purified from cell culture supernatants by affinity chromatography using MabSelectSure-Sepharose™ (GE Healthcare, Sweden) chromatography. Briefly, sterile filtered cell culture supernatants were captured on a MabSelect SuRe resin equilibrated with PBS buffer (10 mM Na_2HPO_4, 1 mM KH_2PO_4, 137 mM NaCl and 2.7 mM KCl, pH 7.4), washed with equilibration buffer and eluted with 25 mM citrate, pH 3.0. After neutralization with 1 M Tris pH 9.0, aggregated protein was separated from monomeric antibody species by size exclusion chromatography (Superdex 200, GE Healthcare) in 20 mM histidine, 140 mM NaCl, pH 6.0. Monomeric protein fractions were pooled, concentrated if required using e.g. a MILLIPORE Amicon Ultra (30KD MWCO) centrifugal concentrator and stored at −80°C. Sample aliquots were used for subsequent analytical characterization e.g. by CE-SDS, size exclusion chromatography, mass spectrometry and endotoxin determination.

1.6 Cellular binding of anti-huFAP antibodies

The binding of anti-FAP antibodies with a human IgG1 P329G LALA Fc to human FAP was determined by flow cytometry using human FAP-transfected HEK cells. Briefly, the target cells were harvested with Cell Dissociation buffer, washed with FACS Buffer (PBS + 2 % FCS + 5 mM EDTA + 0.25 % sodium acide) and seeded into 96-U bottom plates (1x105 cells/well). Unlabeled primary antibodies were added to the cells (final concentrations 10 µg/ml to 0.64 ng/ml; 1:5 dilutions) and incubated for 30 min at 4°C. After washing, cells were incubated with a PE-conjugated AffiPure $F(ab)_2$ Fragment Goat anti-human IgG, Fcγ specific (Jackson Immunoresearch) for 30 min at 4°C in the dark. Afterwards, cells were washed, fixed and measured using a BD FACS LSR Fortessa™.

All anti-FAP antibodies showed similar binding to human FAP as seen before. The EC_{50} values of selected binders are shown in Table 1 below.

Table 1: Cellular binding of anti-FAP antibodies to huFAP expressing cells

Sample ID	clone	EC_{50} [µg/ml] cellular binding to FAP-transfected HEK cells
	4B9	0.089

Sample ID	clone	EC_{50} [µg/ml] cellular binding to FAP-transfected HEK cells
P1AD9427	209	0.145
P1AD9436	210	0.125
P1AD9437	211	0.198
P1AD9438	212	0.118
P1AD9440	214	0.086

1.7 Cellular internalization of anti-huFAP antibodies

Internalization of FAP binders was determined using human FAP-transfected HEK cells as targets. Briefly, the target cells were harvested with Cell Dissociation buffer, washed with cold FACS Buffer (PBS + 2 % FCS + 5 mM EDTA + 0.25 % sodium acide) and resuspended at 1.5 x 10^6 cells/ml in cold FACS Buffer. Cells were distributed in 15 ml tubes (each tube containing 3x10^6 cells in 2 ml). 2 ml of anti-human FAP antibody solutions were added to the cells (final concentration 20 µg/ml) and incubated for 45 min at 4 °C. Afterwards, cells were washed, resuspended in cold FACS Buffer and cells for time point "0" were seeded immediately into 96-U bottom plates (1.5x10^5 cells/well) and kept at 4°C whereas all other cells were centrifuged, resuspended in warm RPMI1640 medium containing 10 % FCS and 1 % Glutamax (1.5x10^6 cells/ml) and shifted to 37°C in a humidified incubator (5 % CO_2). After each indicated time point, 100 µl/tube of cell suspension was transferred to plates, immediately cooled down with cold FACS Buffer and stored in the fridge until all time points have been collected. After collection of all time points, cells were washed with cold FACS Buffer and incubated with PE-labeled secondary antibody for 30 min at 4°C. Afterwards, cells were washed, fixed and and measured using a BD FACS Canto™ II.

The signals caused by the labeled secondary antibody stayed nearly constant over time, which means that no loss of antibody was observed over time, none of the tested anti-hu FAP antibodies was internalized.

1.8 Binding Kinetics of anti-huFAP antibodies

To evaluate human FAP binding kinetics, biotinylated human FAP was immobilized on a Series S Biacore CAPture Chip (GE Healthcare 28-9202-34) according to the manufacturer's instructions, resulting in a surface density of approximately 20 resonance units (RU). As running and dilution buffer, HBS-P+ (10 mM HEPES, 150 mM NaCl pH 7.4, 0.05% Surfactant P20) was used. A dilution series of anti-huFAP Fabs (3.7 – 300 nM,

1:3 dilution) was successively injected for 120s each, dissociation was monitored for 1800s at a flow rate of 30 µl/min (single cycle kinetics). The surface was regenerated by injecting 6 M guanidine-HCl, 0.25 M NaOH for 120s. Bulk refractive index differences were corrected by subtracting blank injections and by subtracting the response obtained from the control flow cell without captured human FAP. Curve fitting was performed using the 1:1 Langmuir binding model within the Biacore evaluation software. The affinity data are shown in Table 2 below.

Table 2: Affinity of anti-FAP Fabs to human FAP as measured by Biacore

Sample ID	clone	ka (1/Ms)	kd (1/s)	**KD**
	4B9_Fab	1.82E+06	7.80E-04	430 pM
P1AD9427_Fab	209	3.50E+06	1.77E-03	510 pM
P1AD9436_Fab	210	1.87E+06	< E-06	< 10 pM
P1AD9437_Fab	211	8.13E+05	4.61E-05	60 pM
P1AD9438_Fab	212	1.06E+06	< E-06	< 10 pM
P1AD9440_Fab	214	1.99E+06	< E-06	< 10 pM

1.9 Competitive Binding of anti-human FAP clones as determined by Biacore

Epitope binning was performed using a surface plasmon resonance (SPR) based assay on a Biacore T200 instrument. FAP antigen was captured by an immobilized anti-His antibody. In a first step the FAP-binder was injected until saturation. A second FAP-binder was injected subsequently. The assay design is schematically shown in Figure 3C. An increase in binding signal after addition of the second antibody indicates its binding to a different epitope from the first antibody. No additional binding indicated that the first and the second antibody recognize the same epitope region.

An anti-His antibody (GE Healthcare Kit 28-9950-56) with a concentration of 20 µg/ml was immobilized by amine coupling (GE Healthcare Kit BR-1000-50) to the surface of a CM5 sensor chip (GE Healthcare BR-1005-30). Injection time was 600 seconds at a flow rate of 10 µl/min to yield 12000 response units (RU) on two flow cells, one used as reference and one used as active flow cell. Running buffer was HBS-N (GE Healthcare BR-1006-70). For the measurement PBS-P+ (GE Healthcare 28-9950-84) was used as

running and dilution buffer. Flow cell temperature was set to 25°C, sample compartment to 12°C. The flow rate was set to 10µl/min for the whole run.

His-tagged FAP antigen was captured with a concentration of 20 µg/ml for 180 seconds on the active flow cell. The first and second antibody (FAP-binder) were injected successively, each for 120 seconds at a concentration of 10 µg/ml over both flow cells. After each cycle the surface was regenerated with 10 mM glycine pH1.5 for 60 seconds (GE Healthcare BR-1003-54).

The results are shown in Table 5 below:

Table 5: Competitive Binding of anti-FAP antibodies to 4B9

	4B9	209	210	211	212	214
4B9	Competitive Binding	Simultaneous Binding	Simultaneous Binding	Simultaneous Binding	Simultaneous Binding	Simultaneous Binding
209	Simultaneous Binding	Competitive Binding	Simultaneous Binding	Simultaneous Binding	Simultaneous Binding	Simultaneous Binding
210	Simultaneous Binding	Simultaneous Binding	Competitive Binding	Competitive Binding	Competitive Binding	Competitive Binding
211	Simultaneous Binding	Simultaneous Binding	Competitive Binding	Competitive Binding	Competitive Binding	Competitive Binding
212	Simultaneous Binding	Simultaneous Binding	Competitive Binding	Competitive Binding	Competitive Binding	Competitive Binding
214	Simultaneous Binding	Simultaneous Binding	Competitive Binding	Competitive Binding	Competitive Binding	Competitive Binding

Thus, three epitope bins were identified. None of the anti-FAP antibodies did compete for binding with antibody 4B9 (Epitope bin 1). Antibodies 210, 211, 212 and 214 competed with each other for binding and thus form one group (Epitope bin 3), whereas antibody 209 did not compete for binding with any other of the antibodies (Epitope bin 2).

1.9 Thermal stability evaluation of anti-FAP antibodies

Samples are prepared at a concentration of 1 mg/mL in 20 mM Histidine/Histidine chloride, 140 mM NaCl, pH 6.0, transferred into an optical 384-well plate by centrifugation through a 0.4 µm filter plate and covered with paraffine oil. The hydrodynamic radius is measured repeatedly by dynamic light scattering on a DynaPro Plate Reader (Wyatt) while the samples are heated with a rate of 0.05 °C/min from 25 °C to 80 °C. Alternatively, samples were transferred into a 10 µL micro-cuvette array and static light scattering data as well as fluorescence data upon excitation with a 266 nm laser were recorded with an Optim1000 instrument (Avacta Inc.), while they were heated at a rate of 0.1 °C/min from 25 °C to 90 °C. The aggregation onset temperature (T_{agg}) is defined as the temperature at which the hydrodynamic radius (DLS) or the scattered light intensity (Optim1000) starts to increase. The melting temperature is defined as the inflection point in a graph showing fluorescence intensity vs. wavelength. The aggregation onset temperatures of selected anti-FAP antibodies is shown in Table 6.

Table 6: Aggregation onset temperatures of anti-FAP antibodies

	4B9	209	210	212	214
T_{agg} (°C)	60	66	61	67	61

The anti-FAP clone 212 was chosen for humanization as it binds with a comparable high affinity to human FAP as antibody 4B9 and showed favorable properties for the development. In silico analysis of its sequences indicated only one predicted degradation hotspot (Trp at position 401). The sequences of murine clone 212 are shown in Table 7.

Table 7: Amino acid sequences of the variable domains of murine anti-FAP clone 212

Description	Sequence	Seq ID No
FAP(212) VH	EVLLQQSGPELVKPGASVKIACKASGYTLTDYNMDWVRQS HGKSLEWIGDIYPNTGGTIYNQKFKGKATLTIDKSSSTAY MDLRSLTSEDTAVYYCTRFRGIHYAMDYWGQGTSVTVSS	9
FAP(212) VL	DIVLTQSPVSLAVSLGQRATISCRASESVDNYGLSFINWF QQKPGQPPKLLIYGTSNRGSGVPARFSGSGSGTDFSLNIH PMEEDDTAMYFCQQSNEVPYTFGGGTNLEIK	10

1.10 Humanization of anti-FAP clone 212

1.10.1 Methodology

Suitable human acceptor frameworks were identified by querying a BLASTp database of human V- and J-region sequences for the murine input sequences (cropped to the variable part). Selective criteria for the choice of human acceptor framework were sequence homology, same or similar CDR lengths, and the estimated frequency of the human germline, but also the conservation of certain amino acids at the VH-VL domain interface. Following the germline identification step, the CDRs of the murine input sequences were grafted onto the human acceptor framework regions. Each amino acid difference between these initial CDR grafts and the parental antibodies was rated for possible impact on the structural integrity of the respective variable region, and "back mutations" towards the parental sequence were introduced whenever deemed appropriate. The structural assessment was based on Fv region homology models of both the parental antibody and the humanization variants, created with an in-house antibody structure homology modeling protocol implemented using the Biovia Discovery Studio Environment, version 17R2. In some humanization variants, "forward mutations" were included, i.e., amino acid exchanges that change the original amino acid occurring at a given CDR position of the parental binder to the amino acid found at the equivalent position of the human acceptor germline. The aim is to increase the overall human character of the humanization variants (beyond the framework regions) to further reduce the immunogenicity risk.

An in silico tool developed in-house was used to predict the VH-VL domain orientation of the paired VH and VL humanization variants (see WO 2016/062734). The results were compared to the predicted VH-VL domain orientation of the parental binders to select for framework combinations which are close in geometry to the original antibodies. The rationale is to detect possible amino acid exchanges in the VH-VL interface region that might lead to disruptive changes in the pairing of the two domains that in turn might have detrimental effects on the binding properties.

1.10.2 Choice of acceptor framework and adaptations thereof

The following acceptor frameworks were chosen:

Table 8: Acceptor framework

	Murine V-region germline	Graft variant	Choice of human acceptor V-region germline	Identity to human V-region germline after grafting

				(BLASTp):
FAP (212) VH	IGHV1-18*01	VH1	IGHV1-46*01	87.8 %
		VH2	IGHV3-23*03	82.7 %
FAP (212) VL	IGKV3-2*01	VL1	IGKV3-11*01	85.1 %
		VL2	IGKV1-39*01	82.8 %

Post-CDR3 framework regions were adapted from human IGHJ germline IGHJ6*01/02 (YYYYYGMDV**WGQGTTVTVSS,** SEQ ID NO:84) and human IGKJ germline IGKJ4*01/02 (LT**FGGGTKVEIK,** SEQ ID NO:85). The part relevant for the acceptor framework is indicated in bold script.

Based on structural considerations, back mutations from the human acceptor framework to the amino acid in the parental binder were introduced at positions H43 (Q>K), H44 (G>S), H48 (M>I), H71 (R>I), H73 (T>K), H93 (A>T) [VH1], H49 (S>G), H71 (R>I), H73 (N>K), H78 (L>A), H93 (A>T), H94 (K>R) [VH2], L36 (Y>F), L43 (A>P), L87 (Y>F) [VL1] and L36 (Y>F), L42 (K>Q), L43 (A>P), L85 (T>M), L87 (Y>F) [VL2].

Furthermore, the positions H60 (N>A), H64 (K>Q) [VH1], H60 (N>A), H61 (Q>D), H62 (K>S), H63 (F>V) [VH2], L33 (I>L), L34 (N>A) [VL1] and L27b (V>I), L33 (I>L) [VL2] were identified as promising candidates for forward mutations. All positions are given in the Kabat EU numbering scheme.

Table 9: list of variants

Variant name	Back/forward mutations	Identity to human V-region germline (BLASTp)
VH1G1a	bM48I, bR71I, bA93T	84.7 %
VH1G2a	bQ43K, bG44S, bM48I, bR71I, bT73K, bA93T	81.6 %
VH1G3a	bM48I, fN60A, fK64Q, bR71I, bA93T	86.7 %
VH2G1a	bS49G, bA93T, bK94R	79.6 %
VH2G2a	bS49G, bR71I, bN73K, bL78A, bA93T, bK94R	76.5 %
VH2G3a	bS49G, fN60A, fQ61D, fK62S, fF63V, bA93T, bK94R	83.7 %
VL1G1a	bY36F, bY87F	83 %
VL1G2a	bY36F, bA43P, bY87F	81.9 %
VL1G3a	fI33L, fN34A, bY36F, bY87F	85.1 %
VL2G1a	bY36F, bY87F	80.8 %
VL2G2a	bY36F, bK42Q, bA43P, bT85M, bY87F	77.8 %
VL2G3a	fV27bI, fI33L, bY36F, bY87F	82.8 %

Note: Back mutations are prefixed with b, forward mutations with f, e.g., bM48I refers to a back mutation (human germline amino acid to parental antibody amino acid) from methionine to isoleucine at position 48 (Kabat).

The resulting VH and VL domains of humanized FAP antibodies based on the acceptor framework can be found in Table 10 below.

Table 10: Amino acid sequences of the VH and VL domains of humanized FAP antibodies

Description	Sequence	Seq ID No
VH1G1a	QVQLVQSGAEVKKPGASVKVSCKASGYTLTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFKGRVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHYAMDYWGQGTTVTVSS	15
VH1G2a	QVQLVQSGAEVKKPGASVKVSCKASGYTLTDYNMDWVRQAPGKSLEWIGDIYPNTGGTIYNQKFKGRVTMTIDKSTSTVYMELSSLRSEDTAVYYCTRFRGIHYAMDYWGQGTTVTVSS	16
VH1G3a	QVQLVQSGAEVKKPGASVKVSCKASGYTLTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYAQKFQGRVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHYAMDYWGQGTTVTVSS	17
VH2G1a	EVQLLESGGGLVQPGGSLRLSCAASGYTLTDYNMDWVRQAPGKGLEWVGDIYPNTGGTIYNQKFKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRFRGIHYAMDYWGQGTTVTVSS	18
VH2G2a	EVQLLESGGGLVQPGGSLRLSCAASGYTLTDYNMDWVRQAPGKGLEWVGDIYPNTGGTIYNQKFKGRFTISIDKSKNTAYLQMNSLRAEDTAVYYCTRFRGIHYAMDYWGQGTTVTVSS	19
VH2G3a	EVQLLESGGGLVQPGGSLRLSCAASGYTLTDYNMDWVRQAPGKGLEWVGDIYPNTGGTIYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRFRGIHYAMDYWGQGTTVTVSS	20
VL1G1a	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFINWFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFTLTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIK	21
VL1G2a	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFINWFQQKPGQPPRLLIYGTSNRGSGIPARFSGSGSGTDFTLTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIK	22
VL1G3a	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFLAWFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFTLTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIK	23
VL2G1a	DIQMTQSPSSLSASVGDRVTITCRASESVDNYGLSFINWFQQKPGKAPKLLIYGTSNRGSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCQQSNEVPYTFGGGTKVEIK	24
VL2G2a	DIQMTQSPSSLSASVGDRVTITCRASESVDNYGLSFINW	25

	FQQKPGQPPKLLIYGTSNRGSGVPSRFSGSGSGTDFTLT ISSLQPEDFAMYFCQQSNEVPYTFGGGTKVEIK	
VL2G3a	DIQMTQSPSSLSASVGDRVTITCRASESIDNYGLSFLNW FQQKPGKAPKLLIYGTSNRGSGVPSRFSGSGSGTDFTLT ISSLQPEDFATYFCQQSNEVPYTFGGGTKVEIK	26

1.10.3 New humanized anti-FAP Fabs

Based on the new humanization variants of VH and VL new anti-FAP Fabs were expressed.

Table 11: Nomenclature for VH/VL combinations expressed as Fabs

	VL1G1a	VL1G2a	VL1G3a	VL2G1a	VL2G2a	VL2G3a
VH1G1a	P1AE1689					
VH1G2a	P1AE1690	P1AE1693				
VH1G3a						
VH2G1a						
VH2G2a					P1AE1702	
VH2G3a						

The affinity of the new humanized anti-FAP variants based on clone 212 was analyzed in comparison with anti-FAP antibody 4B9. Furthermore, the humanness of the humanized variants was calculated and its aggregation onset temperature was measured.

Table 12: Affinity of humanization variants of clone 212 as measured by Biacore

Sample ID	ka (1/Ms)	kd (1/s)	KD (pM)	T 1/2 (min)	Identity to hu V germline	T_{agg} [°C]
P1AE1689 _Fab	4.43E+05	4.21E-05	95	274	83/84,7	72,7
P1AE1690 _Fab	5.51E+05	6.30E-05	114	183	83/81,7	75,4
P1AE1693 _Fab	5.30E+05	7.18E-05	135	161	81,9/81,7	75,4
P1AE1702 _Fab	5.02E+05	1.07E-04	213	108	77,8/76,5	71,6

Sample ID	ka (1/Ms)	kd (1/s)	KD (pM)	T 1/2 (min)	Identity to hu V germline	T_{agg} [°C]
4B9_Fab	7.47E+05	2.08E-04	279	55		60

Antibody P1AE1689 is called FAP antibody 1G1a in the following.

1.11 FcRn/Heparin binding and in silico charge distribution

The charge distribution of antibodies 4B9 and 1G1a in PBS, pH 7.4, was calculated in an in-silico model. According to the model, 4B9 has a large positive patch which is sometimes correlated with increased heparin binding. 1G1a, on the other hand, shows a large negative charge patch which might be indicative for weak heparin interaction.

These predictions were confirmed by chromatography of both antibodies using a FcRn affinity column and pH gradient as well as a heparin affinity column and pH gradient. WO 2015/140126 discloses a method for the prediction of the in vivo half-life of an antibody based on the retention time determined on an FcRn affinity chromatography column, whereas heparin binding correlates with non-specific interactions with cell surface structures.

Example 2

Generation and Production of Bispecific Antigen Binding Molecules Targeting OX40 and Fibroblast Activation Protein (FAP)

2.1 Generation of bispecific antigen binding molecules targeting OX40 and fibroblast activation protein (FAP)

The cDNAs encoding variable heavy and light chain domains of anti OX40 antibodies (clones 49B4, 8H9 and CLC563 as described in WO 2017/055398 A2, or MOXR0916 as described in WO 2015/153513 A1) as well as anti-FAP antibody 1G1a were cloned in frame with the corresponding constant heavy or light chains of human IgG1 in suitable expression plasmids. Expression of heavy and light chain is driven by a chimeric MPSV promoter consisting of the MPSV core promoter and a CMV enhancer element. The expression cassette also contains a synthetic polyA signal at the 3′ end of the cDNAs. In addition the plasmid vectors harbor an origin of replication (EBV OriP) for episomal maintenance of the plasmids.

Bispecific OX40-FAP antibodies were prepared in 2+1, 3+1 and 4+1 format consisting of two, three or four OX40 binding moieties combined with one FAP binding arm at the C-terminus of an Fc (**Figures 1A to 1c**) The bispecific OX40-FAP antibodies included anti-FAP clone 212 (1G1a) as described in Example 1. To generate the 2+1, 3+1 and the 4+1 antigen binding molecules the knob-into-hole technology was used to achieve heterodimerization. The S354C/T366W mutations were introduced in the first heavy chain HC1 (Fc knob heavy chain) and the Y349C/T366S/L368A/Y407V mutations were introduced in the second heavy chain HC2 (Fc hole heavy chain). In the 2+1, 3+1 and 4+1 antigen binding molecules the CrossMab technology as described in WO 2010/145792 Al ensured correct light chain pairing. Independent of the bispecific format, in all cases an effector silent Fc domain (P329G; L234A, L235A) was used to abrogate binding to Fcγ receptors according to the method described in WO 2012/130831 A1. Sequences of the bispecific molecules are shown in **Table 13** below.

All genes were transiently expressed under control of a chimeric MPSV promoter consisting of the MPSV core promoter combined with the CMV promoter enhancer fragment. The expression vector also contained the oriP region for episomal replication in EBNA (Epstein Barr Virus Nuclear Antigen) containing host cells.

Table 13: Amino acid sequences of the bispecific antigen binding molecules

Molecule	Sequence	Seq ID No
P1AE6838 OX40 (49B4) x FAP (1G1a) (4+1)		
OX40(49B4) VHCH1-OX40(49B4) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGG SGGQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAIS WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD KSTSTAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQG TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVED YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSG GGGSGGGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKA SGYTLTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQ	86

Molecule	Sequence	Seq ID No
	KFKGRVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFR GIHYAMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC	
OX40(49B4) light chain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQ KPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTIS SLQPDDFATYYCQQYSSQPYTFGQGTKVEIKRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC	87
FAP(1G1a) VLCH1-light chain	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSC	88
OX40(49B4) VHCH1-OX40(49B4) VHCH1-Fc hole_PGLALA	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGG SGGQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAIS WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD KSTSTAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQG TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVED YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	89
P1AE8786 OX40(49B4) x FAP (1G1a) (3+1)		
OX40(49B4) VHCH1-OX40(49B4) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGG SGGQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAIS WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD KSTSTAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQG TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVED	86

Molecule	Sequence	Seq ID No
	YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSG GGGSGGGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKA SGYTLTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQ KFKGRVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFR GIHYAMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC	
OX40(49B4) light chain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQ KPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTIS SLQPDDFATYYCQQYSSQPYTFGQGTKVEIKRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC	87
FAP(1G1a) VLCH1-light chain	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSC	88
OX40(49B4) VHCH1- Fc hole_PGLALA	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCP PCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKG QPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	90
P1AE6840 OX40(49B4) x FAP (1G1a) (2+1)		
OX40(49B4) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCP	91

Molecule	Sequence	Seq ID No
	PCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKG QPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGG SGGGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKASGY TLTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFK GRVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIH YAMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC	
OX40(49B4) light chain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQ KPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTIS SLQPDDFATYYCQQYSSQPYTFGQGTKVEIKRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC	87
FAP(1G1a) VLCH1-light chain	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSC	88
OX40(49B4) VHCH1- Fc hole_PGLALA	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCP PCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKG QPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	90
P1AF7205 OX40 (CLC563) x FAP (1G1a_EPKSCD) (4+1) C-terminal crossfab fusion		
OX40(CLC563) VHCH1- OX40(CLC563) VHCH1- Fc knob_PGLALA- FAP(1G1a) VHCL	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGGSG GEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWV RQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNS	92

Molecule	Sequence	Seq ID No
	KNTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGS GGGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKASGYT LTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFKG RVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHY AMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC	
OX40(CLC563) light chain	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQ QKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTI SRLEPEDFAVYYCQQYGSSPLTFGQGTKVEIKRTVAAP SVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC	93
FAP(1G1a) VLCH1-light chain (EPKSCD)	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCD	94
OX40(CLC563) VHCH1-OX40(CLC563) VHCH1-Fc hole_PGLALA	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGGSG GEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWV RQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG	95

Molecule	Sequence	Seq ID No
P1AF7217 OX40 (CLC563) x FAP (1G1a_EPKSCS) (4+1) C-terminal crossfab fusion		
OX40(CLC563) VHCH1-OX40(CLC563) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGGSG GEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWV RQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGS GGGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKASGYT LTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFKG RVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHY AMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC	92
OX40(CLC563) light chain	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQ QKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTI SRLEPEDFAVYYCQQYGSSPLTFGQGTKVEIKRTVAAP SVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC	93
FAP(1G1a) VLCH1-light chain (EPKSCS)	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCS	96
OX40(CLC563) VHCH1-OX40(CLC563) VHCH1-Fc hole_PGLALA	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGGSG GEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWV RQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNS	95

Molecule	Sequence	Seq ID No
	KNTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG	
P1AE8874 OX40 (CLC563) x FAP (1G1a) (3+1) C-terminal crossfab fusion		
OX40(CLC563) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSG GGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKASGYTL TDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFKGR VTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHYA MDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	97
OX40(CLC563) light chain	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQ QKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTI SRLEPEDFAVYYCQQYGSSPLTFGQGTKVEIKRTVAAP SVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC	93
FAP(1G1a) VLCH1-light chain	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSC	88
OX40(CLC563) VHCH1-OX40(CLC563) VHCH1-Fc hole_PGLALA	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS	95

Molecule	Sequence	Seq ID No
	SLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGGSG GEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWV RQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG	
P1AF6454 OX40 (CLC563) x FAP (1G1a_EPKSCD) (3+1) C-terminal crossfab fusion		
OX40(CLC563) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSG GGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKASGYTL TDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFKGR VTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHYA MDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	97
OX40(CLC563) light chain	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQ QKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTI SRLEPEDFAVYYCQQYGSSPLTFGQGTKVEIKRTVAAP SVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC	93
FAP(1G1a) VLCH1-light chain (EPKSCD)	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCD	94
OX40(CLC563) VHCH1-	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK	95

Molecule	Sequence	Seq ID No
OX40(CLC563) VHCH1-Fc hole_PGLALA	NTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGGSG GEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWV RQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG	
P1AF6455 OX40 (CLC563) x FAP (1G1a_EPKSCS) (3+1) C-terminal crossfab fusion		
OX40(CLC563) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSG GGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKASGYTL TDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFKGR VTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHYA MDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	97
OX40(CLC563) light chain	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQ QKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTI SRLEPEDFAVYYCQQYGSSPLTFGQGTKVEIKRTVAAP SVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC	93
FAP(1G1a) VLCH1-light chain (EPKSCS)	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG	96

Molecule	Sequence	Seq ID No
	TQTYICNVNHKPSNTKVDKKVEPKSCS	
OX40(CLC563) VHCH1-OX40(CLC563) VHCH1-Fc hole_PGLALA	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGGSG GEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWV RQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG	95
P1AE8871 OX40 (CLC563) x FAP (1G1a) (2+1) C-terminal crossfab fusion		
OX40(CLC563) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSG GGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKASGYTL TDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFKGR VTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHYA MDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	97
OX40(CLC563) light chain	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQ QKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTI SRLEPEDFAVYYCQQYGSSPLTFGQGTKVEIKRTVAAP SVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC	93
FAP(1G1a) VLCH1-light	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT	88

Molecule	Sequence	Seq ID No
chain)	LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSC	
OX40(CLC563) VHCH1 Fc hole_PGLALA	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCALDVGAFDYWGQGALVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPG	98
P1AE8875 OX40 (MOXR0916) x FAP (1G1a) (3+1) C-terminal crossfab fusion		
OX40(MOXR0916) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSWVR QAPGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDTST STAYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGS GGGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKASGYT LTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFKG RVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHY AMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC	99
OX40(MOXR0916) light chain	DIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQ KPGKAPKLLIYYTSRLRSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQGHTLPPTFGQGTKVEIKRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC	100
FAP(1G1a) VLCH1-light chain	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG	88

Molecule	Sequence	Seq ID No
	TQTYICNVNHKPSNTKVDKKVEPKSC	
OX40(MOXR0916) VHCH1-OX40(MOXR0916) VHCH1-Fc hole_PGLALA-	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSWVR QAPGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDTST STAYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGGS GGEVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSW VRQAPGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDT STSTAYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYF PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTC PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAK GQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	101
P1AF4845 OX40 (MOXR0916) x FAP (1G1a_EPKSCD) (3+1) C-terminal crossfab fusion		
OX40(MOXR0916) VHCH1-Fc knob_PGLALA-FAP(1G1a) VHCL	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSWVR QAPGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDTST STAYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGS GGGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKASGYT LTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFKG RVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHY AMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC	99
OX40(MOXR0916) light chain	DIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQ KPGKAPKLLIYYTSRLRSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQGHTLPPTFGQGTKVEIKRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC	100
FAP(1G1a) VLCH1-light	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT	94

Molecule	Sequence	Seq ID No
chain (EPKSCD)	LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCD	
OX40(MOXR0916) VHCH1-OX40(MOXR0916) VHCH1-Fchole_PGLALA-	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSWVR QAPGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDTST STAYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGGS GGEVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSW VRQAPGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDT STSTAYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYF PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTC PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAK GQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	101
P1AF4851 OX40 (MOXR0916) x FAP (1G1a_EPKSCS) (3+1) C-terminal crossfab fusion		
OX40(MOXR0916) VHCH1-Fcknob_PGLALA-FAP(1G1a) VHCL	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSWVR QAPGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDTST STAYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGS GGGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKASGYT LTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFKG RVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHY AMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC	99
OX40(MOXR0916) light chain	DIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQ KPGKAPKLLIYYTSRLRSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQGHTLPPTFGQGTKVEIKRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK	100

Molecule	Sequence	Seq ID No
	VYACEVTHQGLSSPVTKSFNRGEC	
FAP(1G1a) VLCH1-light chain (EPKSCS)	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCS	96
OX40(MOXR0916) VHCH1-OX40(MOXR0916) VHCH1-Fc hole_PGLALA-	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSWVR QAPGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDTST STAYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGGS GGEVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSW VRQAPGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDT STSTAYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYF PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTC PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAK GQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	101
P1AE8872 OX40 (MOXR0916) x FAP (1G1a) (2+1) C-terminal crossfab fusion		
OX40(MOXR0916) VHCH1-Fc knob_PGLALA-FAP(1G1a) VHCL	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSWVR QAPGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDTST STAYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGS GGGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKASGYT LTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFKG RVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHY AMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC	99
OX40(MOXR0916) light chain	DIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQ KPGKAPKLLIYYTSRLRSGVPSRFSGSGSGTDFTLTIS	100

Molecule	Sequence	Seq ID No
	SLQPEDFATYYCQQGHTLPPTFGQGTKVEIKRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC	
FAP(1G1a) VLCH1-light chain	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSC	88
OX40(MOXR0916) VHCH1- Fc hole_PGLALA	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSWVR QAPGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDTST STAYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG	102
P1AF4852 OX40 (MOXR0916) x FAP (1G1a_EPKSCD) (2+1) C-terminal crossfab fusion		
OX40(MOXR0916) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSWVR QAPGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDTST STAYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGS GGGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKASGYT LTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFKG RVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHY AMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC	99
OX40(MOXR0916) light chain	DIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQ KPGKAPKLLIYYTSRLRSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQGHTLPPTFGQGTKVEIKRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK	100

Molecule	Sequence	Seq ID No
	VYACEVTHQGLSSPVTKSFNRGEC	
FAP(1G1a) VLCH1-light chain (EPKSCD)	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCD	94
OX40(MOXR0916) VHCH1-Fc hole_PGLALA	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSWVR QAPGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDTST STAYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG	102
P1AF4858 OX40 (MOXR0916) x FAP (1G1a_EPKSCS) (2+1) C-terminal crossfab fusion		
OX40(MOXR0916) VHCH1-Fc knob_PGLALA-FAP(1G1a) VHCL	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSWVR QAPGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDTST STAYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGS GGGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKASGYT LTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFKG RVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHY AMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC	99
OX40(MOXR0916) light chain	DIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQ KPGKAPKLLIYYTSRLRSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQGHTLPPTFGQGTKVEIKRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC	100
FAP(1G1a) VLCH1-light	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT	96

Molecule	Sequence	Seq ID No
chain (EPKSCS)	LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCS	
OX40(MOXR0916) VHCH1-Fc hole_PGLALA	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSWVR QAPGQGLEWIGDMYPDNGDSSYNQKFRERVTITRDTST STAYLELSSLRSEDTAVYYCVLAPRWYFSVWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG	102
P1AE8873 OX40 (8H9) x FAP (1G1a) (3+1) C-terminal crossfab fusion		
OX40(8H9) VHCH1-Fc knob_PGLALA-FAP(1G1a) VHCL	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKST STAYMELSSLRSEDTAVYYCAREYGWMDYWGQGTTVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSG GGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKASGYTL TDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFKGR VTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHYA MDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	103
OX40(8H9) light chain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQ KPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTIS SLQPDDFATYYCQQYLTYSRFTFGQGTKVEIKRTVAAP SVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC	104
FAP(1G1a) VLCH1-light chain	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG	88

Molecule	Sequence	Seq ID No
	TQTYICNVNHKPSNTKVDKKVEPKSC	
OX40(8H9) VHCH1-OX40(8H9) VHCH1-Fc hole_PGLALA	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKST STAYMELSSLRSEDTAVYYCAREYGWMDYWGQGTTVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGGSG GQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWV RQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKS TSTAYMELSSLRSEDTAVYYCAREYGWMDYWGQGTTVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG	105
P1AE8870 OX40 (8H9) x FAP (1G1a) (2+1) C-terminal crossfab fusion		
OX40(8H9) VHCH1-Fc knob_PGLALA-FAP(1G1a) VHCL	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKST STAYMELSSLRSEDTAVYYCAREYGWMDYWGQGTTVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSG GGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKASGYTL TDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQKFKGR VTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFRGIHYA MDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	103
OX40(8H9) light chain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQ KPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTIS SLQPDDFATYYCQQYLTYSRFTFGQGTKVEIKRTVAAP SVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC	104
FAP(1G1a) VLCH1-light	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT	88

Molecule	Sequence	Seq ID No
chain	LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSC	
OX40(8H9) VHCH1- Fc hole_PGLALA	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKST STAYMELSSLRSEDTAVYYCAREYGWMDYWGQGTTVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPG	106

For comparison, the following molecules were prepared:

Molecule P1AD4524, or OX40 (49B4) x FAP (4B9) (4+1) bispecific molecule, comprises four OX40 (49B4) binding Fab fragments combined with one FAP (4B9) binding moiety as VH and VL domain, wherein the VH domain is fused at the C-terminus of the Fc knob chain and the VL domain is fused at the C-terminus of the Fc hole chain (tetravalent for OX40 and monovalent for FAP). The molecule comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:113, a heavy chain comprising the amino acid sequence of SEQ ID NO:114 and four light chains each comprising the amino acid sequence of SEQ ID NO:115. The molecule P1AD3690 is an untargeted OX40 agonist comprising four OX40 (49B4) binding Fab fragments. This molecule comprises two heavy chains comprising the amino acid sequence of SEQ ID NO:116 and four light chains each comprising the amino acid sequence of SEQ ID NO:115. The generation and production of the molecules is described in WO 2017/060144 A1.

2.2 Production of bispecific antigen binding molecules targeting FAP and OX40

The molecules were produced by co-transfecting either HEK293-EBNA cells growing in suspension with the mammalian expression vectors using polyethylenimine (PEI) or co-transfecting CHO K1 cells growing in suspension with the mammalian expression using eviFECT (Evitria AG, Switzerland). The cells were transfected with the corresponding expression vectors.

Antibody constructs were expressed by transient transfection of HEK cells grown in suspension with expression vectors encoding the 4 different peptide chains. Transfection of

Expi293F™ cells (Gibco™) was performed according to the cell supplier's instructions using Maxiprep (Macherey-Nagel) preparations of the antibody vectors, Expi293F™ Expression Medium (Gibco™), ExpiFectamine™ 293 Reagent, (Gibco™) and an initial cell density of 2-3 million viable cells/ml in Opti-MEM® 1x Reduced Serum Medium (Gibco®). On the day after transfection (Day 1, 18–22 hours post-transfection), ExpiFectamine™ 293 Transfection Enhancer 1 and ExpiFectamine™ 293 Transfection Enhancer 2 was added to the transfected culture. Transfected cultures were incubated at 37°C in a humidified atmosphere of 8% CO_2 with shaking. Cell culture supernatants were harvested after 7 days of cultivation in shake flasks or stirred fermenters by centrifugation at 3000-5000 g for 20-30 minutes and filtered through a 0.22 µm filter.

For production in CHO K1 cells, CHO K1 cells were grown in eviGrow medium (Evitria AG, Switzerland), a chemically defined, animal-component free, serum-free medium and transfected with eviFect (Evitria AG, Switzerland). After transfection the cells were kept in eviMake (Evitria AG, Switzerland), a chemically defined, animal-component free, serum-free medium, at 37° C. and 5% CO_2 for 7 days. After 7 days the cultivation supernatant was collected for purification by centrifugation for 45 min at maximum speed in a Rotanta 460 RC. The solution was sterile filtered (0.22 µm filter) and kept at 4° C. The concentration of the molecules in the culture medium was either determined by Protein A-HPLC or Protein A-Bio-Layer Interferometry (BLI).

Antibodies were purified from cell culture supernatants by affinity chromatography using MabSelectSure-Sepharose™ (GE Healthcare, Sweden) chromatography. Briefly, sterile filtered cell culture supernatants were captured on a MabSelect SuRe resin equilibrated with PBS buffer (10 mM Na_2HPO_4, 1 mM KH_2PO_4, 137 mM NaCl and 2.7 mM KCl, pH 7.4), washed with equilibration buffer and eluted with 100 mM sodium acetate, pH 3.0. After neutralization with 1 M Tris pH 9.0, aggregated protein was separated from monomeric antibody species by ion exchange Chromatography (Poros XS) with equilibration buffer 20 mM His, pH 5.5, 1,47 mS/cm and elution buffer 20 mM His, 500 mM NaCl, pH 5.5, 49.1 mS/cm, (gradient: to 100% elution buffer in 60 CV). In some cases a size exclusion chromatography (Superdex 200, GE Healthcare) in 20 mM histidine, 140 mM NaCl, pH 6.0, was subsequently performed. Monomeric protein fractions were pooled, and if required concentrated using e.g. a MILLIPORE Amicon Ultra (30KD MWCO) centrifugal concentrator. Purified proteins were stored at -80° C. Protein quantification was performed using a Nanodrop spectrophotometer and analyzed by CE-SDS under denaturing and reducing conditions and analytical SEC. Sample aliquots were used for subsequent analytical characterization e.g. by CE-SDS, size exclusion chromatography, mass spectrometry and endotoxin determination..

Purity and molecular weight of the bispecific antigen binding molecule after the final purification step were analyzed by CE-SDS analyses in the presence and absence of a reducing agent. The Caliper LabChip GXII system (Caliper Lifescience) was used according to the manufacturer's instruction.

The aggregate content of the bispecific antigen binding molecule was analyzed using a TSKgel G3000 SW XL analytical size-exclusion column (Tosoh) in 25 mM potassium phosphate, 125 mM sodium chloride, 200 mM L-arginine monohydrocloride, 0.02% (w/v) NaN_3, pH 6.7 running buffer at 25° C.

Table 14: Production yield and Quality of bispecific OX40 antigen binding molecules

Molecule	Monomer [%]	CE-SDS (non- reduced) [%]	Yield [mg/l]
P1AE6838 OX40(49B4) x FAP(1G1a) 4+1	99	94	44
P1AE8786 OX40(49B4) x FAP(1G1a) 3+1	99	93	21
P1AE6840 OX40(49B4) x FAP(1G1a) 2+1	98	98	3.7
P1AF7205 OX40(CLC563) x FAP(1G1a_EPKSCD) 4+1	99	96	25
P1AF7217 OX40(CLC563) x FAP(1G1a_EPKSCS) 4+1	99	98	32
P1AE8874 OX40(CLC563) x FAP(1G1a) 3+1	99	100	35
P1AF6454 OX40(CLC563) x FAP(1G1a_EPKSCD) 3+1	98	98	122
P1AF6455 OX40(CLC563) x FAP(1G1a_EPKSCS) 3+1	100	100	19
P1AE8871 OX40(CLC563) x FAP(1G1a) 2+1	96	98	90
P1AE8875 OX40(MOXR0916) x FAP(1G1a) 3+1	98	97	9
P1AF4845 OX40(MOXR0916) x	86	100	0.2

Molecule	Monomer [%]	CE-SDS (non- reduced) [%]	Yield [mg/l]
FAP(1G1a_EPKSCD) 3+1			
P1AF4851 OX40(MOXR0916) x FAP(1G1a_EPKSCS) 3+1	99	100	0.5
P1AE8872 OX40(MOXR0916) x FAP(1G1a) 2+1	95	98	2.4
P1AF4852 OX40(MOXR0916) x FAP(1G1a_EPKSCD) 2+1	95	100	2.7
P1AF4858 OX40(MOXR0916) x FAP(1G1a_EPKSCS) 2+1	98	99	4.6
P1AE8873 OX40(8H9) x FAP(1G1a) 3+1	100	100	13
P1AE8870 OX40(8H9) x FAP(1G1a) 2+1	98	98	13

2.3 Generation of further bispecific antigen binding molecules targeting OX40 and Fibroblast Activation Protein (FAP) - Charge Patch Variants

In analogy to Example 2.1, different variants of a 4+1 bispecific format consisting of four OX40 binding moieties combined with one FAP binding crossfab at the C-terminus of the Fc domain have been prepared. In all these constructs, the variable heavy and light chain domains of the anti-OX40 antibody correspond to the OX40 clone 49B4 as described in WO 2017/055398 A2. The generation and preparation of the FAP antibody 1G1a is described in Example 1. To generate the 4+1 antigen binding molecules, the knob-into-hole technology was used to achieve heterodimerization. The S354C/T366W mutations were introduced in the first heavy chain HC1 (Fc knob heavy chain) and the Y349C/T366S/L368A/Y407V mutations were introduced in the second heavy chain HC2 (Fc hole heavy chain). Furthermore, the CrossMab technology as described in WO 2010/145792 A1 ensures correct light chain pairing. Independent of the bispecific format, in all cases an effector silent Fc (P329G; L234A, L235A) has been used to abrogate binding to Fcγ receptors according to the method described in WO 2012/130831 Al. Amino acid sequences of the bispecific antigen binding molecules are shown in Table 15.

All genes are transiently expressed under control of a chimeric MPSV promoter consisting of the MPSV core promoter combined with the CMV promoter enhancer fragment. The expression vector also contains the oriP region for episomal replication in EBNA (Epstein Barr Virus Nuclear Antigen) containing host cells.

Table 15: Amino acid sequences of the bispecific antigen binding molecules

Molecule	Sequence	Seq ID No
P1AE9167 OX40 (49B4_K73E) x FAP (1G1a) (4+1)		
OX40(49B4_K73E) VHCH1-OX40(49B4_K73E) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGG SGGQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAIS WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD ESTSTAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQG TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVED YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSG GGGSGGGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKA SGYTLTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQ KFKGRVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFR GIHYAMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC	107
OX40(49B4_K73E) light chain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQ KPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTIS SLQPDDFATYYCQQYSSQPYTFGQGTKVEIKRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC	87
FAP(1G1a) VLCH1-light chain	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSC	88
OX40(49B4_K73E) VHCH1-OX40(49B4_K73E) VHCH1-Fc hole_PGLALA	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGG SGGQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAIS	108

Molecule	Sequence	Seq ID No
	WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD ESTSTAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQG TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVED YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	
P1AE9169 OX40 (49B4_K23T_K73E) x FAP (1G1a) (4+1)		
OX40(49B4_K23T_K73E) VHCH1-OX40(49B4_K23T_K73E) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	QVQLVQSGAEVKKPGSSVKVSCTASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGG SGGQVQLVQSGAEVKKPGSSVKVSCTASGGTFSSYAIS WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD ESTSTAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQG TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVED YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSG GGGSGGGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKA SGYTLTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQ KFKGRVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFR GIHYAMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC	109
OX40(49B4_K23T_K73E) light chain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQ KPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTIS SLQPDDFATYYCQQYSSQPYTFGQGTKVEIKRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC	87
FAP(1G1a) VLCH1-light chain	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV	88

Molecule	Sequence	Seq ID No
	SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSC	
OX40(49B4_K23T_K73E) VHCH1-OX40(49B4_K23T_K73E) VHCH1-Fc hole_PGLALA	QVQLVQSGAEVKKPGSSVKVSCTASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGG SGGQVQLVQSGAEVKKPGSSVKVSCTASGGTFSSYAIS WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD ESTSTAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQG TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVED YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	110
P1AE9176 OX40 (49B4_K23E_K73E) x FAP (1G1a) (4+1)		
OX40(49B4_K23E_K73E) VHCH1-OX40(49B4_K23E_K73E) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	QVQLVQSGAEVKKPGSSVKVSCEASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGG SGGQVQLVQSGAEVKKPGSSVKVSCEASGGTFSSYAIS WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD ESTSTAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQG TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVED YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSG GGGSGGGGSGGSGGGQVQLVQSGAEVKKPGASVKVSCKA SGYTLTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQ KFKGRVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFR GIHYAMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC	111
OX40(49B4_K2	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQ	87

Molecule	Sequence	Seq ID No
3E_K73E) light chain	KPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTIS SLQPDDFATYYCQQYSSQPYTFGQGTKVEIKRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC	
FAP(1G1a) VLCH1-light chain	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSC	88
OX40(49B4_K23E_K73E) VHCH1-OX40(49B4_K23E_K73E) VHCH1-Fc hole_PGLALA	QVQLVQSGAEVKKPGSSVKVSCEASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGG SGGQVQLVQSGAEVKKPGSSVKVSCEASGGTFSSYAIS WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD ESTSTAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQG TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVED YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	112
P1AF6456 OX40 (49B4_K23E_K73E) x FAP (1G1a_EPKSCD) (4+1)		
OX40(49B4_K23E_K73E) VHCH1-OX40(49B4_K23E_K73E) VHCH1- Fc knob_PGLALA-FAP(1G1a) VHCL	QVQLVQSGAEVKKPGSSVKVSCEASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGG SGGQVQLVQSGAEVKKPGSSVKVSCEASGGTFSSYAIS WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD ESTSTAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQG TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVED YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK	111

Molecule	Sequence	Seq ID No
	SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSG GGGSGGGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKA SGYTLTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQ KFKGRVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFR GIHYAMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC	
OX40(49B4_K23E_K73E) light chain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQ KPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTIS SLQPDDFATYYCQQYSSQPYTFGQGTKVEIKRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC	87
FAP(1G1a) VLCH1-light chain (EPKSCD)	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCD	94
OX40(49B4_K23E_K73E) VHCH1-OX40(49B4_K23E_K73E) VHCH1-Fc hole_PGLALA	QVQLVQSGAEVKKPGSSVKVSCEASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGG SGGQVQLVQSGAEVKKPGSSVKVSCEASGGTFSSYAIS WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD ESTSTAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQG TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVED YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	112
P1AF6457 OX40 (49B4_K23E_K73E) x FAP (1G1a_EPKSCS) (4+1)		
OX40(49B4_K23E_K73E) VHCH1-OX40(49B4_K23E_K73E) VHCH1- Fc knob_PGLALA-FAP(1G1a)	QVQLVQSGAEVKKPGSSVKVSCEASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGG SGGQVQLVQSGAEVKKPGSSVKVSCEASGGTFSSYAIS WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD	111

Molecule	Sequence	Seq ID No
VHCL	ESTSTAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQG TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVED YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSG GGGSGGGGSGGSGGQVQLVQSGAEVKKPGASVKVSCKA SGYTLTDYNMDWVRQAPGQGLEWIGDIYPNTGGTIYNQ KFKGRVTMTIDTSTSTVYMELSSLRSEDTAVYYCTRFR GIHYAMDYWGQGTTVTVSSASVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC	
OX40(49B4_K23E_K73E) light chain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQ KPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTIS SLQPDDFATYYCQQYSSQPYTFGQGTKVEIKRTVAAPS VFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC	87
FAP(1G1a) VLCH1-light chain (EPKSCS)	EIVLTQSPATLSLSPGERATLSCRASESVDNYGLSFIN WFQQKPGQAPRLLIYGTSNRGSGIPARFSGSGSGTDFT LTISSLEPEDFAVYFCQQSNEVPYTFGGGTKVEIKSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCS	96
OX40(49B4_K23E_K73E) VHCH1-OX40(49B4_K23E_K73E) VHCH1-Fc hole_PGLALA	QVQLVQSGAEVKKPGSSVKVSCEASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQGTTV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDEKVEPKSCGGGGSGG SGGQVQLVQSGAEVKKPGSSVKVSCEASGGTFSSYAIS WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITAD ESTSTAYMELSSLRSEDTAVYYCAREYYRGPYDYWGQG TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVED YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK AKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	112

2.4 Production of bispecific antigen binding molecules targeting FAP and OX40 (Charge patch variants)

Antibodies were expressed by transient transfection of HEK cells grown in suspension with expression vectors encoding the 4 different peptide chains. Transfection into HEK293-F cells (Invitrogen) was performed according to the cell supplier's instructions using MaxiPREP (Qiagen) preparations of the antibody vectors, Freestyle™ F17 medium (Invitrogen, USA), PEIpro® transfection reagent (Polyscience Europe GmbH) and an initial cell density of 1-2 million viable cells/ml in serum free FreeStyle 293 expression medium (Invitrogen). Cell culture supernatants were harvested after 7 days of cultivation in shake flasks or stirred fermenters by centrifugation at 14000xg for 30 minutes and filtered through a 0.22 µm filter.

Antibodies were purified from cell culture supernatants by affinity chromatography using MabSelectSure-Sepharose™ (GE Healthcare, Sweden) chromatography. Briefly, sterile filtered cell culture supernatants were captured on a MabSelectSure resin equilibrated with PBS buffer (10 mM Na_2HPO_4, 1 mM KH_2PO_4, 137 mM NaCl and 2.7 mM KCl, pH 7.4), washed with equilibration buffer and eluted with 25 mM citrate, pH 3.0. After neutralization with 1 M Tris buffer pH 9.0, aggregated protein was separated from monomeric antibody species by size exclusion chromatography (Superdex 200, GE Healthcare) in 20 mM histidine, 140 mM NaCl, pH 6.0. Monomeric protein fractions were pooled, concentrated if required using e.g. a MILLIPORE Amicon Ultra (30KD MWCO) centrifugal concentrator and stored at −80° C. Sample aliquots were used for subsequent analytical characterization e.g. by CE-SDS, size exclusion chromatography, mass spectrometry and endotoxin determination.

Table 16: Production yield and Quality of bispecific OX40 antigen binding molecules

Molecule	Monomer [%]	CE-SDS (non- reduced) [%]	Yield [mg/l]
P1AE9167 OX40(49B4_K73E) x FAP(1G1a) 4+1	99	92	2.7
P1AE9169 OX40(49B4_K23T_K73E) x FAP(1G1a) 4+1	95	98	6.7
P1AE9176 OX40(49B4_K23E_K73E) x FAP(1G1a) 4+1	99	99	32
P1AF6456 OX40(49B4_K23E_K73E) x FAP(1G1a_EPKSCD) 4+1	93	94	2.6

Molecule	Monomer [%]	CE-SDS (non- reduced) [%]	Yield [mg/l]
P1AF6457 OX40(49B4_K23E_K73E) x FAP(1G1a_EPKSCS) 4+1	98	96	0.9

Example 3

Characterization of bispecific antigen binding molecules targeting OX40 and FAP

3.1 Binding to naïve versus activated human PBMCs of FAP-targeted anti-OX40 bispecific antigen binding molecules

Human PBMCs were isolated by ficoll density gradient centrifugation. 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 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 frozen for later use. PBMC were thawed, washed and resuspended in AIM-V medium (ThermoFischer, Cat. No. 12055091). PBMCs were used unstimulated (binding on resting human PBMCs) or they were stimulated to receive a strong human Ox40 expression on the cell surface of T cells (binding on activated human PBMCs). Therefore thawed PBMCs were cultured for three days at 37°C/5% CO_2 in Aim-V media in 6-well tissue culture plate pre-coated for 2 hours with [2 µg/mL] anti-human CD3 (clone OKT3) and [2 µg/mL] anti-human CD28 (clone CD28.2).

For detection, OX40 naïve human PBMCs and activated human PBMCs were mixed. To enable distinction of naïve from activated human PBMCs, naïve cells were labeled prior to the binding assay using the eFluor670 cell proliferation dye (eBioscience, Cat.-No.65-0840-85). A 1 to 1 mixture of 1 x 10^5 naïve, eFluor670 labeled human PBMC and unlabeled activated human PBMCs were then added to each well of a round-bottom 96-well plate (TPP, Cat. No. 92097) and the binding assay was performed.

Cells were first stained 10 min with Zombie Aqua Fixable viability dye (Biolegend, Cat.No. 423102) in DPBS, followed by a 90 minutes incubation at 4°C in the dark in 50

µL/well FACS buffer containing titrated anti–OX40 bispecific antibody constructs. After three times washing with excess FACS buffer, cells were stained for 30 minutes at 4°C in the dark in 25 µL/well FACS buffer containing a mixture of fluorescently labeled anti-human CD4 (clone OKT-4, mouse IgG2b, BioLegend, Cat.-No. 317434), anti-human CD8 (clone RPA-T8, mouse IgG1k, BioLegend, Cat.-No. 301042) and Fluorescein isothiocyanate (FITC)-conjugated AffiniPure anti-human IgG Fcγ-fragment-specific goat IgG F(ab`)2 fragment (Jackson ImmunoResearch, Cat.-No. 109-096-098). Samples were finally resuspended in 20 µL/well FACS-buffer and acquired the same day using iQue Cell Screener and ForeCyt software (Sartorius).

As can be seen in **Figures 2A** and **2C**, the bispecific OX40 (49B4) x FAP antigen binding molecule in tetravalent format (4+1) bound better to OX40 than as a trivalent (3+1) or bivalent (2+1) format (avidity of the 49B4 clone). Along the natural prevalence of OX40 on T cells, the bispecific formats bound stronger to activated CD4 than to CD8 T cells, and had no binding to target negative cells (resting CD4 and CD8 T cells, Figures 2B and 2D). As shown in **Figures 3A** and **3C**, the bispecific antigen binding molecules comprising clone 8H9 bound with subnanomolar affinity to OX40 positive cells and with comparable strength as tri- and bivalent antibody. Along the natural prevalence of OX40 on T cells, the constructs bound stronger to activated CD4 than CD8 T cells, and had no binding to target negative cells (resting CD4 and CD8 T cells, Figures 3B and 3D). In **Figures 4A** and **4C** it is shown that bispecific antibodies comprising clone MOX0916 bound with subnanomolar affinity to OX40 positive cells, with comparable strength as trivalent or bivalent antibody. Along the natural prevalence of OX40 on T cells, the constructs bound stronger to activated CD4 than to CD8 T cells, and had no binding to target negative cells (resting CD4 and CD8 T cells, see Figures 4B and 4D). Bispecific antibodies comprising clone CLC-563 bound with nanomolar affinity to OX40 positive cells, with comparable strength as trivalent and bivalent antibody as is shown in **Figures 5A** and **5C**. Along the natural prevalence of OX40 on T cells, the constructs bound stronger to activated CD4 than to CD8 T cells, and had no binding to target negative cells (resting CD4 and CD8 T cells, see Figures 5B and 5D).

In **Figures 6A** and **6C** it is shown that all bispecific antigen binding molecules comprising 49B4 amino acid variant based clones showed slightly improved binding to OX40 positive cells compared to the parental antibody 49B4. Along the natural prevalence of OX40 on T cells, the constructs bound stronger to activated CD4 than CD8 T cells, and had no binding to target negative cells (resting CD4 and CD8 T cells, see Figures 6B and 6D).

In another experiment, the binding of bispecific antigen binding molecules targeting OX40 and FAP with different C-terminal variants was tested. PBMC were used that were thawed, washed and resuspended in RPMI medium (Gibco, Cat. No. 72400-021) with (10% (v/v) Fetal calf serum (FCS, SIGMA, Cat.-No. F4135). PBMCs were stimulated to receive a strong human Ox40 expression on the cell surface of T cells (binding on activated human PBMCs). Therefore thawed PBMCs were cultured for three days at 37°C/5% CO_2 in in RPMI medium with 10% FCS in 6-well tissue culture plate pre-coated for 2 hours with [2 µg/mL] anti-human CD3 (clone OKT3) and [2 µg/mL] anti-human CD28 (clone CD28.2). For detection, 2 x 10^5 activated human PBMCs were added to each well of a round-bottom 96-well plate (TPP, Cat. No. 92097) and the binding assay was performed. Cells were first stained 20 min with LIVE/DEAD™ Fixable Aqua Dead Cell Stain (Molecular probes, Cat.No. L34957) in DPBS, followed after one washing step (200 µL 4°C FACS buffer) by incubation of 90 minutes at 4°C in the dark in 50 µL/well FACS buffer containing titrated anti–OX40 bispecific antigen binding molecules. After one washing with excess FACS buffer, cells were stained for 30 minutes at 4°C in the dark in 50 µL/well FACS buffer containing a mixture of fluorescently labeled anti-human CD4 (clone A161A1, BioLegend, Cat.-No. 357406), anti-human CD8 (clone SK1, BioLegend, Cat.-No. 344742) and Phycoerythrin (PE)-conjugated AffiniPure anti-human IgG Fcγ-fragment-specific goat IgG F(ab`)2 fragment (Jackson ImmunoResearch, Cat. No. 109-116-098). Samples were finally resuspended in 100 µL/well FACS-buffer and acquired the same day using with BD Fortessa running FACS Diva software.

As can be seen in **Figures 34A** to **34F,** all tested bispecific FAP-OX40 bispecific antibodies bound to activated CD4 T cells. Along the natural prevalence of OX40 on T cells, the bispecific formats bound stronger to activated CD4 than to CD8 T cells (**Figures 34A, 34C, 34E** versus **34B, 34D** and **34F**). For all tested compounds the D and the S variant showed comparable binding properties (compare for each plot open vs closed symbols).

3.2 Binding to human FAP-expressing tumor cells

The binding to cell surface FAP was tested using human fibroblast activating protein (huFAP) expressing NIH/3T3-huFAP clone 19 cells. This cell line was generated by the transfection of the mouse embryonic fibroblast NIH/3T3 cell line (ATCC CRL-1658) with the expression vector pETR4921 to express huFAP under 1.5µg/mL Puromycin selection. The lack of binding to OX40 negative FAP negative tumor cells was tested using A549 NucLight™ Red Cells (Essen Bioscience, Cat. No. 4491) expressing the NucLight Red fluorescent protein restricted to the nucleus to allow separation from unlabeled human FAP positive NIH/3T3-huFAP clone 19 cells. Parental A549 (ATCC CCL-185) were

transduced with the Essen CellPlayer NucLight Red Lentivirus (Essen Bioscience, Cat. No. 4476; EF1α, puromycin) at an MOI of 3 (TU/cell) in the presence of 8 µg/ml polybrene following the standard Essen protocol. This resulted in ≥70% transduction efficiency. Alternatively, the lack of binding to OX40 negative FAP negative tumor cells was tested using the HeLa cell line (ATCC, CCL2), a human cervix adenocarcinoma cell line. Enzyme free cell dissociation buffer was used for detachment to preserve trypsin sensitive surface proteins.

A mixture of 5 x 10^4 unlabeled NIH/3T3-huFAP clone 19 cells and A549 NucLight™ Red Cells in FACS buffer were added to each well of a round-bottom 96-well plates (TPP, Cat. No. 92097) and the binding assay was performed. Cells were first stained 10 min with Zombie Aqua Fixable viability dye (Biolegend, Cat.No. 423102) in DPBS, followed by a 75 minutes incubation at 4°C in the dark in 50 µL/well FACS buffer containing titrated anti-OX40 bispecific antibody constructs. Afterwards the cells were washed three times with 200 µL 4°C FACS buffer and resuspended by a short vortex. Cells were further stained with 25 µL/well of 4 °C cold secondary antibody solution containing Fluorescein isothiocyanate (FITC)-conjugated AffiniPure anti-human IgG Fcγ-fragment-specific goat IgG F(ab`)2 fragment (Jackson ImmunoResearch, Cat. No. 109-096-098) and incubated for 30 minutes at 4 °C in the dark. Samples were finally resuspended in 20 µL/well FACS-buffer and acquired the same day using iQue Cell Screener and ForeCyt software (Sartorius).

As can be seen in **Figures 2E, 3E, 4E, 5E** and **6E**, all bispecific antigen binding molecules, sharing the FAP (1G1a) antigen binding domain, had comparable binding to human FAP positive fibroblasts (NIH/3T3-huFAP-clone 19). Only in Figure 3E it is shown, that there was a slightly enhanced binding to human FAP positive fibroblasts (NIH/3T3-huFAP-clone 19) when clone 8H9 was incorporated in the bispecific antigen binding molecules, despite of sharing the same FAP (1G1a) antigen binding domain. No binding was observed to target negative cells (A549-NLR cells, Figures 2F, 3F, 4F, 5F and 6F).

In another experiment, a mixture of 2 x 10^5 NIH/3T3-huFAP clone 19 cells and HeLa cells in FACS buffer were added to each well of a round-bottom 96-well plates (TPP, Cat. No. 92097) and the binding assay was performed. Cells were first stained 20 min with LIVE/DEAD™ Fixable Aqua Dead Cell Stain (Molecular probes, Cat.No. L34957) in DPBS, followed after one washing step with 200 µL 4 °C FACS buffer by a 75 minutes incubation at 4 °C in the dark in 50 µL/well FACS buffer containing titrated anti-OX40 bispecific antibody constructs. Afterwards the cells were washed once with 200 µL FACS buffer at 4 °C and resuspended by a short vortex. Cells were further stained with 50 µL/well of 4 °C cold secondary antibody solution containing Phycoerythrin (PE)-

conjugated AffiniPure anti-human IgG Fcγ-fragment-specific goat IgG F(ab`)2 fragment (Jackson ImmunoResearch, Cat. No. 109-116-098) and incubated for 30 minutes at 4 °C in the dark. Samples were finally resuspended in 100 µL/well FACS-buffer and acquired the same day using with BD Fortessa running FACS Diva software.

All bispecific antigen binding molecules sharing the FAP (1G1a) antigen binding domain, but comprising a C-terminal S or D variant, had comparable binding to human FAP positive fibroblasts (NIH/3T3-huFAP-clone 19), meaning that the variants had no impact on the binding to FAP (see data in Table 17 below). No binding was observed when targeting negative cells (HeLa cells).

3.3 Summary of cellular binding properties of the bispecific antigen binding molecules

For evaluation of the binding properties of the FAP-targeted OX40 antibodies human FAP negative tumor cells (A549- NLR or HeLa), FAP positive fibroblasts (NIH/3T3-huFAP-clone 19), OX40 positive activated PBMC (activated CD4 and CD8 T cells) as well as OX40 negative resting PBMC (resting CD4 and CD8 T cells) were incubated with indicated serial dilutions of test antibody detected then by fluorescently labeled 2nd antibody against human Fcγ.

All FAP-targeted OX40 antigen binding molecules bound efficiently to human FAP-expressing target cells and had no binding to target negative cells. This is expected to translate in patients to direct tumor-targeting and enrichment of the molecules. Along the natural prevalence of OX40 on T cells, all constructs bound stronger to activated CD4 than to CD8 T cells. The bispecific antigen binding molecules comprising clone 49B4 bound better to OX40 in a tetravalent format (4+1) than in a trivalent (3+1) or bivalent (2+1) format (avidity of the clone; see Figures 2A and 2C). All 49B4 amino acid variant based bispecific antigen binding molecules showed slightly improved binding to OX40 positive cells compared to the parental antibody in a tetravalent format (see Figures 6A and 6C). The clones 8H9 (Figures 3A and 3C) and MOX0916 (Figures 4A and 4C) bound with subnanomolar affinity, whereas clone CLC-563 (Figures 5A and 5C) bound with nanomolar affinity to OX40 positive cells, with comparable strength as tri- and bivalent formats. For all tested C-terminal variants, the D and the S variant showed comparable binding properties. FAP binding was for all bispecific antigen binding molecules in a comparable nanomolar range.

EC_{50} values of binding to activated human CD4 T cells and FAP positive tumor cells are summarized in Table 17.

Table 17: EC_{50} values for binding of FAP-targeted OX40 antigen binding molecules to cell surface human FAP and human Ox40 (on $CD4^+$ T-cells)

Molecule ID	anti-Ox40 clone	Format	Ox40	FAP
			EC_{50} [nM]	
P1AE6838	49B4	4+1	0.03	2.63
P1AE9167	49B4 AA variant K73E	4+1	0.11	2.51
P1AE9169	49B4 AA variant K23T_K73E	4+1	0.12	2.47
P1AE9176	49B4 AA variant K23E_ K73E	4+1	0.20	2.97
P1AE8786	49B4	3+1	17.08	2.91
P1AE6840	49B4	2+1	73.57	2.26
P1AE8873	8H9	3+1	0.11	1.01
P1AE8870	8H9	2+1	0.17	0.95
P1AE8875	MOX0916	3+1	0.69	2.70
P1AE8872	MOX0916	2+1	0.90	1.78
P1AE8874	CLC-563	3+1	3.62	2.52
P1AE8871	CLC-563	2+1	3.19	2.51
P1AF6455	CLC-563, S-variant	3+1	1.47	1.71
P1AF6454	CLC-563, D-variant	3+1	1.64	1.22
P1AF7217	CLC-563, S-variant	4+1	1.46	1.33
P1AF7205	CLC-563, D-variant	4+1	2.11	1.04
P1AF6457	49B4 AA variant K23E_ K73E, S-variant	4+1	0.05	0.69
P1AF6456	49B4 AA variant K23E_ K73E, D variant	4+1	0.07	0.36

3.4 Biophysical and biochemical characterization of bispecific antigen binding molecules targeting OX40 and FAP

3.4.1 Determination of thermal stability

Thermal stability of the FAP-targeted OX40 antigen binding molecules prepared in Example 2 was monitored by Dynamic Light Scattering (DLS) and by monitoring of temperature dependent intrinsic protein fluorescence by applying a temperature ramp using an Optim 2 instrument (Avacta Analytical, UK). 10 µg of filtered protein sample with a

protein concentration of 1 mg/ml was applied in duplicate to the Optim 2 instrument. The temperature was ramped from 25°C to 85°C at 0.1°C/min, with the ratio of fluorescence intensity at 350 nm/330 nm and scattering intensity at 266 nm being collected. The results are shown in Table 18. The aggregation temperature (T_{agg}) of all the tested FAP-Ox40 molecules produced in Example 2 is favorable than for the previously described OX40 (49B4) x FAP (4B9) (4+1) bispecific molecule (molecule P1AD4524) as described in WO 2017/060144 A1.

3.4.2 Hydrophobic interaction chromatography (HIC)

Apparent hydrophobicity was determined by injecting 20 µg of sample onto a HIC-Ether-5PW (Tosoh) column equilibrated with 25 mM Na-phosphate, 1.5 M ammonium sulfate, pH 7.0. Elution was performed with a linear gradient from 0 to 100% buffer B (25 mM Na-phosphate, pH 7.0) within 60 minutes. Retention times were compared to protein standards with known hydrophobicity. High HIC retention times were obtained for FAP x OX40 bispecific antigen binding molecules containing the clone 8H9 and charged patch variants of clone 49B4. Increased nonspecific interactions have been shown to correlate with high HIC retention time.

3.4.3 FcRn affinity chromatography

FcRn was expressed, purified and biotinylated as described (Schlothauer et al., MAbs 2013, 5(4), 576-86). For coupling, the prepared receptor was added to streptavidin-sepharose (GE Healthcare). The resulting FcRn-sepharose matrix was packed in a column housing. The column was equilibrated with 20 mM 2-(N-morpholine)-ethanesulfonic acid (MES) and 140 mM NaCl, pH 5.5 (eluent A) at a 0.5 ml/min flow rate. 30 µg of antibody samples were diluted at a volume ratio of 1: 1 with eluent A and applied to the FcRn column. The column was washed with 5 column volumes of eluent A followed by elution with a linear gradient from 20 to 100% 20 mM Tris/HCl and 140 mM NaCl, pH 8.8 (eluent B) in 35 column volumes. The analysis was performed with a column oven at 25 °C. The elution profile was monitored by continuous measurement of the absorbance at 280 nm. Retention times were compared to protein standards with known affinities.

3.4.4 Heparin affinity chromatography

Heparin affinity was determined by injecting 30-50 µg of sample onto a TSKgel Heparin-5PW (Tosoh) column equilibrated with 50 mM Tris, pH 7.4. Elution was performed with a linear gradient from 0 to 100% buffer B (50 mM Tris, 1M NaCl, pH 7.4 mM) within 37 minutes. Retention times were compared to protein standards with known affinities.

Table 18: Biophysical and biochemical properties of tested FAP x OX40 bispecific antibodies

Sample		Thermal stability DLS T_{agg}	Apparent hydro-phobicity	FcRn affinity	Heparin affinity
P1AE8870	OX40 (8H9) x FAP (1G1a) 2+1	57.6	0.83	1.81	0.62
P1AE8872	OX40 (MOXR0916) x FAP (1G1a) 2+1	67.5	0.31	0.15	0.58
P1AE8873	OX40 (8H9) x FAP (1G1a) 3+1	56.9	0.89	2.02	0.65
P1AE8874	OX40 (CLC563) x FAP (1G1a) 3+1	66.2	0.15	-0.14	0.51
P1AE8875	OX40 (MOXR0916) x FAP (1G1a) 3+1	67.4	0.32	-0.22	0.6
P1AE9176	OX40 (49B4_K26E_K73E) x FAP (1G1a) 4+1	62	0.61	-0.48	0.5
P1AF7217	OX40 (CLC563) x FAP (1G1a_EPKSCS) 4+1	66	0.14	-0.3	0.5
P1AF7205	OX40 (CLC563) x FAP (1G1a_EPKSCD) 4+1	67	0.14	-0.3	0.5
P1AF6455	OX40 (CLC563) x FAP (1G1a_EPKSCS) 3+1	67	0.14	-0.1	0.5
P1AF6454	OX40 (CLC563) x FAP (1G1a_EPKSCD) 3+1	66	0.14	-0.1	0.5
P1AF6457	OX40 (49B4_K23E_K73E) x FAP (1G1a_EPKSCS) 4+1	62	0.61	-0.5	0.5
P1AF6456	OX40 (49B4_K23E_K73E) x FAP (1G1a_EPKSCD) 4+1	62	0.61	-0.5	0.5
P1AD4524	OX40 (49B4) x FAP (4B9) (4+1)	48	0.56	0	0.68

3.5 Characterization of binding potency by surface plasmon resonance (SPR) after stress

The reduction in binding potency caused by incubation of the molecules for 14 days at 37°C, pH 7.4 and at 40°C, pH 6 was quantified by surface plasmon resonance using a Biacore T200 instrument (GE Healthcare). Samples stored at -80°C and pH 6 were used as reference. The reference samples and the samples stressed at 40°C were in 20 mM Histidine buffer, 140 mM NaCl, pH 6.0, and the samples stressed at 37°C in PBS buffer, pH 7.4, all at a concentration of 1.0 mg/ml. After the stress period (14 days) samples in PBS buffer were dialyzed back to 20 mM Histidine buffer, 140 mM NaCl, pH 6.0 for further analysis.

All SPR experiments were performed at 25°C with HBS-P+ buffer (10 mM HEPES, 150 mM NaCl, pH 7.4, 0.05% Surfactant P20) as running and dilution buffer. Biotinylated human OX40 and FAP, as well as biotinylated anti-hu IgG (Capture Select, Thermo Scientific, #7103262100) were immobilized on a Series S Sensor Chip SA (GE Healthcare, #29104992), resulting in surface densities of at least 1000 resonance units (RU). FAP-OX40 bispecific antibodies with a concentration of 2 µg/ml were injected for 30 s at a flow rate of 5 µl/min, and dissociation was monitored for 120 s. The surface was regenerated by injecting 10 mM glycine buffer, pH 1.5, for 60 s. Bulk refractive index differences were corrected by subtracting blank injections and by subtracting the response obtained from a blank control flow cell. For evaluation, the binding response 5 seconds after injection end was taken.

To normalize the binding signal, the OX40 and FAP binding was divided by the anti-hu IgG response (the signal (RU) obtained upon capture of the FAP x OX40 bispecific antibody on the immobilized anti-hu IgG antibody). The relative binding activity was calculated by referencing each temperature stressed sample to the corresponding, non-stressed sample. As shown in Table 19, all FAP x OX40 bispecific antibodies prepared in Example 2 show an improved binding upon stress to OX40 and FAP, as compared to a previously described FAP-OX40 bispecific antibody as described in WO 2017/060144 A1.

Table 19: Binding activity of FAP-OX40 bispecific antibodies to human to Ox40 and FAP after incubation at pH 6/40°C or pH 7.4/37°C for 2 weeks

Sample		binding activity [%]			
		2 weeks at pH 6.0/40°C		2 weeks at pH 7.4/37°C	
		FAP	Ox40	FAP	Ox40
P1AE8870	OX40 (8H9) x FAP (1G1a) 2+1	> 90	> 90	> 90	> 90
P1AE8872	OX40 (MOXR0916) x FAP (1G1a) 2+1	> 90	> 90	> 90	> 90
P1AE8873	OX40 (8H9) x FAP (1G1a) 3+1	> 90	> 90	> 90	> 90
P1AE8874	OX40 (CLC563) x FAP (1G1a) 3+1	> 90	> 90	> 90	> 90
P1AE8875	OX40 (MOXR0916) x FAP (1G1a) 3+1	> 90	> 90	> 90	> 90
P1AE9176	OX40 (49B4_K26E_K73E) x FAP (1G1a) 4+1	> 90	> 90	> 90	> 90
P1AF7217	OX40 (CLC563) x FAP (1G1a_EPKSCS) 4+1	98	100	94	100
P1AF7205	OX40 (CLC563) x FAP (1G1a_EPKSCD) 4+1	98	100	94	100
P1AF6455	OX40 (CLC563) x FAP	98	100	96	100

	(1G1a_EPKSCS) 3+1				
P1AF6454	OX40 (CLC563) x FAP (1G1a_EPKSCD) 3+1	99	100	95	99
P1AF6457	OX40 (49B4_K23E_K73E) x FAP (1G1a_EPKSCS) 4+1	99	99	99	99
P1AF6456	OX40 (49B4_K23E_K73E) x FAP (1G1a_EPKSCD) 4+1	99	100	99	100
P1AD4524	OX40 (49B4) x FAP (4B9) (4+1)	~90	> 90	~90	> 90

Example 4

Functional properties of FAP-targeted anti-human OX40 antigen binding molecules

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

Agonistic binding of OX40 to its ligand induces downstream signaling via activation of nuclear factor kappa B (NFκB) (A. D. Weinberg et al., J. Leukoc. Biol. 2004, 75(6), 962-972). The recombinant reporter cell line HeLa_hOx40_NFkB_Luc1 was generated to express human OX40 on its surface. Additionally, it harbors a reporter plasmid containing the luciferase gene under the control of an NFκB-sensitive enhancer segment. OX40 triggering induces dose-dependent activation of NFκB, which translocates to the nucleus, where it binds on the NFκB sensitive enhancer of the reporter plasmid to increase expression of the luciferase protein. Luciferase catalyzes luciferin-oxidation resulting in oxyluciferin which emits light. This can be quantified by a luminometer.

Thus, the capacity of the various FAP-targeted OX40 antigen binding molecules to induce NFκB activation in HeLa_hOx40_NFkB_Luc1 reporter cells was analyzed as a measure for bioactivity.

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

Adherent HeLa_hOX40_NFkB_Luc1 cells were cultured over night at a cell density of 0.2 x 10^5 cells per well and were stimulated for 6 hours with assay medium containing titrated anti-OX40 antigen binding molecules. For testing the effect of hyper-crosslinking

by secondary antibodies, 25 µL/well of medium containing secondary antibody anti-human IgG Fcγ-fragment-specific goat IgG F(ab')2 fragment (Jackson ImmunoResearch, 109-006-098) was added in a 1:2 ratio (primary to secondary antibodies). To test the effect of hyper-crosslinking by cell surface FAP binding, 25 µL/well of medium containing FAP^+ tumor cells (NIH/3T3-huFAP clone 19) were co-cultured in a 3 to 1 ratio (three times more FAP^+ tumor cells than reporter cells per well).

After incubation, assay supernatant was aspirated and plates washed two times with DPBS. Quantification of light emission was done using the luciferase 1000 assay system and the reporter lysis buffer (both Promega, Cat.-No. E4550 and Cat-No: E3971) according to manufacturer instructions. Briefly, cells were lysed for 30 minutes on dry ice by addition of 30 µL per well 1x lysis buffer. Cells were thawed for 20 minutes at 37 °C before 100 µL per well provided luciferase assay reagent was added. Light emission was quantified immediately with a Spark10M Tecan microplate reader using 500 ms integration time, without any filter to collect all wavelengths. Emitted relative light units (URL) were corrected by basal luminescence of HeLa_hOx40_NFkB_Luc1 cells and were plotted against the logarithmic primary antibody concentration using Prism7 (GraphPad Software, USA). Curves were fitted using the inbuilt sigmoidal dose response.

In **Figures 7A** to **7C** is shown the NFκB activation of bispecific antigen binding molecules comprising OX40 clone 49B4 in a 4+1, 3+1 and 2+1 format, either crosslinked with human FAP expressing NIH/3T3 fibroblasts, a secondary antibody at a 2 to 1 ratio or w/o further crosslinking. **Figures 8A** to **8C** show the NFκB activation of bispecific antigen binding molecules comprising OX40 clone 8H9 in a 3+1 and 2+1 format. **Figures 9A** to **9C** show the NFκB activation of bispecific antigen binding molecules comprising OX40 clone MOXR0916 in a 3+1 and 2+1 format and in **Figures 10A** to **10C** the NFκB activation of bispecific antigen binding molecules comprising OX40 clone CLC563 in a 3+1 and 2+1 format is shown. **Figures 11A** to **11C** demonstrate the NFκB activation of bispecific antigen binding molecules comprising amino acid variants of OX40 clone 49B4 in 4+1 format.

Thus, we tested the NFκB activating capacity of selected bispecific OX40 antigen binding molecules in a bivalent, trivalent and tetravalent FAP-targeted cross-Fab format alone and with hyper-crosslinking of the molecules by either a secondary antibody or a FAP^+ fibroblast cell line. The crosslinking of FAP-binding antibodies by cell surface FAP was tested using human fibroblast activating protein (huFAP) expressing NIH/3T3-huFAP clone 19. All OX40 antigen binding molecules induced dose dependent NKκB activation. The tetravalent and trivalent use of OX40 antibodies induced a certain NFκB activation due to the assembly of the trimeric core OX40 receptor-signaling unit already in the

absence of further external crosslinking of the constructs. OX40 antigen binding molecules with bivalent format (2+1) showed accordingly less bioactivity (49B4: Figure 7; 8H9: Figure 8, MOXR0916: Figure 9; CLC-563: Figure 10). Additional crosslinking by human FAP expressing fibroblasts via the FAP binding moiety, or by a secondary crosslinking antibody via the Fc region of the OX40 antigen binding molecule further increased the NFκB activation of all antigen binding molecules. For low-nanomolar bivalent OX40 antibodies (49B4, CLC-563), a higher valency of the OX40 resulted also with respect to bioactivity in an avidity gain (4+1 > 3+1 > 2+1), whereas no benefit of a 3+1 over 2+1 format was observed for sub-nanomolar bivalent OX40 binders (8H9, MOXR0916). All amino acid variants of 49B4 in a 4+1 format induced dose dependent NKκB activation to a similar extent than the parental antibody in the 4+1 format (Figure 11).

EC_{50} values of NFκB induction dose response curves w/o further crosslinking and w/ crosslinking by cell surface human FAP (NIH/3T3 huFAP clone 19) are summarized in Table 20.

Table 20: EC_{50} values for dose responses of NFκB activation by OX40 antigen binding molecules in a FAP targeted format in the presence or absence of cell surface human FAP^+ fibroblasts

Molecule ID	anti-Ox40 clone	Format	w/ hu FAP	w/o	AUC compared	w/ hu FAP	w/o
			EC50 [nM]		to	% of AUC	
P1AD3690	49B4	4+0	0.18	0.23	49B4 4+0	-	-
P1AE6838	49B4	4+1	0.04	0.38		225	89
P1AE9167	49B4 AA variant K73E	4+1	0.04	0.57		214	75
P1AE9169	49B4 AA variant K23T_K73E	4+1	0.04	0.39		221	84
P1AE9176	49B4 AA variant K23E_ K73E	4+1	0.04	0.45		206	78
P1AE8786	49B4	3+1	0.20	0.83	49B4 4+1	100	29
P1AE6840	49B4	2+1	0.12	-		77	17
P1AE8873	8H9	3+1	0.14	0.44	8H9 3+1	-	-
P1AE8870	8H9	2+1	0.21	-		75	76
P1AE8875	MOXR0916	3+1	0.31	3.94	MOXR09 16 3+1	-	-
P1AE8872	MOXR0916	2+1	0.19	-		73	no curve
P1AE8874	CLC563	3+1	0.24	0.79	CLC563 3+1	-	-
P1AE8871	CLC563	2+1	0.21	-		63	27

In another experiment, adherent HeLa_hOX40_NFkB_Luc1 cells were cultured over night at a cell density of 0.3 x 10^5 cells per well and were stimulated for 6 hours with assay medium containing titrated anti-OX40 antigen binding molecules. To test the effect of hyper-crosslinking by cell surface FAP binding, 25 µL/well of medium containing FAP+ tumor cells (NIH/3T3-huFAP clone 19) were co-cultured in a 3 to 1 ratio (three times more FAP+ tumor cells than reporter cells per well).

After incubation, assay supernatant was aspirated and plates washed two times with DPBS. Quantification of light emission was done using the luciferase 1000 assay system and the reporter lysis buffer (both Promega, Cat.-No. E4550 and Cat-No: E3971) according to manufacturer instructions. Briefly, cells were lysed for 30 minutes on dry ice by addition of 30 µL per well 1x lysis buffer. Cells were thawed for 20 minutes at 37 °C before 100 µL per well provided luciferase assay reagent was added. Light emission was quantified immediately with a Spark10M Tecan microplate reader using 500 ms integration time, without any filter to collect all wavelengths. Emitted relative light units (URL) were corrected by basal luminescence of HeLa_hOx40_NFkB_Luc1 cells and were plotted against the logarithmic primary antibody concentration using Prism7 (GraphPad Software, USA). Curves were fitted using the inbuilt sigmoidal dose response.

Thus, we tested the NFκB activating capacity of selected bispecific OX40 antigen binding molecules comprising clones OX40(49B4_K23E_K73E) or OX40(CLC563) in 3+1 and 4+1 formats as D and S variant alone and with hyper-crosslinking of the molecules by FAP+ fibroblast cell line. The results are shown in **Figures 35A** to **35F**. All OX40 antigen binding molecules induced dose dependent NKκB activation. The tetravalent and trivalent use of OX40 antibodies induced a certain NFκB activation due to the assembly of the trimeric core OX40 receptor-signaling unit already in the absence of further external crosslinking of the constructs **(Figures 35B, 35D** and **35F).** Additional crosslinking by human FAP expressing fibroblasts via the FAP binding moiety further increased the NFκB activation of all antigen binding molecules **(Figures 35A, 35C** and **35E).** The bioactivity of the D and S variant was of comparable strength.

EC_{50} values of NFκB induction dose response curves without further crosslinking and with crosslinking by cell surface human FAP (NIH/3T3 huFAP clone 19) are summarized in Table 21.

Table 21: EC_{50} values for dose responses of NFκB activation by OX40 antigen binding molecules (D and S variants) in a FAP targeted format in the presence or absence of cell surface human FAP^+ fibroblasts

Molecule ID	anti-Ox40 clone	Variant	Format	w/ hu FAP	w/o
				EC_{50} [nM]	
P1AF6455	CLC-563	S	3+1	0.2	0.8
P1AF6454	CLC-563	D	3+1	0.2	1.9
P1AF7217	CLC-563	S	4+1	0.18	0.7
P1AF7205	CLC-563	D	4+1	0.2	0.5
P1AF6457	49B4 AA variant K23E_ K73E	S	4+1	0.02	0.06
P1AF6456	49B4 AA variant K23E_ K73E	D	4+1	0.03	0.08

4.2 OX40 mediated co-stimulation of sub-optimally TCR triggered resting human PBMCs and hyper-crosslinking by cell surface FAP

It was shown in Example 4.1 that addition of FAP^+ tumor cells can strongly increase the NFκB activity induced by FAP targeted OX40 antigen binding molecules in human OX40 positive reporter cell lines by providing strong oligomerization of OX40 receptors. Likewise, we tested all constructs in a primary T cell assay for their ability to rescue suboptimal TCR stimulation of resting human PBMC cells in the presence of NIH/3T3-huFAP clone 19 cells.

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

Resting human PBMC were stimulated for four days with a suboptimal concentration of anti-CD3 antibody in the presence of irradiated FAP^+ NIH/3T3-huFAP clone 19 cells and titrated bispecific OX40 antigen binding molecules. Effects on T-cell survival and proliferation were analyzed through monitoring of total cell counts ($CD4^+$ or

$CD8^+$ T cells) and co-staining with fluorescently-labeled antibodies against T-cell activation marker (CD25 expression on $CD4^+$ T cells) by flow cytometry. Mouse embryonic fibroblast NIH/3T3-huFAP clone 19 cells were harvested using cell dissociation buffer (Invitrogen, Cat.-No. 13151-014) for 10 minutes at 37 °C. Cells were washed once with DPBS. NIH/3T3-huFAP clone 19 cells were irradiated in an xRay irradiator using a dose of 4500 RAD to prevent later overgrowth of human PBMCs by the tumor cell line. Irradiated cells were cultured at a density of 0.2×10^5 cells per well in T cell media in a sterile 96-well round bottom adhesion tissue culture plate (TPP, Cat. No 92097) over night at 37 °C/5% CO_2.

Human PBMCs were isolated from fresh blood by ficoll density centrifugation. Cells were added to each well at a density of 0.6×10^5 cells per well. Anti-human CD3 antibody (clone V9, human IgG1) at a final concentration of [10 nM] and FAP-targeted OX40 antigen binding molecules were added at the indicated concentrations. Cells were incubated for four days at 37 °C/5% CO_2 prior to analysis.

Cells were first stained 10 min with Zombie Aqua Fixable viability dye (Biolegend, Cat.No. 423102) in DPBS, followed by surface staining with fluorescent dye-conjugated antibodies anti-human CD4 (clone RPA-T4, BioLegend, Cat.-No. 300532), CD8 (clone RPa-T8, BioLegend, Cat.-No. 3010441) and CD25 (clone M-A251, BioLegend, Cat.-No. 356112) for 20 min at 4°C. Cell pellets were washed twice with FACS buffer. Samples were finally resuspended in 20 µL/well FACS-buffer and acquired the same day using iQue Cell Screener and ForeCyt software (Sartorius). Table 22 summarizes the EC_{50} values for dose responses of CD25 upregulation on $CD4^+$ T cells of bispecific OX40 antigen binding molecules in a FAP targeted format following suboptimal TCR stimulation of primary human PBMCs.

Table 22: EC_{50} values for dose responses of CD25 upregulation on $CD4^+$ T cells following suboptimal TCR stimulation of primary human PBMCs

MoleculeID	anti-Ox40 clone	Format	EC_{50} [nM]		AUC compared to reference compound	
					[%]	+/- SEM
P1AD3690	49B4	4+0	no curve fit		13	5
P1AE6838	49B4	4+1	0.02/0.003		107	31
P1AE9167	49B4 AA variant K73E	4+1	0.001		137	9
P1AE9169	49B4 AA variant K23T_K73E	4+1	0.001		128	10

P1AE9176	49B4 AA variant K23E_ K73E	4+1	0.001		128	10
P1AE8786	49B4	3+1	0.04		97	16
P1AE6840	49B4	2+1	0.09		59	22
P1AE8873	8H9	3+1	0.01		134	31
P1AE8870	8H9	2+1	0.01		113	32
P1AE8875	MOX0916	3+1	0.02		100	18
P1AE8872	MOX0916	2+1	0.01		106	22
P1AE8874	CLC-563	3+1	0.04		100	0
P1AE8871	CLC-563	2+1	0.06		99	15

As shown in **Figures 12** to **17**, co-stimulation with non-targeted anti-OX40 (49B4) 4+0 format barely rescued sub-optimally TCR stimulated CD4 and CD8 T cells. Hyper-crosslinking of the FAP targeted tetravalent, trivalent and bivalent OX40 antigen binding molecules in the presence of NIH/3T3-huFAP clone 19 cells strongly promoted survival and induced an enhanced activated phenotype in human CD4 T cells for all OX40 clones tested (49B4: **Figures 12** and **13**; 8H9: **Figure 14**, CLC563: **Figure 16**, MOXR0916: **Figure 15**). All amino acid variants of 49B4 in a 4+1 format supported T cell activation to an at least similar, if not even slightly improved extent than the parental antibody in the 4+1 format (**Figure 17**).

The normalized areas under the curve for the bioactivity (as measured as CD25 upregulation on $CD4^+$ T cells) of the various antigen binding molecules are shown in **Figure 18**. The major enhancement of OX40 supported T cell activation was achieved by FAP mediated hyper-crosslinking and thus cell surface immobilization of the OX40 antigen binding molecules (compare normalized AUC of 49B4 4+0 vs 4+1 antigen binding molecules in Figure 18 and Table 19). An increased valency for OX40 still added to the agonistic capacity of the targeted OX40 agonist antigen binding molecules, but to a lower extent than FAP crosslinking (compare normalized AUC of 4+1 vs 3+1 vs 2+1 formats in Figure 18 and Table 19). The strongest increase of agonistic power of a higher valency was observed for the avidity clone 49B4, where the tetravalency doubled the normalized AUC over the one of the bivalent format. This indicates that when selecting a high affinity –and less avidity driven OX40 clone for targeted OX40 agonists, bivalent and trivalent molecules can maintain similar activity than tetravalent 49B4 in a primary T cell assay *in vitro*.

The molecule design requirement for optimal T cell costimulation seems to be slightly different to that of pure NFκB activation downstream of the OX40 receptor. The first requires strong surface immobilization of multiple OX40 agonistic antibodies, which

cannot be compensated fully by antibody intrinsic valency of OX40 antibodies. The latter requires a high degree of OX40 receptor oligomerization by antibody intrinsic multivalent OX40 engagement, which cannot fully be compensated by surface immobilization of the agonists.

This might reflect the requirement for spatial restriction of response modulating phosphorylation and dephosphorylation events within primary T cells (termed T cell synapse) for optimal TCR engagement. A fully functional T cell synapse assembles the various signaling components (e.g. via lipid rafts) to achieve full functionality. The decreased lateral mobility of synapse component favors this, as might the cell surface immobilization of OX40 by FAP-targeted OX40 antigen binding molecules.

In a further experiment testing the C-terminal S and D variants, human PBMCs were labeled with the CFSE proliferation kit (Thermo Fisher, Cat-No. C34554) for 10 minutes at room temperature according to manufacturer's instruction at a final concentration of 0.2 [µM] CFSE. Cells were added to each well at a density of 0.6 x 105 cells per well in T cell media. Anti-human CD3 antibody (clone V9, human IgG1) at a final concentration of [10 nM] and the FAP-targeted OX40 antigen binding molecules, both prepared in T cell media, were added at the indicated concentrations. Cells were incubated for four days at 37 °C/5% CO_2 prior to analysis.

Cells were first stained 20 min with LIVE/DEAD™ Fixable Aqua Dead Cell Stain (Molecular probes, Cat.No. L34957) in DPBS, followed by one washing step (200 µL 4°C FACS buffer). Thereafter, surface staining with fluorescent dye-conjugated antibodies anti-human CD4 (clone OKT4, BioLegend, Cat.-No. 317440), CD8 (clone SK-1, BioLegend, Cat.-No. 344714) and CD25 (clone BC96, BioLegend, Cat.-No. 302626) for 30 min at 4°C. Cell pellets were washed twice with dPBS. Samples were finally resuspended in 100 µL/well FACS-buffer and acquired the same day using with BD Fortessa running FACS Diva software. Table 23 summarizes the EC_{50} values for dose responses of CD25 upregulation on CD4+ T cells of bispecific OX40 antigen binding molecules in a FAP targeted format following suboptimal TCR stimulation of primary human PBMCs.

Table 23: EC_{50} values for dose responses of CD25 upregulation on $CD4^+$ T cells following suboptimal TCR stimulation of primary human PBMCs

Molecule ID	anti-Ox40 clone	Variant	Format	EC_{50} [nM]
P1AF6455	CLC-563	S	3+1	0.003
P1AF6454	CLC-563	D	3+1	0.001
P1AF7217	CLC-563	S	4+1	0.002
P1AF7205	CLC-563	D	4+1	0.001

Molecule ID	anti-Ox40 clone	Variant	Format	EC_{50} [nM]
P1AF6457	49B4 AA variant K23E_ K73E	S	4+1	0.001
P1AF6456	49B4 AA variant K23E_ K73E	D	4+1	0.001

As shown in **Figures 36A** to **36F**, co-stimulation with a non-targeted tetravalent anti-OX40 (49B4) (4+0 format) rescued sub-optimally TCR stimulated CD4 and CD8 T cells only at concentrations higher than [1nM]. Hyper-crosslinking of the FAP targeted tetravalent and trivalent OX40 antigen binding molecules in the presence of NIH/3T3-huFAP clone 19 cells strongly promoted proliferation and induced an enhanced activated phenotype in human CD4 (**Fig. 36A, 36C** and **36E**) and CD8 (**Fig. 36B, 36D** and **36F**). The S variant performed slightly, but not statistically significant, worse than the D variant for the CLC563 constructs (**Fig. 36A** to **36D**). All amino acid variants of 49B4 in a 4+1 format supported T cell activation to an at least similar, if not even slightly improved extent than the parental antibody in the 4+1 format. Here, no difference was seen between the S and D variant (**Fig. 36E** and **36F**).

4.3 OX40 mediated co-stimulation increases the secretion of cytokines of CECAM5 TCB redirected PBMC that lyse CEA+ tumor cells

One clinically exploited way to recruit the patient's own immune system to fight cancer are T cell bispecific antibodies (TCB). These molecules are comprised of an agonistic anti-CD3 unit, specific for the T cell receptor (TCR) on T cells, and a targeting moiety specific for a unique cancer antigen. TCBs redirect polyclonal T cells to lyse cancer cells expressing the respective target antigen on their cell surface. No T cell activation occurs in the absence of such target antigen. The TCB used in this example is the CEACAM5 TCB targeting the carcinoembryonic antigen (CEA) and is described in detail in WO 2016/079076 A1. Triggering of the TCR increases, depending on the strength and duration of this primary stimulus, the expression of costimulatory molecules, e.g. OX40, a member of the Tumor necrosis factor receptor (TNFR) superfamily. Concomitant agonistic ligation of this receptor by its respective ligand promotes in turn hallmark T cell effector functions like proliferation, survival and secretion of certain proinflammatory cytokines (GM-CSF, IFN-γ, IL-2, TNF-α) (M. Croft et al., Immunol. Rev. 2009, 229(1), 173-191, I. Gramaglia et al., J. Immunol. 1998, 161(12), 6510-6517; S. M. Jensen et al., Seminars in Oncology 2010, 37(5), 524-532). This co-stimulation is needed to raise the full potential of T cells against tumor cells, especially in the context of weak tumor antigen priming, and to sustain the anti-tumor response beyond the first attack allowing for protective memory formation. In certain patients with a strong immune suppressed or exhausted phenotype, only the combination of polyclonal, yet tumor specific T cell recruitment (signal 1) and the

restoration of tumor-restricted positive co-stimulation (signal 2) might facilitate sufficient anti-tumor efficacy and prolonged adaptive immune protection. This can persistently drive the tumor microenvironment towards a more immune activating and less immunosuppressive state. FAP-dependent costimulation of OX40 may also facilitate TCB mediated killing of tumor cells at lower intratumoral concentrations which would allow reduction of systemic exposure and correlated side effects. Additionally, the treatment intervals might be prolonged as lower TCB concentration could still be active.

MKN45 NucLight Red (NLR) cells, which naturally express the CEA antigen were used as target cells. MKN-45 (DSMZ; ACC409) were transduced with the Essen CellPlayer NucLight Red Lentivirus Reagent (Essenbioscience, Cat. No. 4476; EF1α, puromycin) at an MOI of 5 (TU/cell) in the presence of 8 µg/mL polybrene following the manufacturer's instructions to stable express a nuclear-restricted NucLight Red fluorescent protein. This enabled easy separation from non-fluorescent effector T cells or fibroblasts and monitoring of the tumor cell growth by high throughput life fluorescence microscopy.

The crosslinking of FAP-binding antibodies by cell surface FAP was provided by human fibroblast activating protein (huFAP) expressing NIH/3T3-huFAP clone 19 (see Example 3.2). Human PBMCs were isolated from fresh blood by ficoll density centrifugation (see Example 3.1).

MKN45 NucLight Red (NLR) cells were added in each well at a density of 0.1 x 10^5 cells per well in T cell media. NIH/3T3-huFAP clone 19 were pre-irradiated at 4600 RAD and were then added in each well at a density of 0.1 x 10^5 cells per well in T cell media. Human PBMC were added to each well at a density of 0.5 x 10^5 cells per well in T cell media. The CEACAM5 TCB at a final concentration of 2nM and titrated dilutions of FAP-targeted OX40 antigen binding molecules, both prepared in T cell media, were added at the indicated concentrations. Cells were incubated for three days at 37 °C/5% CO_2 prior to analysis. Samples were performed as triplicates.

After 72 hours, the supernatant was collected for subsequent analysis of selected cytokine using the Bio-Plex Pro Human Cytokine 8-Plex Assay Catalogue No. BIO-RAD M50000007A according to manufacturer's instructions. Triplicate samples were united to equal parts for each tested compound at the respective tested concentration and the mix was analyzed for the cytokine content. The fold increase of a respective cytokine (GM-CSF, IL-2, TNF-α, IFN-γ) compared to the concentration in the TCB-only control sample was calculated and plotted against the FAP-OX40 antibody concentration. Dose-response curves were calculated using GraphPAD Prism, AUC and the EC_{50} values calculated and are reported in Table 24. Dose response curves for GM-CSF and TNF-α are depicted in

Fig. 37A to **37F**, and that for IFN-γ and IL-2 in **Fig. 38A** to **38F**. The AUC was normalized for each cytokine to the AUC of the FAP-OX40 antigen binding molecule OX40(CLC563) x FAP(1G1a_EPKSCD) 3+1 (called 3+1 CLC563/H212 –D in the Figure) and plotted for each compound as Box-Whisker plot in **Fig. 39**.

Table 24: EC_{50} values for dose responses of increased TCB mediated cytokine secretion following FAP-OX40 costimulation of primary human PBMCs

Molecule ID	anti-Ox40 clone	Variant	Format	EC_{50} [nM]
P1AF6455	CLC-563	S	3+1	0.058
P1AF6454	CLC-563	D	3+1	0.144
P1AF7217	CLC-563	S	4+1	0.058
P1AF7205	CLC-563	D	4+1	0.034
P1AF6457	49B4 AA variant K23E_K73E	S	4+1	0.022
P1AF6456	49B4 AA variant K23E_K73E	D	4+1	0.017

As shown in **Fig. 37A** to **37F** and **Fig. 38A** to **38F**, co-stimulation with non-targeted anti-OX40 (49B4) 4+0 format did not enhance the cytokine secretion of PBMC induced by CEACAM5 TCB mediated lysis of tumor cells. Hyper-crosslinking of the FAP targeted tetravalent and trivalent OX40 antigen binding molecules in the presence of NIH/3T3-huFAP clone 19 cells strongly promoted the secretion of GM-CSF (**Fig. 37A**, **37C** and **37E**) and of TNF-α (**Fig. 37B, 37D** and **37F**), as well as that of IFN-γ (**Fig. 38A, 38C** and **38E**) and of IL-2 (**Fig. 38B, 38D** and **38F**). The S-variant performed slightly, but not statistically significant, worse than the D-variant for all tested constructs (**Fig. 39**). The amino acid variants of 49B4 in a 4+1 format supported T cell activation stronger than the parental clone in the 4+1 format. Here, the CLC563 showed stronger agonistic potential as tetra than as trivalent FAP targeted OX40 agonist (**Fig. 39**).

4.4 OX40 mediated co-stimulation reduces TGFβ induced FoxP3 expression

CD4+Foxp3+ T regulatory cells (Tregs) play a critical role in immune homeostasis and peripheral tolerance (Sakaguchi S, Yamaguchi T, Nomura T, Ono M, Cell 2008, 133(5), 775-87). Their development, lineage stability, and suppressor functions are dependent on the expression of the transcription factor FoxP3, which is a ‘‘master’’ regulator of Treg identity (Hori S, Nomura T, Sakaguchi S, Science 2003, 299(5609), 1057-61). In addition to their thymic origin, CD4+FoxP3+ Treg cells can also be induced in the periphery from naive CD4+ T cells following activation, which are often called inducible Tregs (iTregs) or peripheral Tregs (pTregs) (Curotto de Lafaille MA, Lafaille J, Immunity 2009, 30(5), 626-35). The best characterized conditions for the induction of iTregs *in vitro* is the combination of transforming growth factor β (TGF-β) and CD28

costimulation. This cytokine potently induces de novo FoxP3 expression, which programs the conversion of activated conventional T cells to iTregs (Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM, J Exp. Med. 2003, 198(12), 1875-86). OX40 signaling has been described to inhibit FoxP3 expression and Treg induction (Zhang X, Xiao X, Lan P, et al., Cell Rep. 2018, 24(3), 607-618). It was shown in examples 4.1 to 4.3 that all FAP-OX40 bispecific antigen binding molecules were able to induce NFκB, and promote TCR stimulation resulting in enhanced activation phenotype and increased cytokine secretion. Likewise, we tested all constructs in a primary T cell assay for their ability to suppress TGF-β mediated FoxP3 induction.

Human PBMC preparations containing naive CD4 T cells were cultured in the presence of TGFβ during T cell activation with antibodies against CD28 and CD3. FoxP3 induction as well as OX40 expression occurs within several hours. Functional agonistic compounds against OX40, e.g. bispecific antigen binding molecules comprising OX40 clones OX40(49B4_K23E_K73E) or OX40(CLC563) n 3+1 and 4+1 formats as D- and S-variant, can signal via the OX40 receptor present on the activated CD4+ T cells, when crosslinking by FAP is provided (here FAP antigen coated to beads). This interferes with Treg induction visible by reduced FoxP3 expression.

Sterile 96-well round bottom adhesion tissue culture plates (TPP, Cat. No 92097) were pre-coated with anti-human CD3 antibodies (eBioscience, Cat.No. 16-0037-85) in dPBS at a concentration of 3 µg/mL for two hours 37°C/5% CO_2. Dynabeads® M-280 Streptavidin (ThermoFisher, Cat. No. 11205D) were coated with biotinylated human FAP antigen (Roche, P1AD8986; 0.01 µg protein for 1 µg beads) in dPBS for 30 minutes at room temperature according to manufacturer's instructions before storage at 4°C for long-term in dPBS containing 0.1% (w/v) BSA. Human PBMCs were isolated from fresh blood by ficoll density centrifugation. Cells were labeled with the CFSE proliferation kit (Thermo Fisher, Cat-No. C34554) for 10 minutes at room temperature according to manufacturer's instruction at a final concentration of 0.4 µM CFSE. Cells were then added to each well of the pre-coated plates at a density of 1 x 10^5 cells per well in serum-free X-Vivo15 medium (Lonza, Cat. No. BE02-054Q). Recombinant human TGF-β (2 ng/mL, R&D Systems, Cat.No. 240-B-010), monoclonal anti-human CD28 (1 µg/mL, eBioscience, Cat.No. 16-0289-85) and FAP coated beads (2 x 10^5 cells per well) were added. Titrated dilutions of FAP-targeted OX40 antigen binding molecules were added at the indicated concentrations. Cells were incubated for three days at 37 °C/5% CO_2 prior to analysis.

Cells were first stained 10 min with LIVE/DEAD™ Fixable Aqua Dead Cell Stain (Molecular probes, Cat.No. L34957) in DPBS, followed by one washing step (200 µL 4°C FACS buffer). Thereafter, surface staining with fluorescent dye-conjugated antibodies anti-

human CD4 (clone RPA-T4, BioLegend, Cat.-No. 300532), CD8 (clone RPA-T8, BioLegend, Cat.-No. 301042) and CD25 (clone BC96, BioLegend, Cat.-No. 302610) for 30 min at 4°C. Cell pellets were washed twice with FACS buffer, before cells were fixed and permeabilized in FoxP3 Fixation/Permeabilization working solution (FoxP3 staining Kit, eBioscience, Cat.No. 00-5521) for 60 minutes at room temperature in the dark according to manufacturer's instructions. After washing twice with 1x Perm buffer solution (FoxP3 staining Kit, eBioscience, Cat.No. 00-5521), cells were stained with fluorescent dye-conjugated antibody against FoxP3 (clone 29D6, BioLegend, Cat.-No. 20108) in 1x Perm buffer for 40 minutes at room temperature in the dark. Cell pellets were washed twice with 1x Perm buffer and finally resuspended in 100 µL/well FACS-buffer and acquired the same day using with BD Fortessa running FACS Diva software. Alive CD4+ CD25+Treg singlet cells were gated and the MFI of the αFoxP3 antibody reported. The FoxP3 MFI of each concentration was corrected by the MFI of the sample without OX40 bispecific antibody, thus only TGBβ, present.

Fig. 40A to **40C** show that FAP-OX40 bispecific antigen bispecific antigen binding molecules suppressed FoxP3 induction on resting CD4 T cells activated in the presence of TGFβ in a dose dependent manner. The D- and S-variant of each FAP-OX40 bispecific antibody showed similar bioactivity properties. In Table 25 the EC_{50} values were summarized for the FAP-OX40 agonist dose-responses of FoxP3 suppression (FoxP3 MFI) on CD4+ CD25+Treg cells.

Table 25: EC_{50} values of FAP-OX40 suppressed FoxP3 induction on TGFβ-exposed resting CD4 T cells

Molecule ID	anti-Ox40 clone	Variant	Format	EC_{50} [nM]
P1AF6455	CLC-563	S	3+1	0.25
P1AF6454	CLC-563	D	3+1	0.11
P1AF7217	CLC-563	S	4+1	0.01
P1AF7205	CLC-563	D	4+1	0.02
P1AF6457	49B4 AA variant K23E_ K73E	S	4+1	0.01
P1AF6456	49B4 AA variant K23E_ K73E	D	4+1	0.02

Summary of Results

For evaluation of the binding properties of targeted OX40 antigen binding molecules human FAP negative tumor cells (A549- NLR), FAP positive fibroblasts (NIH/3T3-huFAP-clone 19), OX40 positive activated PBMC (activated CD4 and CD8 T cells) as well as OX40 negative resting PBMC (resting CD4 and CD8 T cells) were incubated with

indicated serial dilutions of test antibody detected then by fluorescently labeled 2nd antibody against human Fcγ. All FAP-targeted OX40 antigen binding molecules bound efficiently to human FAP-expressing target cells and had no binding to target negative cells. FAP binding was for all constructs in a comparable nanomolar range. This is expected to translate in patients to direct tumor targeting and enrichment of the molecules. Along the natural prevalence of OX40 on T cells, all FAP-targeted OX40 antigen binding molecules bound stronger to activated CD4 than CD8 T cells. Antigen binding molecules harboring the avidity binder OX40 (49B4) bound better to OX40 in a tetravalent format (4+1) than in a trivalent or bivalent format (> 1000 x shift in EC_{50}). The mutated amino acid variants of clone 49B4 showed a comparable behavior. All other evaluated OX40 clones did not show an avidity gain in a trivalent format (3+1) compared to a bivalent format (2+1). The clones 8H9 and MOXR0916 bound with subnanomolar affinity, whereas clone CLC-563 bound with nanomolar affinity to OX40 positive T cells.

The tetravalent and trivalent OX40 antigen binding molecules induced NFκB activation in human OX40 positive NFκB reporter cells (HeLa_hOx40_NFkB_Luc1 reporter cells). This is due to the assembly of the trimeric core OX40 receptor-signaling unit already in the absence of further external crosslinking of the constructs. Bivalent formats per se showed accordingly less bioactivity. Additional crosslinking of the bispecific OX40 agonists by human FAP expressing fibroblasts via the FAP binding moiety, or by a secondary crosslinking antibody via the Fc region of the OX40 antigne binding molecule further increased the NFκB activation of all antigen binding molecules. For low nanomolar bivalent OX40 clones (49B4, CLC-563), a higher valency of the OX40 resulted also with respect to bioactivity in an avidity gain (4+1 > 3+1 > 2+1), whereas no benefit of a 3+1 over 2+1 format was observed for sub-nanomolar bivalent OX40 clones (8H9, MOXR0916). All amino acid variants of 49B4 in a 4+1 format induced dose dependent NKκB activation to a similar extent than the parental antibody in the 4+1 format. The bi- and trivalent OX40 antigen binding molecules did not reach the NFκB activation level of the tetravalent 4+1 format even at plateau concentration and at optimal crosslinking conditions.

Other than in the NFκB reporter assay, co-stimulation with non-targeted anti-OX40 (49B4) 4+0 format barely rescued sub-optimally TCR stimulated CD4 and CD8 T cells. Hyper-crosslinking of the FAP-targeted tetravalent, trivalent and bivalent OX40 antigen binding molecules in the presence of NIH/3T3-huFAP clone 19 cells, however, strongly promoted survival and induced an enhanced activated phenotype in human CD4 T cells for all OX40 clones tested. All amino acid variants of 49B4 were equally bioactive than the parental antibody in the same format.

The major enhancement of OX40 supported T cell activation was achieved by FAP-mediated hypercrosslinking and thus cell surface immobilization of the OX40. An increased valency for OX40 still added to the agonistic capacity of the FAP-targeted OX40 agonists, but to a lower extent. Selection of a higher affinity, and less avidity driven OX40 clone for targeted OX40 agonists, allowed reaching similar bioactivity than the tetravalent OX40 agonist already with bi- and trivalent OX40 antigen binding molecules. This discrepancy in molecule design requirements to achieve optimal OX40 receptor stimulation compared to T cell activation might reflect the requirement for spatial restriction of response modulating phosphorylation and dephosphorylation events within primary T cells (termed T cell synapse) for optimal TCR engagement. A fully functional T cell synapse assembles the various signaling components (e.g. via lipid rafts) to achieve full functionality. The decreased lateral mobility of synapse component favors this, as might the cell surface immobilization of OX40 by FAP-targeted OX40 agonists.

Example 5

Engineering of PK properties

5.1 Background and Properties of the reference compound

The bispecific antigen binding molecule OX40 (49B4) x FAP (4B9) (4+1) (molecule P1AD4524) as described in WO 2017/060144 A1 was explored in single intravenous dose huFcRn and in single intravenous dose PK studies in the cynomolgus monkey and atypically high clearances (>10-12 mL/min/kg) were observed as shown in Table 26. The *in vivo* protocols and the bioanalytical assays are described in Example 6.

Hu FcRn mice carry the human FcRn instead of the mouse FcRn and are, therefore, considered more predictive for the clearance in human as in wild-type mice (C57/Bl6). Indeed, the clearance in wild-type mice (11.0 mL/day/kg) had been relevantly lower as for huFcRn mice (33.0 mL/day/kg), which was consistent with the usual assumption that clearance in cynomolgus monkeys may be more predictive for human as e.g. clearance in wild-type mice following allometric transformation. There were no signs indicative of target mediated disposition (TMDD) in the construct plasma concentration-time profiles (**Figure 19**, very similar for monkey), as it is shown by similar clearances for increasing doses in the monkey (see Table 26).

Table 26: Single dose PK parameters of P1AD4524

Dose [mg/kg]	Clearance [mL/day/kg]			
	Mouse			Monkey
	C57/Bl6	Hu FcRn	FcRn KO	Cynom. (M/F)
5	11.9	33.0	190	30.7/25.7
25	--	--	--	29.5/19.7
100	--	--	--	34.3/31.1

Table 26 shows in addition the clearance in FcRn knockout mice, which is relevantly higher as expected for a usual human antibody (usually about 60-80 mL/day/kg are observed for a wild-type human antibody), although clearances in FcRn KO mice are always higher due to the lack of the FcRn rescue mechanism.

5.2 Surface Charge Patch exploration

The pharmacokinetics of an IgG antibody is dependent on FcRn binding, however it has been found that also the variable domains of an antibody have an influence on the clearance of the antigen binding molecule. Thus, it has been found that an antibody with a lower isoelectric point (pI) has a longer half-life (Igawa et al., Protein Engineering, Design & Selection 2010. 23, 385-392). A method on how to reveal positive charge patches on antibodies has been described by Zhang et al, Anal. Chem. 2011, 83, 8501-8508. Unspecific clearance of antibody constructs is often associated with so-called charge patches, i.e. areas on the antibody construct surface, which show primarily a positive charge and bind then to the negatively charged glycocalix of the cell membranes, followed by internalisation and degradation without rescue via FcRn.

5.2.1 Surface charge patch exploration of the FAP clone

The observed *in vivo* results, especially the high clearance in the FcRn knockout (KO) mice point to a high unspecific clearance of molecule P1AD4524, an antigen binding molecule with a fourfold OX40 (49B4) Fab fragment and a single FAP (4B9) antigen binding domain in the format of single domains VH and VL that are each connected to a C-terminal end of the Fc region, respectively.

Therefore it was explored by an iso-potential surface simulation of the molecule P1AD4524, whether its surface would show such positive charge patches. In analogy to Example 1.11, the FAP(4B9) antigen binding domain shows such a positive charge patch of prominent size (Figure 20), which could contribute to the unwanted high clearance of P1AD4524.

Furthermore, a similar VH/VL fragment as used in P1AD4524 was also included in a study with an unrelated molecule as published by Holland et al., J. Clin. Immunol. 2013, 33, 1192–1203. This VH/VL fragment was considered to be associated with immunological findings and potentially unspecific binding properties. Therefore, a replacement of the VH/VL fragment by a Fab fragment carrying the FAP binder was implemented for the antigen binding molecules of the present invention.

An antigen binding molecule P1AE6836 similar to P1AD4524, but comprising a C-terminal crossfab instead of the VH/VL fragment was then tested in HuFcRn mice to check, whether the omission of the VH/VL fragment would decrease the clearance relevantly.

The results are shown in Figure 21 and Table 27. The omission of the VH/VL fragment reduced clearance by about 7 mL/day/kg, but clearance was still at about 26 mL/day/kg and consequently above the desired range of not more than 10-12 mL/day/kg.

Table 27: Single dose PK parameters of P1AD4524

	Clearance [mL/day/kg]	
Dose	huFcRn mouse	
[mg/kg]	P1AD4524	P1AE6836
5	33.0	25.7

Therefore, further changes in the antigen binding molecule are necessary to reduce the clearance of the molecule.

5.2.2 Surface charge patch exploration of the OX40 (49B4) clone

Since the elimination of the positive charge patch on the FAP (4B9) clone was not sufficient to reach a clearance at least below 12 mL/day/kg in HuFcRn mice, the OX40 (49B4) clone was also explored for potential charge patches and the simulated isopotential surface of its Fab region is shown in Figures 22A-22C. The positive charge patches on the OX40 (49B4) clone may be smaller as on the 4B9 FAP binder, but since they occur four times in the antigen binding molecule due to its 4+1 valency, they might nevertheless be relevant for the high clearance of P1AE4524.

Therefore, it was decided to explore also other OX40 antibodies and to try to reduce the positive charge patches in P1AE4524.

5.3 Change of OX40 clones and effects on *in vitro* PK parameters

5.3.1 Surface charge patch exploration of alternative FAP and OX40 clones

Three different alternative OX40 clones were available and tested apart from two sequence variants of the 49B4 binder to avoid such charge patches. The simulated isopotential surface area of the Fab regions are shown in **Figures 23A-23C** (clone 8H9), **Figures 24A-24C** (clone CLC563) and Figures **25A-25C** (clone MOXR0916). The isopotential surface area of the Fab region for the alternative FAP clone 1G1a is shown in **Figure 26**.

A very crude ranking in order of potentially increasing clearance due to existing charge patches was probably CLC563 < 8H9 < MOXRO916, but this needed a test and confirmation by independent pharmacokinetic data, since the visual inspection of the isopotential surface is not quantitative. The three different binders were then explored by additional *in vitro* data and a selection needs to take also the bioactivity of the resulting construct into account to reach a balance between pharmacological bioactivity and clearance.

The FAP clone 4B9 on the VH/VL fragment was replaced by FAP clone 1G1a in a crossFab fragment connected to the C-terminal end of the antigen binding molecule. This FAP clone showed no prominent positive charge patch as shown in **Figures 20D** to **20F**.

5.3.2 ARC *in vitro* Assay Results

It was not feasible to perform with each potential antigen binding molecule of the available clones a new huFcRn mouse PK study. Therefore a preselection was performed by an *in vitro* assay, which explores the differences in recovery from compounds loaded to epithelial cells at neutral pH (pH 7.4, low binding to FcRn) and at pH 6.0 when binding to FcRn is strong. The shift in recovery between the two pH values to a better rescue by FcRn should be high (wild-type antibody > 10) and the value at pH 7.4 should be low (for a normal human wild-type antibody < 0.10- 0.20), otherwise there is unspecific binding with subsequent clearance expected. The assay is always controlled by a wild-type antibody and antibody not binding to FcRn, to have an internal control in each experiment.

The results of the *in vitro* ARC Assay are tabulated below in Table 28.

Table 28: Results of the ARC *in vitro* assay for various antigen binding molecules

		1st and 2nd experiment				3rd experiment			
Molecule ID	Valency	Shift	pH 6.0	pH 7.4	Recovery	Shift	pH 6.0	pH 7.4	Recovery
Wild-type IgG	2+0	10 >10	1.17 0.86	0.12 <0.06	Not rep. Not rep.	29	1.77	0.06	Not rep.
Non-binder	2+0	1.5 1.0	0.18 0.06	0.12 0.06	Not rep. Not rep.	1.0	0.06	0.06	Not rep.
P1AD4524	4+1 (VH/VL)	2.3 1.4	1.97 0.74	0.84 0.54	Not rep. Not rep.	4.6	0.92	0.20	83%
P1AE6838	4+1 (cross Fab)	2.0	0.40	0.20	83%	2.7	0.64	0.24	100%
P1AE8786	3+1	3.0	0.36	0.12	75%				
P1AE6840	2+1	11	0.67	0.06	60%				
P1AE9167	4+1					3.3	0.40	0.12	100%
P1AE9176	4+1					2.9	0.40	0.14	78%
P1AE9169	4+1					2.3	0.40	0.20	100%
P1AE8873	3+1	5.2	2.31	0.44					
P1AE8870	2+1	10	2.11	0.21	100%	24	6.99	0.28	88%
P1AE8874	3+1	1.7	0.32	0.20	83%	4.2	0.50	0.12	100%
P1AE8871	2+1	2.8	0.34	0.12	75%				
P1AE8875	3+1	1.1	0.18	0.16	80%				
P1AE8872	2+1	6.7	0.40	0.06	100%	29	1.72	0.06	100%

It is evident from Table 28 that the valency (how many OX40 binders are present in the antigen binding molecule) has a profound influence on the pH shift in the ARC assay.

Higher valencies (4+1 vs 3+1 and 3+1 vs 2+1) lead to a lower shift and presumably a higher clearance in the same homologues series.

The highest pH shift values were obtained for the 2+1 8H9 construct, 4+1 compounds had always the lowest pH shift, suggesting lower FcRn rescue and higher clearances.

The value at pH7.4 is suggestive of a high unspecific binding and the original compound P1AD4524 showed indeed the highest values at pH 7.4. The result of the ARC assay is therefore consistent with the high clearance of P1AD4524 in FcRn KO mice and the observed charge patches. Similarly, also the 3+1 8H9 construct may have a high unspecific clearance according to these *in vitro* results.

Consistent with the at least partial removal of the charge patches in the 4+1 antigen binding molecules containing OX40 (49B4) amino acid variants, compounds P1AE9167, P1AE9176 and to a less degree P1AE9169 had lower values at pH 7.4 suggesting lower unspecific binding and added further evidence for the negative impact of positive charge patches on unspecific antibody clearance.

More generally, it can be concluded from the results of the ARC assay that the removal of charge patches may reduce unspecific clearance and that higher valency leads to a higher clearance, which may be in line with recent observations published by Pyzik et al, Front. Immunol. 2019, 10:1540, doi: 10.3389/fimmu.2019.01540.

5.3.3 Exploration of selected antigen binding molecules *in vivo* in huFcRn

Three compounds were selected for an *in vivo* study in huFcRn mice based on the *in vitro* ARC assay and the bioactivity data: P1AE8870 (OX40 (8H9) x FAP (1G1a) 2+1), P1AE8873 (OX40 (8H9) x FAP (1G1a) 3+1) and P1AE9176 (OX40 (49B4_K23E_K73E) x FAP (1G1a) 4+1). The results are shown in Table 29. The results of the ARC assay were in good correlation to the *in vivo* results in huFcRn mice.

Table 29: Results of the HuFcRn mouse PK results in three selected antigen binding molecules vs. the original compound P1AD5424

Molecule ID	hu FcRn mice CL [mL/day/kg]	hu FcRn KO mice CL [mL/day/kg]	ARC score	ARC ratio
P1AD4524	34	190	0.80	1.9

P1AE9176	21	31	0.14	2.9
P1AE8873	18	85	0.44	5.3
P1AE8870	9.5	59	0.21	10
Roughly usual for wild-type antibody	<5-10	60-80	<0.1-0.2	>10-15

Based on these *in vitro* and animal *in vivo* results it can be concluded that the removal of the charge patch on the FAP antibody reduced clearance in huFcRn mice by about 7 mL/day/kg and that a higher valency (e.g. 4+1 vs 2+1) of the OX40 antibodies is associated with higher clearance in huFcRn mice, which may be a good surrogate for human patients.

5.4 Comparison of the PK properties of selected antigen binding molecules in 4+1 and 3+1 format

5.4.1 Background and Properties of the selected antigen binding molecules

The 4+1 variant of the OX40 (CLC563) x FAP (1G1a) bispecific antigen binding molecule (4x CLC563) tended to show improved activity properties in *in-vitro* assays in comparison to the respective 3+1 constructs (3x CLC563). Therefore, it was of interest to compare the pharmacokinetic (PK) properties of the two variants.

The bispecific antigen binding molecule OX40 (CLC563) x FAP (1G1a) 3+1 (molecule P1AF6454, S variant) was explored in single intravenous dose PK studies in human FcRn mice (Hu FcRn) and FcRn KO mice and relevantly improved lower clearances (target: <10-12 mL/day/kg for HuFcRn mice) were observed in comparison to the original 4+1 construct P1AD4524 as shown in Table 29. The *in vitro* and *in vivo* protocols as well as the bioanalytical assays are described in Example 6.5.

Hu FcRn mice carry the human FcRn instead of the mouse FcRn and are, therefore, considered more predictive for the clearance in humans as in wild-type mice (C57/Bl6). There were no signs indicative of target mediated disposition (TMDD) as e.g. a faster clearance below a certain concentration threshold in the construct plasma concentration-time profiles (**Figure 41**).

Table 30 shows in addition the clearance in FcRn knockout mice, which is relevantly higher as expected for a usual human antibody (usually about 60-80 mL/day/kg are observed for a wild-type human antibody), although clearances in FcRn KO mice are always higher due to the lack of the FcRn rescue mechanism.

Table 30: Single dose PK parameters of P1AF6455 / P1AF6454 (3+1) vs P1AD4524 (4+1)

Molecule ID	anti-Ox40 clone	huFcRn mice CL [ml/day/kg]	FcRn KO mice CL [ml/day/kg]
P1AD4524	4+1 49B4	34	190
P1AF6455	3+1 CLC563 (S)	--	28
P1AF6454	3+1 CLC563 (D)	6.1	--

5.4.2 Change of valency from 3+1 to 4+1 and effects on *in vitro* PK parameters

It was not feasible to perform with each potential antigen binding molecule of the available clones a new huFcRn mouse PK study. Therefore, a preselection was performed by two *in vitro* assays, the ARC assay and the LUCA assay. The ARC assay is already described in Example 5.3.2.

The LUCA assay makes use of a pH dependent difference in fluorescence intensity of a dye attached covalently to the antibody construct of interest during the *in vitro* procedure. The construct with the attached dye is then incubated with human primary liver endothelial cells which express human FcRn. The fluorescence in the endothelial cell remains low, if the labelled construct is well recycled by the FcRn in the endothelial cell, but increases, if the labelled construct is not well recycled and is processed in late endosomes and degraded in the lysosome, since the pH is decreasing in these late endosomes and the lysosome. The higher the LUCA value, the higher may be the *in-vivo* clearance.

The results of the *in vitro* ARC and LUCA Assay are tabulated below in Table 31.

Table 31: Results of the ARC and LUCA *in vitro* assays for various antigen binding molecules

Molecule ID	Valency & anti-OX40 clone	pH 7.4 ARC score	ARC ratio	LUCA
P1AF6455	3+1 CLC563 (D)	0.40	1.6	2.1
P1AF6454	3+1 CLC563 (S)	0.34	1.8	--

P1AF7205	4+1 CLC563 (D)	0.38	1.3	2.7
P1AF7217	4+1 CLC563 (S)	0.36	1.1	2.7

It is evident from Table 31 that the valency (how many OX40 binders are present in the antigen binding molecule) has a subtle influence on the results of the ARC assay, showing a higher ARC ratio for the 3+1 variants in comparison to the 4+1 construct, i.e. a lower *in vivo* clearance is theoretically expected for the 3+1 construct, if the unspecific uptake (pH7.4 ARC score is comparable), which was indeed the case here.

In contrast to the ARC assay results, the LUCA assay suggested minor advantages for the 4+1 construct, since here the LUCA value should be correlated to the fraction of construct handed over to the late endosome, indicating higher clearance for higher LUCA values.

Overall, the differences in the *in vitro* assays tended be rather subtle, therefore an *in vivo* study with the constructs was performed in hu FcRn and FcRn KO mice, since according to Pyzig et al., Front. Immunol. 2019, 10, 1540 (doi: 10.3389/fimmu.2019.01540) higher valency may be associated with higher clearance, as indicated by the - although moderate - difference in the ARC assay.

5.4.3 Exploration of selected antigen binding molecules *in vivo* in hu FcRn and FcRn KO mice

The following compounds were selected for an *in vivo* study in huFcRn mice based on the *in vitro* ARC assay and the bioactivity data: P1AE6455 (OX40(CLC563) x FAP(1G1a) 3+1 as C-terminal S variant,) P1AF7217 (OX40(CLC563) x FAP(1G1a) 4+1 as C-terminal S-variant) and P1AF7205 (as the respective C-terminal D-variant of the 4+1 construct). The results are shown in Table 32. For the in vivo study in FcRn KO mice P1AE6455 was replaced by P1AE6454 (OX40(CLC563) x FAP(1G1a) 3+1 as C-terminal D-variant). The *in vivo* results in huFcRn mice were in good rank order correlation with the results of the ARC assay.

Table 32: Results of the HuFcRn mouse PK results for three selected antigen binding molecules vs. P1AD5424

Molecule ID	hu FcRn mice CL [mL/day/kg]	hu FcRn KO mice CL [mL/day/kg]	pH7.4 ARC score	ARC ratio pH 6/7.4

P1AE6454 3+1 **D**-variant	--	28	0.34	1.8
P1AE6455 3+1 **S**-variant	6.1	--	0.40	1.6
P1AE7217 4+1 **S**-variant	30	49	0.36	1.1
P1AE7205 4+1 **D**-variant	23	51	0.38	1.3
Roughly usual for wild-type antibody	<5(-10)	60-80	<0.1-0.2	>10-15

Based on these *in vitro* and animal *in vivo* results it can be concluded that the higher valency (4+1) lead to a higher clearance of the more bulky 4+1 construct vs the 3+1 construct in line with the higher values for the ARC ratio. The HuFcRn clearance values were relevantly higher as the target value of <10-12 mL/day/kg and therefore the 4+1 construct is less preferred than the 3+1 construct due to high clearance.

Example 6

Method description of the PK *in vitro* and *in vivo* assays

6.1 Approach to detect possible PK liabilities of the OX40 (49B4) clone and antibody engineering to improve the antibody

Briefly, the isopotential surface area of the variable region of the OX40 binder was generated and assessed for large positively charged patches, which can be a cause of high unspecific clearance. One positively charged patch consisting of three positively charged amino acids were identified of which two were mutated to negatively charged amino acids. These amino acids are located in the framework and not in the CDRs and have therefore been predicted to preserve the ability of the antibody to bind to its target. Details to the approach can be found in patent application WO 2018/197533.

The amino acid sequence of the variable domain VH of the OX40 clone 49B4 was used to create a homology model using the MoFvAb software version 9 (MoFvAb is a Modeling tool for Antibody Fv regions, built internally by Roche). By using an *in silico* calculation method starting with the homology model, followed by pH- protonation of acidic and basic side-chains, we calculated the 3D charge distribution using the software

CHARMM and Delphi as implemented in the software suite Discovery Studio (vendor: Dassault Systems). By visual inspection of the resulting three dimensional charge distribution, a positively charged patch was identified which can be a cause of high unspecific clearance. It is composed of three amino acids in the VH, of which two are in the framework and not in the CDRs. Choosing framework residues and avoiding CDRs increases the probability of preserving target-binding affinity. Subsequently, these two framework residues (K23 and K73) were chosen to be mutated to e.g. glutamic acid, carrying a negative charge, which should decrease unspecific clearance.

6.2 ARC assay for the determination of pH dependent uptake and recovery *in vitro*

A cell-based FcRn transcytosis assay was established as screening assay and ranking tool that might help to predict the clearance of therapeutic antigen binding molecules (IgG molecule) and help in the selection of lead candidates.

Human FcRn-transfected Madin-Darby Canine Kidney (MDCK) cells were cultured as monolayers on Transwell® polycarbonate filters to assess IgG recycling and transcytosis at 37°C either at pH 6.0 or pH 7.4. **Figure 26** shows a scheme illustrating the transwell system. The assay is conducted in a "Pulse-Chase" format. First, the cells are incubated with the IgG molecule at either pH 6.0 or pH 7.4 (Pulse). Both compartments are subsequently washed to remove non-loaded IgG molecule and then the buffer is replaced with pH 7.4 for the "Chase". The release of the IgG molecule from the cells is measured after 2 hours (Chase) and quantified by IgG ELISA where a mean amount in ng is calculated for each condition. Both the apical and basolateral compartment are sampled to represent recycling and transcytosis, respectively. At pH 7.4, IgG is uptaken only via fluid-phase pinocytosis. This investigation reveals the susceptibility of an IgG to undergo nonspecific uptake for example due to its physicochemical properties and subsequent interactions with the cellular surface.

A final ARC score is calculated at both pH values:

$ARC\ Score\ pH\ 6.0$ = ng IgG recycled pH 6.0 + ng IgG transcytosed pH 6.0
$ARC\ Score\ pH\ 7.4$ = ng IgG recycled pH 7.4 + ng IgG transcytosed pH 7.4

Equation 1 ARC score formula for both pH values.

6.3 Analysis of serum samples for analyte concentrations for the huFcRn mouse studies

For the huFcRn and FcRn KO mouse studies, the concentration of analytes in mice serum was determined by an exploratory ELISA assay for mice serum which uses capture

and detection reagents specific for a human antibody (Ckappa and CH1 region). Limit of quantification was 69 ng/mL. The assay was described for cynomolgus monkey serum by Stubenrauch et al., Journal of Pharmaceutical and Biomedical Analysis 2013, 72, 208-215, and adapted for mouse plasma.

6.4 Analysis of serum for anti-drug antibodies for the huFcRn mouse studies

For the huFcRn and FcRn KO mouse studies, the measurement of anti-drug antibodies directed against the drug in mouse serum was performed by an exploratory ELISA assay which uses an anti-human IgG Fab fragment (Ckappa and CH1 region) as capture and an anti-Mouse-IgG antibody as detection reagent in mouse serum. The assay was described for cynomolgus monkey serum by Stubenrauch et al., Journal of Pharmaceutical and Biomedical Analysis 52 (2010) 249–254, and adapted for mouse plasma.

6.5 In vivo studies: Hu FcRn mouse and FcRn KO mouse PK studies

The test compounds were administered (5 mg/kg) intravenously to four male huFcRn Tg32 +/+ mice as a slow bolus and 20 microliter K_3EDTA blood was collected by microsampling at 0.0833, 7, 24, 48, 72, 168, 336, 408 and 504 h after the dose. Plasma was prepared by centrifugation and stored frozen until shipped on dry ice to be analyzed for human antibody concentrations and antidrug antibodies occurence by generic assays described in section 6.3 and 6.4.

Furthermore, the test compounds were administered (5 mg/kg) intravenously to three male FcRn KO mice as a slow bolus and 20 microliter K_3EDTA blood was collected by microsampling at 0.167, 2, 7, 24, 30, 48, 72 and 96 hours after the dose. Plasma was prepared by centrifugation and stored frozen until shipped on dry ice to be analyzed for human antibody concentrations and antidrug antibodies occurrence by generic assays described in section 6.3 and 6.4. The pharmacokinetic evaluation was performed by established noncompartmetal procedures.

6.6 LUCA assay for the estimation of unspecific clearance of antibodies

An *in vitro* assay was established for the prediction of unspecific clearance of therapeutic antigen binding molecules in primary human liver endothelial cells. Data is acquired by labeling the antigen binding molecules with a pH-sensitive dye exhibiting high fluorescence, when accumulating in the lysosome (acidic pH 5.5) and low fluorescence when remaining outside the cell (neutral pH 7.4). Human or animal endothelial cells are incubated with labeled antibodies for 2 and 4 hours and the fluorescent readout is recorded

using a flow cytometer. The geo-mean intensities are used for linear regression analysis. The extracted slopes form, when normalized to standard antibodies, the so-called relative LUCA rate.

To test whether the clearance detected within the LUCA assay is only mediated by unspecific uptake mechanisms such as pinocytosis, the assay was modified to also test for potential target-mediated (or off-target mediated) drug disposition (TMDD). Therefore, cells were pre-incubated with the equivalent unlabeled antibody counterpart to saturate the clearance-contributing target. To monitor the effect, different concentrations of the unlabeled antibody are applied to the cells prior as well as together with the labeled antibody. If the molecule exhibits potential TMDD, the relative LUCA rate would decrease with increasing concentrations of unlabeled antibody.

Antibody Labeling: The Antibodies were labeled using the SiteClick™ Antibody Azido Modification Kit (Thermo Fisher Scientific) according to the manufactures instructions. Briefly, N-linked galactose residues of the Fc-region were removed by β-galactosidase and replaced by an azide-containing galactose (GalNaz) via β-1,4-galactosyltransferase (GalT). This azide modification enables a copper-free conjugation of sDIBO-modified dyes. The pH-sensitive amine-reactive dye (523 nm) was purchased from Promega and coupled to a sulfo DBCO PEG4 amine. Antibodies were labeled with a molar dye excess of 2. Excess dye was removed using the Amicon® Ultra-2 Centrifugal Filter with a MWCO of 50 kD (EMD Millipore, # UFC200324) and antibodies were re-buffered in 20 mM histidine buffer (pH 5.5). The concentration of the labeled antibodies [1] as well as the dye to antibody ratio (DAR) [2] was determined with a Nanodrop spectrometer at 280 nm and 532 nm.

$$CAB = [A280nm - [A280nm * CFDye]] / \varepsilon mAb \quad [1]$$

$$DAR = [A532nm * MWmAb] / [cmAb * \varepsilon Dye] \quad [2]$$

$\varepsilon Dye = 47225$

$CFDye = 0.36$

Cell Maintenance and Preparation: Cryopreserved human liver-derived endothelial Cells (HLEC-P2) were purchased from Lonza (Lonza, #HLECP2). Cell were maintained in EBM™-2 Endothelial Cell Growth Basal Medium-2 (Lonza, #CC-3156) supplemented with EGM™-2 MV Microvascular Endothelial Cell Growth Medium SingleQuots™ (Lonza, # CC-4176). Five days prior antibody treatment, cells were plated onto collagen I coated 100 mm culture dishes (Corning® BioCoat™, #354450) and two days prior treatment sub-cultured into collagen I coated 96-well plates (Corning®

BioCoat™, #354407) at a cell density of $4x10^4$ cells/well to allow adherence for 48 hours. Medium was changed after 24 hours and cells were kept at 37 °C and 5 % CO_2.

On the day of the experiment, cells were washed twice with 200 µl pre-warmed medium and subsequently incubated with 400 nM labeled antibody or 20 mM histidine buffer (pH 5.5) as negative control in medium. If potential TMDD was tested, cells were pre-incubated with unlabeled counterparts (0, 0.2, 0.6, 1.2, 3, 6,2 µM) for 30 min at 37°C before the labeled antibody was added. After 2 and 4 hours, the antibody solution was removed and cells were washed once with 200 µl ice-cold DPBS (without Mg and Ca) and detached by applying 100 µl Trypsin (with EDTA) for 2.5 minutes at 37 °C. Trypsin was inactivated by the addition of 100 µl FACS Buffer (20 % FCS, 1 mM EDTA in DPBS).

Quality control: Biophysical binding properties are key determinants affecting clearance mechanisms. Therefore, it was important to assess, whether the binding affinities of the antibodies changed during the labeling process. Heparin chromatography and neonatal Fc receptor binding has been previously shown to predict antibody clearance in vitro (Kraft et al, mAbs 2020). Herein, this method was used to account for potential aberrant binding properties introduced by the click label. To confirm the absence of unbound dye and to verify the concentration measured at the spectrometer, a size exclusion chromatography of the labeled antibodies was performed. Samples were separated using a BioSuite Diol (OH) column (Waters, 186002165) with a potassium dihydrogen phosphate buffer (pH 6.2) as the mobile phase at a flow rate of 0.5 ml/min. Detectors at 280 nm and 532 nm were used to quantify and analyze the labeled antibodies. The area under the curve (AUCs) at 280 nm and 532nm was extracted to calculate the concentration. The geo-mean of the AUCs from all antibodies was computed and the deviation from each antibody to this geo-mean was identified. For an antibody to be reliable within this assay, the difference from the geo-mean was expected to be below 15 %.

Flow Cytometry and Pharmacokinetic Analysis: The mean fluorescent intensity (MFI, more specifically the geometric mean (geo-mean)) of the internalized antibodies was acquired using the MACSQuant® Analyzer 10 (Miltenyi Biotec) equipped with a laser to excite at 488 nm and a filter to collect emitted light at 585 nm / 540 nm. The exact same conditions, gains and gates were used for both times points (2 hours and 4 hours). Data extraction was performed using the FloJo_V10 software. Values of the negative control was subtracted from all geo-mean values followed by normalization to the DAR. The normalized geo-mean values from each antibody were plotted as linear regression curve using GraphPad Prism to extract the slope (Geo Mean MFI/min for 120 and 240 min). Two standard antibodies were selected to normalize the slopes: Motavizumab-YTE (G.J. Robbie et al., Antimicrob. Agent Chemother. 2013, 57(12), 6147) was set to 0 and CD20-CD3

TCB (Hutchings et al., Blood 2019, 134, 2871) was set to 1. The final slopes were plotted against published *in vivo* human, cynomolgus and hFcRn Tg32 +/+ mouse clearance values using the TIBCO Spotfire software.

***In vivo* Pharmacokinetic data**: Human and cynomologus clearance values were compiled from study reviews published by FDA, EMA and NCBI or personal communication with clinical pharmacologists. If several clearance values were available, dose linear clearance describing the unspecific clearance of molecules, was used. In case of linear pharmacokinetics parameter were determined by standard non-compartmental methods. Clearance was calculated according to following formula:

Clearance = Dose / Area under concentration-time curve

In cases of non-linear pharmacokinetics the linear fraction of the clearance was determined via following alternative methods: Either clearance values were estimated following IV administration at high dose levels, at which additional non-linear clearance pathways are virtually saturated. Alternatively, PK models comprising a linear and a non-linear, saturable clearance term were established. In these cases, the model-determined linear clearance fraction was used for correlations.

Murine clearance was obtained from internally performed studies as follows:

Mice: B6.Cg-Fcgrt tm1Dcr Tg(FCGRT)276Dcr mice deficient in mouse FcRn α-chain gene, but hemizygous transgenic for a human FcRn α-chain gene (muFcRn-/-huFcRn tg +/-, line 276) were used for the pharmacokinetic studies. Mouse husbandry was carried out under specific pathogen free conditions. Mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) (female, age 4-10 weeks, weight 17-22 g at time of dosing). All animal experiments were approved by the Government of Upper Bavaria, Germany (permit number 55.2-1-54-2532.2-28-10) and performed in an AAALAC accredited animal facility according to the European Union Normative for Care and Use of Experimental Animals. The animals were housed in standard cages and had free access to food and water during the whole study period.

Pharmacokinetic study: A single dose of antibody was injected i.v. via the lateral tail vein at a dose level of 5 mg/kg. The mice were divided into 3 groups of 6 mice each to cover 9 serum collection time points in total (at 0.08, 2, 8, 24, 48, 168, 336, 504 and 672 hours post dose). Each mouse was subjected twice to retro-orbital bleeding, performed under light anesthesia with Isoflurane™ (CP-Pharma GmbH, Burgdorf, Germany); a third blood sample was collected at the time of euthanasia. Blood was collected into serum tubes (Microvette 500Z-Gel, Sarstedt, Nümbrecht, Germany). After 2 h of incubation, samples

were centrifuged for 3 min at 9.300 g to obtain serum. After centrifugation, serum samples were stored frozen at -20 °C until analysis.

Determination of human antibody serum conditions: Concentrations of Ustekinumab, Briakinumab, mAb 8 and mAb 9 antibodies in murine serum were determined by specific enzyme-linked immunoassays. Biotinylated Interleukin 12 specific to the antibodies and digoxigenin-labeled anti-human-Fc mouse monoclonal antibody (Roche Diagnostics, Penzberg, Germany) were used for capturing and detection, respectively. Streptavidin-coated microtiter plates (Roche Diagnostics, Penzberg, Germany) were coated with biotinylated capture antibody diluted in assay buffer (Roche Diagnostics, Penzberg, Germany) for 1 h. After washing, serum samples were added at various dilutions followed by another incubation step for 1 h. After repeated washings, bound human antibodies were detected by subsequent incubation with detection antibody, followed by an anti-digoxigenin antibody conjugated to horseradish peroxidase (HRP; Roche Diagnostics, Penzberg, Germany). ABTS (2,2'Azino-di[3-ethylbenzthiazoline sulfonate]; Roche Diagnostics, Germany) was used as HRP substrate to form a colored reaction product. Absorbance of the resulting reaction product was read at 405 nm with a reference wavelength at 490 nm using a Tecan sunrise plate reader (Männedorf, Switzerland). All serum samples, positive and negative control samples were analyzed in duplicates and calibrated against reference standard.

PK analysis: The pharmacokinetic parameters were calculated by non-compartmental analysis using WinNonlin™ 1.1.1 (Pharsight, CA, USA). Briefly, area under the curve (AUC0-inf) values were calculated by logarithmic trapezoidal method due to non-linear decrease of the antibodies and extrapolated to infinity using the apparent terminal rate constant λz, with extrapolation from the observed concentration at the last time point. Plasma clearance was calculated as Dose rate (D) divided by AUC0-inf. The apparent terminal half-life (T1/2) was derived from the equation $T1/2 = \ln 2/\lambda z$.

Example 7

Evaluation and improvement of preexisting Anti-Drug Antibody Reactivity

7.1 Evaluation of preexisting Anti-Drug Antibody (ADA) Reactivity

For the evaluation of the root cause of preexisting IgG interference, an exploratory ELISA in human individual plasma samples to detect anti-drug antibodies (ADA) was performed. It uses a biotinylated anti-PGLALA antibody against the amino acid mutations L234A, L235A and P329G ("PGLALA modification") in the Fc domain of the drug as a capture reagent and a digoxigenin labeled FcγReceptor I (CD64) as detection reagent,

which binds to a human IgG with no PGLALA modification that is part of the ADA-drug complex. To compare preexisting IgG interference to different drug molecules, this assay was performed on the same panel of naïve human individual plasma samples.

2 µg/mL biotinylated anti-PGLALA antibody is coated to the streptavidin coated microtiterplate in the first step. In parallel, naïve human individual plasma samples (BioIVT) were pre-incubated with a buffer containing $6.7x10^{-9}$ mol/L of the respective drug molecule for 30 minutes at room temperature to allow the formation of the Drug-anti-drug antibody complex. After incubation of the samples and a washing step, human IgG with no PGLALA modification that is part of the immune complexes and bound to the surface can be detected with 0.5 µg/mL digoxigenin labeled FcγReceptor I (CD64). After washing, a polyclonal anti-digoxigenin-horseradish peroxidase (HRP) conjugate (50 mU/mL) was added and incubated. After another washing step and the addition of the ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) substrate solution to the microtiterplate, the HRP of the antibody enzyme conjugate catalyzes the color reaction. An ELISA reader measures the absorption at 405nm wavelength. [(Wessels et al, Bioanalysis 2017, 9(11), 849-859).

7.2 Improvement of preexisting Anti-Drug Antibody (ADA) Reactivity

The bispecific antigen binding molecule OX40 (49B4) x FAP (4B9) (4+1) (molecule P1AA1119) as described in WO 2017/060144 A1 was explored in naive human plasma samples for preexisting IgG interference. High incidence with high signals was observed as shown in **Fig. 27A**. Holland et al., J. Clin. Immunol. 2013, 33, 1192–1203 published that a similar VH/VL fragment as included in P1AA1119 in another type of molecule has shown reactivity to human anti-VH autoantibodies. Therefore, it was questioned if a replacement of the VH/VL fragment by a Fab fragment carrying the FAP binder can lead to less ADA reactivity in the antigen binding molecules of the present invention.

An antigen binding molecule P1AE6836, similar to P1AA1119, but with a Fab fragment carrying the FAP binder, was produced and investigated for preexisting IgG reactivity in the same panel of naïve human plasma samples, as shown in **Figures 27A to 27C**. P1AE6836 still showed preexisting IgG Interference, with reduced signals compared to P1AA1119, and in different individual human samples, indicating a different type of preexisting anti-drug antibodies against the Fab fragment carrying the FAP binder (**Fig. 27B**). A similar molecule, but comprising the humanized FAP clone 1G1a (P1AE6838, **Fig. 27C**), confirmed the results obtained with the molecule comprising the FAP 4B9 clone (P1AE6836), indicating that the FAP clone is not the root cause of the preexisting IgG reactivity.

As can be seen in **Figure 28,** all bispecific antigen binding molecules, sharing the FAP (1G1a) antigen binding domain, but different anti-OX40 Clones (49B4, 8H9, MOX0916 and CLC-563) showed the same preexisting IgG interference, indicating that the OX40 Clone is not the root cause of preexisting IgG reactivity.

In **Figure 29A** a subset of human individual plasma samples was tested with control molecules comprising the OX40(49B4) clone (P1AD3690) (4+0), an untargeted molecule comprising four OX40(49B4) Fab fragments, a FAP(1G1a) molecule (P1AE1689) comprising the humanized FAP(1G1a) Fab fragment), and the Germline control antibody (P1AD5108, DP47). These molecules do not cause preexisting IgG reactivity and therefore show low background signals. **Figure 29B** shows that all valences for OX40 (2+1, 3+1 and 4+1) result in preexisting IgG interference with a slight increasing trend of signal height 2+1>3+1>4+1, presumably due to sterical hindrance.

Literature (Kim et al, MABS 2016, 8, 1536–1547) suggests that several proteases associated with invasive diseases are able to cleave antibodies in the hinge-region, thus generating neoepitopes for anti-hinge antibodies. We thus produced molecules with different C-terminal amino acids in the CH1 domain of the Fab fragment that is fused to the C-terminus of the Fc domain and thus has a "free" hinge-like region. Whereas the original bispecific antibody has a C-terminal amino acid sequence of EPKSC (SEQ ID NO:163), variants with C-terminal amino acid sequences of EPKSCD (SEQ ID NO:164) and EPKSCS (SEQ ID NO:165) were produced.

To evaluate the C-terminal extension variants of antigen binding molecules OX40 (49B4) x FAP (1G1a) (3+1), the same panel of human individual serum samples was tested (**Fig. 30A**) and its individual background signal substracted (**Fig. 30C**). The individual background signal was measured by performing the assay without the drug molecule (**Fig. 30B**).

An extension of the C-terminus by naturally occurring aspartate at this position of the upper hinge region was generated in the molecule OX40 (MOXR0916) x FAP (1G1a) (3+1) (P1AF4845) (**Fig. 31B**). This modification resulted in a reduction of preexisting IgG reactivity compared to the molecule P1AE8786 with a free C-terminus (**Fig. 31A**). To eliminate the preexisting IgG reactivity completely, a variant with a C-terminal serine was generated. This serine is not naturally located at this position of the upper hinge region. The extension of the C-terminus by a serine of molecule P1AF4851 led to a complete elimination of the preexisting IgG reactivity, as shown in **Fig. 31C.**

Figures 32A to 32C show a respective molecule set in 2+1 format, and confirm the previous results that a C-terminal extension of an aspartate (Molecule OX40 (MOXR0916)

x FAP (1G1a) (2+1) with EPKSCD terminus, P1AF4852, **Fig. 32B**) reduces, while a C-terminal serine (molecule OX40 (MOXR0916) x FAP (1G1a) (2+1) with EPKSCS terminus, P1AF4858, **Fig. 32C**) eliminates the reactivity with preexisting antibodies in plasma compared to a molecule OX40 (49B4) x FAP (1G1a) (2+1) with a free C-terminus EPKSC (P1AE6840, **Fig. 32A**).

As can be seen in **Figure 33**, three further molecule examples strengthen the finding that the additional C-terminal serine abolishes the preexisting ADA reactivity completely (**Figures 33B, 33D** and **33F**), whereas the C-terminal aspartate reduces the unwanted interference (**Figures 33A, 33C** and **33E)**.

Example 8

Evaluation of FAP-targeted anti-OX40 bispecific antigen binding molecules for the Risk of Cytokine Release in an *in vitro* 24-Hour Human Whole Blood Assay

In order to evaluate potential safety risks relating to cytokine release upon first dosing in humans, *in vitro* non-GLP testing for FAP x OX40 bispecific -mediated cytokine secretion was conducted with fresh undiluted human whole blood samples from 10 healthy donors. The blood samples were incubated for 24 hours with concentrations of 0.1, 1, 10 and 50µg /mL of FAP x OX40 bispecific molecules and subsequent release of cytokines in the plasma was measured. Erbitux®, an anti-EGFR IgG1 mAb, was used as a negative comparator and Lemtrada®, a humanized anti-CD52 IgG1, known to induce first infusion reactions (IRRs) in greater than 90% of recipients, was used as a positive comparator.

8.1 Material and Methods

8.1.1. Tested Molecules

The following FAP-targeted anti-OX40 bispecific antigen binding molecules were tested:

C1: OX40(CLC563) x FAP(1G1a_EPKSCD) 3+1 (P1AF6454), concentration c = 5.8 mg/ml, 20 mM His/HisCl, 140 mM NaCl, pH 6.0, received at -80°C and after thawing stored at 4°C,

C2: OX40(CLC563) x FAP(1G1a_EPKSCS) 3+1 (P1AF6455), c = 2.0 mg/ml, 20 mM His/HisCl, 140 mM NaCl, pH 6.0, received at -80°C and after thawing stored at 4°C,

C3: OX40(49B4) x FAP (4B9) 4 +1 (P1AD4524, RO7194691), c= 10.0 mg/ml, 20 mM His/HisCl, 140 mM NaCl, pH 6.0, received at -80°C and after thawing stored at 4°C,

C4: OX40(CLC563) x FAP(1G1a_EPKSCS) 4+1 (P1AF7217), c = 9.73 mg/ml, 20 mM His/HisCl, 140 mM NaCl, pH 6.0, received at -80°C and after thawing stored at 4°C,

C5: OX40(CLC563) x FAP(1G1a_EPKSCD) 4+1 (P1AF7205), c = 10.28 mg/ml, 20 mM His/HisCl, 140 mM NaCl, pH 6.0, received at -80°C and after thawing stored at 4°C,
C6: OX40(49B4_K23E_K73E) x FAP(1G1a_EPKSCS) 4+1 (P1AF6457), c = 10.40 mg/ml, 20 mM His/HisCl, 140 mM NaCl, pH 6.0, received at -80°C and after thawing stored at 4°C, and
C7: OX40(49B4_K23E_K73E) x FAP(1G1a_EPKSCD) 4+1 (P1AF6456), c = 10.17 mg/ml, 20 mM His/HisCl, 140 mM NaCl, pH 6.0, received at -80°C and after thawing stored at 4°C.

Table 33: FAP-targeted anti-OX40 bispecific antigen binding molecules tested

Molecule ID	anti-Ox40 clone	Variant	Format	Molecule name in figures
P1AF6454	CLC-563	D	3+1	C1
P1AF6455	CLC-563	S	3+1	C2
P1AD4524	49B4	-	4+1	C3
P1AF7217	CLC-563	S	4+1	C4
P1AF7205	CLC-563	D	4+1	C5
P1AF6457	49B4 AA variant K23E_K73E	S	4+1	C6
P1AF6456	49B4 AA variant K23E_K73E	D	4+1	C7

8.1.2. Control Substances

The following molecules were used as control:
Low Risk Comparator: Erbitux®, cetuximab, recombinant anti-EGFR, chimeric IgG1, Merck Serono, USA, stock 5 mg/mL, sterile liquid, stored at 4°C.
High Risk Comparator: Lemtrada®, alemtuzumab, recombinant anti-CD52, humanized IgG1, Genzyme, USA, stock 10 mg/mL, sterile liquid, stored at 4°C.
LPS: lipopolysaccharide derived from Salmonella abortus, Sigma, Product. No. L5886, stock conc: 1 mg/mL
PBS: Dulbecco's phosphate-buffered saline, Gibco No.14190

8.1.3. Human Whole Blood Assay

Venous blood from healthy donors was collected in vacutainer tubes containing lithium heparin as anticoagulant (Roche Medical Center, Basel, Switzerland) and kept at room temperature until initiation of the assay (within 1-3 hours).

Pre-validation experiments revealed optimal performance conditions with blood processed within 3 hours, as otherwise lysis of erythrocytes occurred. Final test items concentrations ranged from 0,1 to 50 µg/ml of antigen binding molecule by adding 195 µl

of blood in triplicates to U-bottom wells of 96-well plates containing 5 µl of the items to be tested (1:40). This concentration range was selected to cover the foreseen exposure following administration of P1AD4524 as known from earlier studies.

These conditions ensure optimal performance with respect to practicality and efficiency to gain at least 70 µl of plasma and sufficient cells to conduct multi-cytokine analysis and enumeration of cell subsets respectively. Endogenous activation of blood cells and responsiveness was assessed by including controls containing PBS or vehicle and LPS, respectively. After incubation for 24 hours at 37°C with 5% CO_2, cells and plasma were separated by centrifugation at 1800g for 5 min. Plasma samples were stored at -80°C until analysis of cytokine content.

8.1.4. Multiplex Cytokine Assay

Determination of cytokine concentrations was performed on frozen plasma samples diluted 1:5. Pre-tests revealed that levels of cytokine did not differ between fresh and thawed samples. Analyte concentrations were determined by ELISA using the Human Cytokine chemiluminescent assay kit (Aushon Ciraplex, Cat. No 101-269-1-AB) with the SignaturePLUS™ imaging system and the Cirasoft analysis software. Results were expressed as pg/ml. Values above ULOQ concentration were assigned the concentration of the highest standard as follows: IFNγ, 500 pg/mL; IL-6, 2000 pg/mL; IL-8, 4000 pg/mL; TNFα, 1000 pg/ml. Sample values below LLOQ level were assigned LLOQ concentrations: IFNγ, 0.24 pg/mL; IL-6, 0.98 pg/mL; IL-8, 1.95 pg/mL; TNFα, 1.95 pg/ml.

8.2 Results and conclusion

In this assay, the OX40(49B4) x FAP (4B9) 4 +1 (P1AD4524, C3) led to the secretion of IL-6, IL-8 with maximum positive response of 33% and 33%, and maximum median cytokine upregulation of 1.0 fold (IL-6) and 2.73 fold (IL-8), respectively, relative to the negative comparator Erbitux®. The highest frequencies of responders and maximum median upregulation were observed at 10 µg/ml P1AD4524 for IL-6 and at 50 µg/ml P1AD4524 for IL-8.

Comparatively, less IL-6 and IL-8 release was observed for the panel of the bispecific FAP x OX40 antigen binding molecules as described herein (C1, C4, C5, C6 and C7), except for C2 which had even slightly elevated frequency of IL-8 responders (44%) at 50 µg/ml. C1 had the best cytokine profile (lowest IL-6 and IL-8 release) among all test molecules. C1 and C2 showed no IFNγ responders whereas C3 had 22% responders. TNF-α responses were low (0-11%) among all tested constructs.

Figures 42A and **42B** show the IL-6 secretion in every donor's blood sample after the addition of different concentrations of the tested molecules, the IL-8 secretion is shown in **Figures 43A** and **43B**.

Example 9

Immunogenicity risk evaluation

The sequence-related risk of immunogenicity was evaluated using an *in vitro* DC:$CD4^+$ T cells re-stimulation assay and the immunogenicity related to the mode of action was evaluated using transgenic mice that are tolerant to human IgG1 antibodies.

9.1 DC-T cell assay

The sequence-related immunogenicity of the FAP x OX40 bispecific antigen binding molecules was carried out using the DC:$CD4^+$ T cells re-stimulation assay for the assessment of T cell activation with PBMCs from 30 healthy human donors. All proteins were tested in the same 30 healthy donors and the $CD4^+$ T cell response induced by each condition was assessed by IFNγ FluoroSpot.

9.1.1. Materials

As controls, the Keyhole limpet haemocyanin (KLH) was reconstituted and stored at -80°C in single use aliquots according to the manufacturer's recommendations under sterile conditions. Bevacizumab (Avastin®) and/or adalimumab (Humira®) was included as a benchmark protein. Avastin® was supplied by F. Hoffmann-La Roche AG and stored at +4°C in single-use aliquots. Avastin® was used at a final concentration of 300 nM for both the DC stimulation stage and for the APC restimulation stage.

The tested FAP x OX40 bispecific antigen binding molecules were OX40(CLC563) x FAP(1G1a_EPKSCD) 3+1 (P1AF6454) and OX40(CLC563) x FAP(1G1a_EPKSCS) 3+1 (P1AF6455). In an earlier experiment, P1AD4524 was tested as well.

For the donor cells, all samples were collected under an ethical protocol approved by a local REC (research ethics committee) and written informed consent was obtained from each donor prior to sample donation. PBMC from healthy donors were prepared from whole blood within six hours of blood withdrawal. Cells were cryopreserved in vapour phase nitrogen until use in the assays. The quality and functionality of each PBMC preparation was analyzed by 7 day activation with positive controls such as KLH to assess naïve T cell responses. The MHC-Class II allotypes of the 30 healthy donors broadly cover for diversity and the HLA-DRB1 frequencies in the study match the world populations.

9.1.2. Methods

9.1.2.1 Epibase® DC:CD4+ T cells re-stimulation assay (according to Lonza protocol)

Monocytes were isolated from frozen PBMC samples by magnetic bead selection (Miltenyi Biotec) and differentiated into immature DC (iDC) using GM-CSF and IL4. iDC were then harvested, washed and loaded with each individual test protein for 4 hours at 37°C. A DC maturation cocktail containing TNFα and IL-1B was then added for further 40-42 hours to activate/mature the DC (mDC). The expression of key DC surface markers (CD11c, CD14, CD40, CD80, CD83, CD86, CD209 and HLA-DR) at both the immature and mature stage were assessed by flow cytometry to ensure the DC were activated prior to T cell interaction. $1x10^5$ mDC were then co-cultured with $1x10^6$ autologous CD4+ T cells (isolated by negative magnetic selection for 6 days at 37°C, 5% CO_2 in a humidified atmosphere. On day 6, autologous monocytes were isolated from PBMC using negative magnetic bead selection and loaded with the selected protein/peptide that were initially used to load the DC. After incubation at 37°C, 5% CO_2 in a humidified atmosphere for 4 hours. $5x10^4$ monocytes/well were added to anti-INFγ/anti-IL-5 pre-coated FluoroSpot plates (Mabtech) along with the corresponding DC:CD4 co-culture in quadruplicate ($2.5x10^5$ $CD4^+$ T cells/well). The FluoroSpot plates were incubated for 40-42 hours at 37°C, 5% CO_2 in a humidified atmosphere. After incubation the FluoroSpot plates were developed using an in-house procedure and the spot-forming cells (SFC) per well assessed for each cytokine in each test condition using an IRISTM FluoroSpot Reader (Mabtech).

Surface marker QC checks were also performed on the monocyte derived DC at both the immature and mature stage to determine any possible influence of the test compound on the DC differentiation and allows for the assessment of the quality of the DCs before subsequent co-culture with $CD4^+$ T cells. Surface markers are assessed by flow cytometry using fluorescently labelled antibodies and the Guava® easyCyte™ 8HT flow cytometer.

9.1.2.2 Data management and QC

Documented QC and QA procedures were performed on all experimental data. Subsequent data analysis was carried out using Excel and the statistical program “R” with GraphPad Prism used for graphical representation of data.

9.1.2.3 Statistical analysis

Response frequency: Data were analysed at donor level to determine if each individual test condition induces a significant T cell response in each donor. Spot forming units (SFU) per well (i.e. the number of cytokine-secreting cells per well) in response to

each test condition were evaluated with the distribution free resampling (DFR) method (Moodie et al. 2010). The DFR method is a non-parametric statistical test that compares each test condition against the reference condition for each donor and indicates if the difference between test condition and the reference condition is statistically significant. The two described variations of this method are DFR(eq) and DFR(2x).

DFR(eq) utilizes permutation resampling and allows for a maximum false positive rating of 5% ($p \leq 0.05$) and a minimum of 1% ($p \leq 0.01$). DFR(2x) replaces permutation resampling with the Bootstrap test and controls false positive rating to < 1% ($p < 0.01$) through application of a stricter null hypothesis which incorporates a minimum response threshold in which the experimental results must be a minimum of 2-fold higher than the related negative controls in order to be judged statistically significant. These two DFR methods are coupled to Westfall-Young step down max T approach (onesided) to calculate p value and account for multiple comparisons. In this project, DFR(2x) was used to determine a statistically significant $CD4^+$ T cell response.

Response Intensity: A stimulation index (SI) was calculated for each test condition in each donor by dividing the SFU/well in the test condition by the SFU/well in the blank (assay medium only) to highlight the magnitude of the T cell response in each donor. The Geometric mean along with the median and interquartile range over the 30 donor population was then calculated for each test condition.

9.1.2.4 Population analysis of Immunogenicity

To assess the impact of each sample on the whole 30 donor population, the stimulation index (SI) was calculated for each test condition in each donor. The stimulation index was calculated by dividing the SFU/well in the test condition by the blank (assay medium only) to highlight the magnitude of the T cell response in each donor. The Geometric mean along with the median and interquartile range over the 30 donor population was then calculated for each test condition.

9.1.3. Results and Conclusion

KLH (positive control) was a potent stimulator of CD4+ T cells and induced high IFNγ (100%) and IL-5 (93.3%) responses in the majority of the donors, whereas the bevacizumab (Avastin, negative control) showed a low response based on IFNγ and IL-5 readouts. Both compounds, P1AF6454 and P1AF6455, induced a low donor T cell response frequency for IFNγ (P1AF6454: 6.6% and P1AF6455: 10%) (**Figures 44A and 44B**) as well as for IL-5 (6.6% for both). Based on previous validation experiments the threshold for low responder frequencies has been set at higher than 10%; therefore both

compounds tested are considered to be associated with a low risk of sequence-related immunogenicity.

Comparatively, P1AD4524 induced a higher donor T cell response frequency for INFγ (33%) and a higher INFγ stimulation (stimulation index of 2.5) (Figures 45A and 45B) compared to the new FAP x OX40 antigen binding molecules P1AF6454 and P1AF6455.

9.2 A 4-week immunogenicity study to assess the potential of FAP-Ox40 candidates in eliciting antidrug antibodies (ADA) in transgenic mice that are immunologically tolerant to human IgG1 antibodies

The aim of the study is to investigate the immunogenic potential of the FAP-OX40 molecules by testing them in a C57BL/6-Tg (hIgG1,k,l) transgenic mouse model. In contrast to C57BL/6 wild type mice the C57BL/6-Tg (hIgG1,k,l) transgenic mice harbor transgenic non-rearranged human Ig-heavy and light chain gene loci. Upon B-cell differentiation these transgenic gene loci undergo functional Ig gene rearrangement with subsequent B-cell expression of soluble human IgG1. Therefore, the immune system of C57BL/6-Tg (hIgG1,k,l) transgenic mice is tolerant to a broad range of human IgG1 antibodies and can serve as an *in vivo* model to assess immunogenicity of IgG1 based drug compounds and potentially predict drug specific immunogenicity in humans. For each test item, 10 wild type mice and 10 transgenic mice were subcutaneously administered 10 µg per mouse per injection on days 1, 5, 8, 12, 15, 19, and 22. Blood samples were prepared for IgG measurements on days 1, 8, 15, 22 and 29 to monitor the progression of potential ADA responses. ELISA-based detection of mouse IgG antibodies specific to the compound injected was used as immunological read out.

9.2.1. Materials

The tested FAP x OX40 bispecific antigen binding molecules were OX40(CLC563) x FAP(1G1a_EPKSCD) 3+1 (P1AF6454), OX40(CLC563) x FAP(1G1a_EPKSCS) 3+1 (P1AF6455) and OX40(49B4) x FAP(4B9) 4+1 (P1AD4524).

The mouse strains used in the experiment were C57BL/6 wild type mice and - C57BL/6-Tg (hIgG1,k,l) transgenic mice.

9.2.2. Methods

ELISA testing: Nunc Maxisorp flat-bottom 96-well ELISA plates were coated with 100 µL per well test compound at 1µg/mL in $NaHCO_3$ 100 mM buffer overnight at +4°C. The next day the ELISA plates were washed three times with PBS + 0.05% Tween. For

blocking, 100 µL of PBS + 2% BSA were added to each well and the ELISA plates were incubated two hours at room temperature. The sera were diluted 1 to 50 in PBS + 1% FBS in the first row of round bottom dilution plate, following by a 1 to 3 serial dilution step, seven times in PBS + 1% FBS. The ELISA plates were washed three times with PBS + 0.05% Tween and 100 µL of the diluted sera were transferred from the dilution plate to the ELISA plate. After two hours of incubation at room temperature, the ELISA plates were washed three times with PBS + 0.05% Tween. For detection of mouse anti-drug antibodies (ADA) in the blood serum of the immunized mice, 100 µL per well of goat anti-mouse IgG Alkaline Phosphatase conjugated (Jackson Cat n° 115-055-071) diluted 1:2000 in PBS +1% BSA were added. After one hour of incubation at room temperature, the ELISA plates were washed three times with PBS + 0.05% Tween. 100 µL of substrate P-nitrophenyl phosphate ready-touse (Life Technologies Cat n°002212) were added per well and after ten minutes of incubation at room temperature, the optical density (OD) at 405 nm was read as an endpoint measurement with a Versamax ELISA reader.

Statistical analysis: The principal readout of this assay is considered binary in nature (distinct immune response vs. no clear response) since OD changes from ELISA cannot be compared across different antibody detection assays and thus be easily translated into a universal quantitative measure. It was decided to focus on response frequencies in the two cohorts of ten mice per test item. For reproducible identification of an immune response, we defined a six-sigma criterion above baseline to call a positive response. Animals that reached an OD value 6 Standard Deviation (SD) above background in at least one-time point were counted as responders. For the determination of mean background level and background standard deviation, we pooled the transgenic and wild type group readouts at day 1 (before treatment, n=20), after verifying there is no evidence for a clear initial difference in baseline level or spread for the two genotypes (t- and F-test, respectively, p-values>0.05). Responders identified by this method correspond very well with those identified by human intuition from the graphical plots, as confirmed in a few sample data sets. 95% confidence intervals for response rates were calculated based on a binomial distribution model, taking the observed response numbers and group size (n=10) into account. ELISA OD values were taken at a dilution of 1:50, going for maximum signal after ensuring a clean baseline and no (matrix effects) from the performed dilution curves. OD values were imported directly from the plate reader format using a custom R script for processing, analyzing and visualizing the in vivo immunogenicity data.

9.2.3. Results and Conclusion

The ELISA results of the two individual mouse groups treated with P1AF6455 are shown in **Figure 46**. These ELISA analyses revealed that 6/10 (60%) C57BL/6 wild type

mice elicited an Ig-immune response, as measured by the presence of ADAs in immunized mouse sera against this compound. This prominent immune response was anticipated, given that P1AF6455 is a fully human IgG1 antibody and is therefore expected to be regarded as foreign by the murine immune system. In contrast, 0/10 (0%) C57BL/6-Tg (hIgG1,k,l) transgenic mice mounted an antibody response against P1AF6455.

The ELISA results of the two individual mouse groups treated with P1AF6454 are shown in **Figure 47**. These ELISA analyses revealed that 0/10 (0%) C57BL/6 wild type mice elicited an Ig-immune response, as measured by the presence of ADAs in immunized mouse sera against this compound. Comparable to the situation in C57BL/6 wild type mice no ADA-responders (0/10 (0%)) against P1AF6454 were seen in C57BL/6-Tg (hIgG1,k,l) transgenic mice. This result indicates that P1AF6454 does not mounted an antibody response neither in C57BL/6 wild type mice nor in C57BL/6-Tg(hIgG1,k,l) transgenic mice.

The ELISA results of the two individual mouse groups treated with P1AD4524 are shown in **Figure 48**. These ELISA analyses revealed that 5/8 (62.5%) C57BL/6 wild type mice elicited an Ig-immune response, as measured by the presence of ADAs in immunized mouse sera against this compound. This prominent immune response was anticipated, given that P1AD4524 is a fully human IgG1 antibody and is therefore expected to be regarded as foreign by the murine immune system. 6/9 (66.7%) C57BL/6-Tg(hIgG1,k,l) transgenic mice mounted an antibody response against RO7194691, highlighting a high risk for immunogenicity in human.

The in vivo immunogenicity studies demonstrate a high risk of immunogenicity for the P1AD4524 and in contrast a low propensity for induction of humoral immune responses for the compounds P1AF6454 and P1AF6455. The difference seen may be attributed to altered sequence properties among the three tested compounds. These sequence alterations in P1AF6454 and P1AF6455 may either impact the MHC class II presentation of compound derived peptides or may lead to non-immunogenic peptide sequences which fail to induce immunogenic T-cell and B-cell responses.

Example 10

Efficacy study with FAP x OX40 bispecific antigen binding molecules in combination with CEACAM5 TCB in MKN45 Xenograft in humanized mice

The efficacy study described herein was aimed to select the most potent FAP x OX40 bispecific antigen binding molecule *in vivo* in terms of tumor regression in fully humanized NSG mice.

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 % CO_2. In vitro passage 7 was used for subcutaneous injection at a viability of 98%. 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 1.5 µg/ml Puromycin. Clone 39 was used at an in vitro passage number 5 and at a viability of > 95%.

50 microliters cell suspension ($1x10^6$ MKN45 cells + $1x10^6$ 3T3-huFAP) mixed with 50 microliters Matrigel were injected subcutaneously in the flank of anaesthetized mice with a 22G to 30G needle.

Female NSG mice, age 4-5 weeks at start of the experiment (Jackson Laboratory) 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 (P 2011/128). 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.

Female NSG mice were injected i.p. with 15 mg/kg of Busulfan followed one day later by an i.v. injection of $1x10^5$ human hematopoietic stem cells isolated from cord blood. At week 14-16 after stem cell injection mice were bled sublingual and blood was analyzed by flow cytometry for successful humanization. Efficiently engrafted mice were randomized according to their human T cell frequencies into the different treatment groups. At that time, mice were injected with the tumor/fibroblast cell mixture as described (**Figure 49**) and treated once weekly with the compounds or Histidine buffer (Vehicle) when tumor size reached approximately 200 mm^3 (day29). All mice were injected i.v. with 200 µl of the appropriate solution. To obtain the proper amount of compounds per 200 µl, the stock solutions (Table 34) were diluted with Histidine buffer when necessary.

Table 34: Compositions used in the *in vivo* experiment

Compound	Stock Solution concentration (mg/ml)	Formulation buffer
CEACAM5-TCB	19.2	20mM Histidine, 140mM NaCl pH6.0
FAP x OX40 (49B CPV; 4+1) (P1AE6456)	1.43	20mM Histidine, 140mM NaCl pH6.0

Compound	Stock Solution concentration (mg/ml)	Formulation buffer
FAP-OX40 (CLC563; 3+1) (P1AF6454)	4.4	20mM Histidine, 140mM NaCl pH6.0
FAP-OX40 (8H9; 2+1) (P1AE8870)	3.99	20mM Histidine, 140mM NaCl pH6.0
FAP-OX40 (49B4, 4+1) (P1AD4524)	24.32	20mM Histidine, 140mM NaCl pH6.0

Tumor growth was measured twice weekly using a caliper and tumor volume was calculated as followed:

Tv: (W2/2) x L (W: Width, L: Length)

At day 50 the study was terminated.

Figure 50A shows the tumor growth kinetics (Mean, +SEM) and **Figure 50B** shows the percentage of change of tumor volume at study termination as compared to baseline (start of treatment) per individual mouse and group. As described here, the FAP x OX40 construct, containing the CLC563 clone in a 3+1 format, reveals best anti-tumor activity in combination with CEACAM5-TCB. In that treatment group, five out of 8 mice, showed tumor regression (Waterfall plot).

***

Claims

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(ac) a third Fab fragment capable of specific binding to OX40,

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

(c) the Fc region composed of a first and a second subunit capable of stable association, wherein the second Fab fragment (ab) is fused at the C-terminus of the VH-CH1 chain to the N-terminus of the VH-CH1 chain of the first Fab fragment (aa), which is in turn fused at its C-terminus to the N-terminus of the first subunit, and the third Fab fragment (ac) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit.

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

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

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

(ac) a third Fab fragment capable of specific binding to OX40,

(ad) a fourth Fab fragment capable of specific binding to OX40,

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

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

18. Isolated nucleic acid encoding the bispecific antigen binding molecule of any one of claims 1 to 17.

19. An expression vector comprising the isolated nucleic acid of claim 18.

20. A host cell comprising isolated nucleic acid of claim 18 or the expression vector of claim 19.

21. A method of producing a bispecific antigen binding molecule, comprising culturing the host cell of claim 20 under conditions suitable for the expression of the bispecific antigen binding molecule, and isolating the bispecific antigen binding molecule.

22. A pharmaceutical composition comprising the bispecific antigen binding molecule of any one of claims 1 to 17 and a pharmaceutically acceptable carrier.

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

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

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

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

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

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

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

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

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

***

Fig.1

FIG.1A

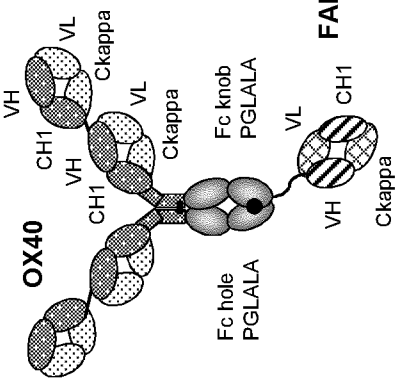


FIG.1B

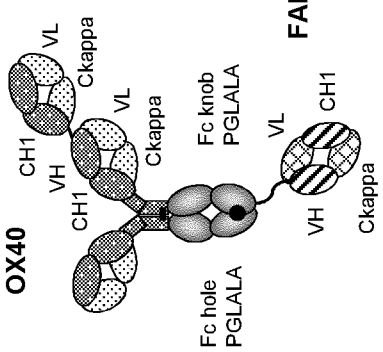


FIG.1C

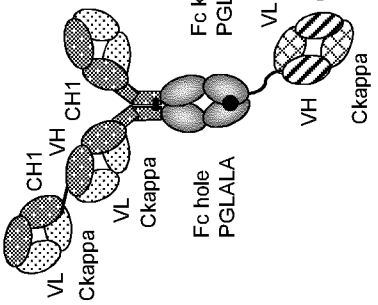


FIG.1D

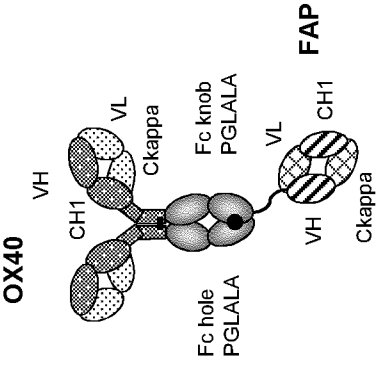


FIG.1E

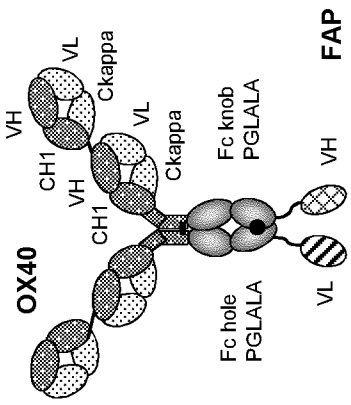


Fig.2

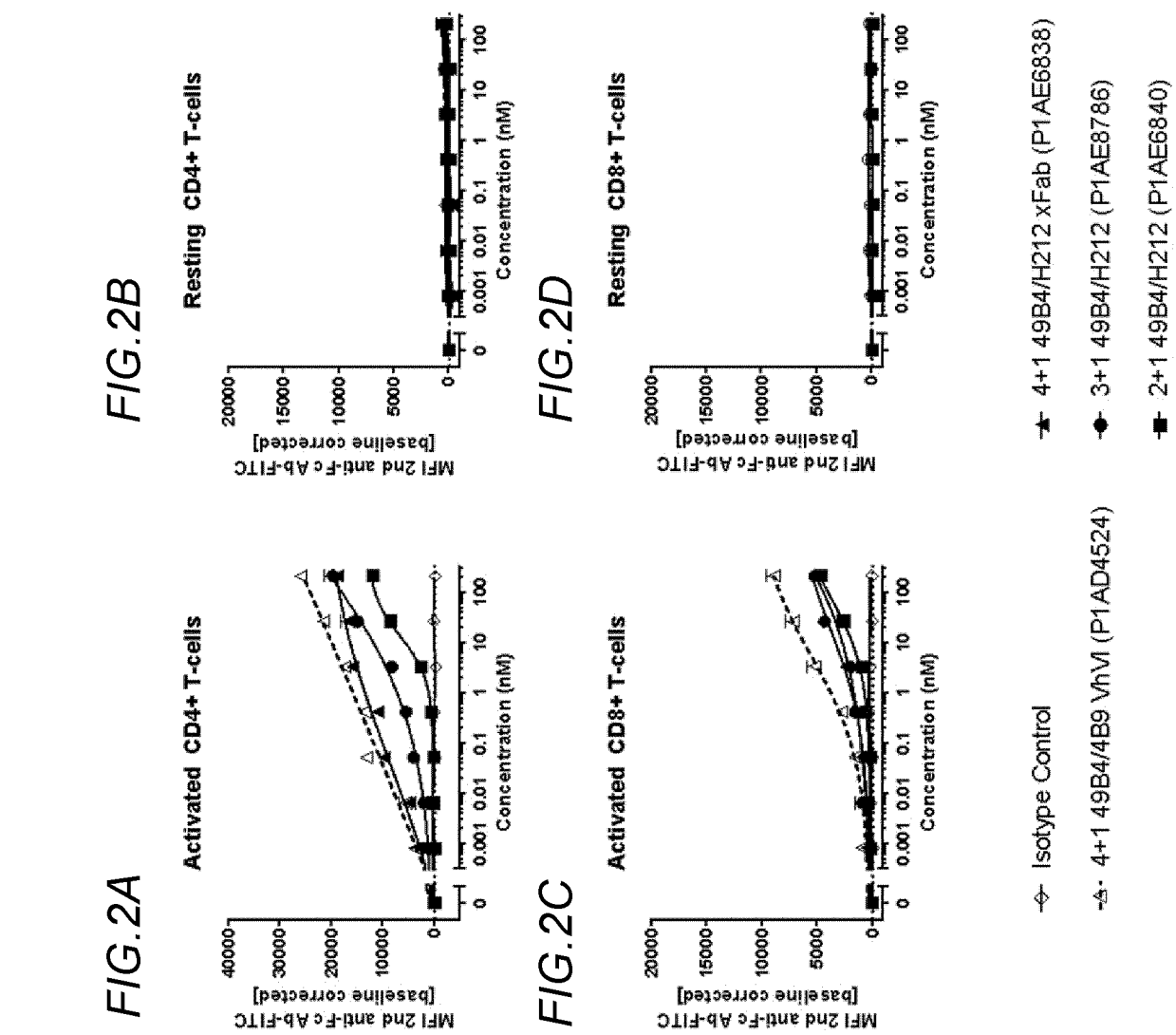

FIG.2A
Activated CD4+ T-cells
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
Concentration (nM)
FIG.2B
Resting CD4+ T-cells
FIG.2C
Activated CD8+ T-cells
FIG.2D
Resting CD8+ T-cells
Isotype Control
4+1 49B4/4B9 VhVl (P1AD4524)
4+1 49B4/H212 xFab (P1AE6838)
3+1 49B4/H212 (P1AE8786)
2+1 49B4/H212 (P1AE6840)

Fig.2

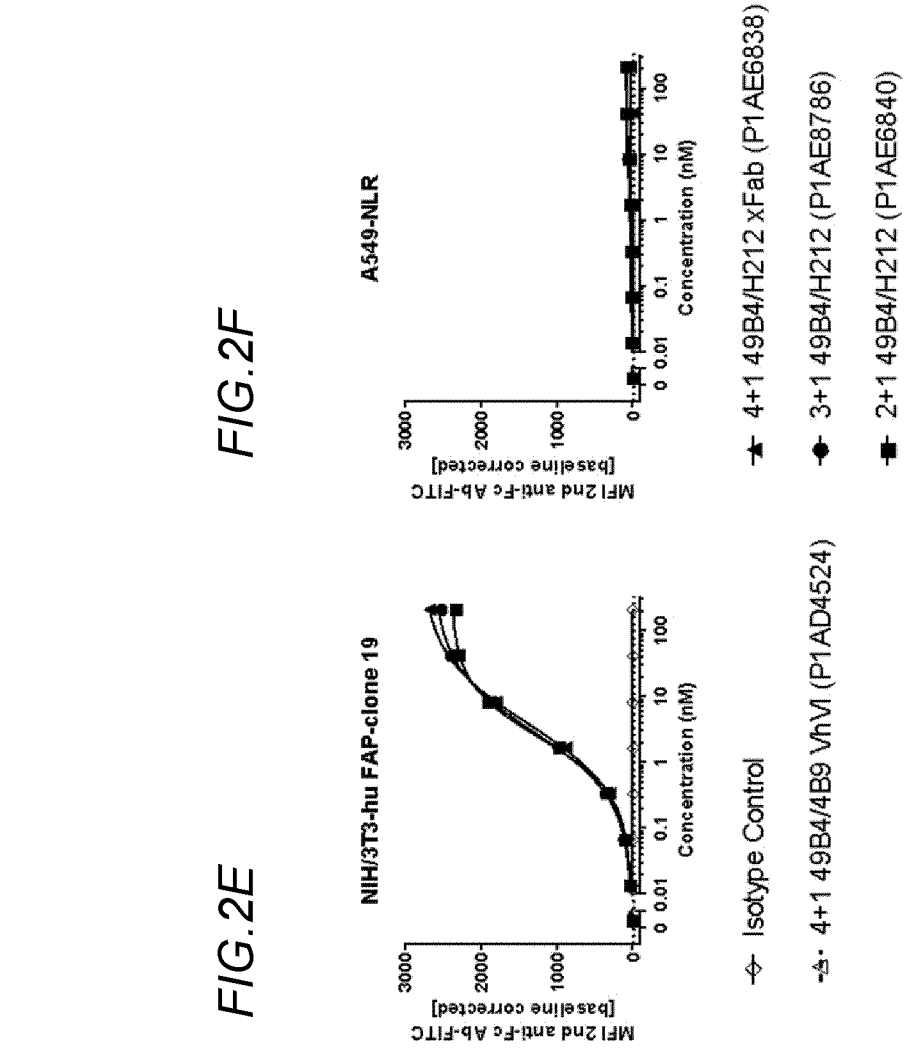

FIG.2E
NIH/3T3-hu FAP-clone 19
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
Concentration (nM)
FIG.2F
A549-NLR
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
Concentration (nM)
Isotype Control
4+1 49B4/4B9 VhVl (P1AD4524)
4+1 49B4/H212 xFab (P1AE6838)
3+1 49B4/H212 (P1AE8786)
2+1 49B4/H212 (P1AE6840)

Fig.3

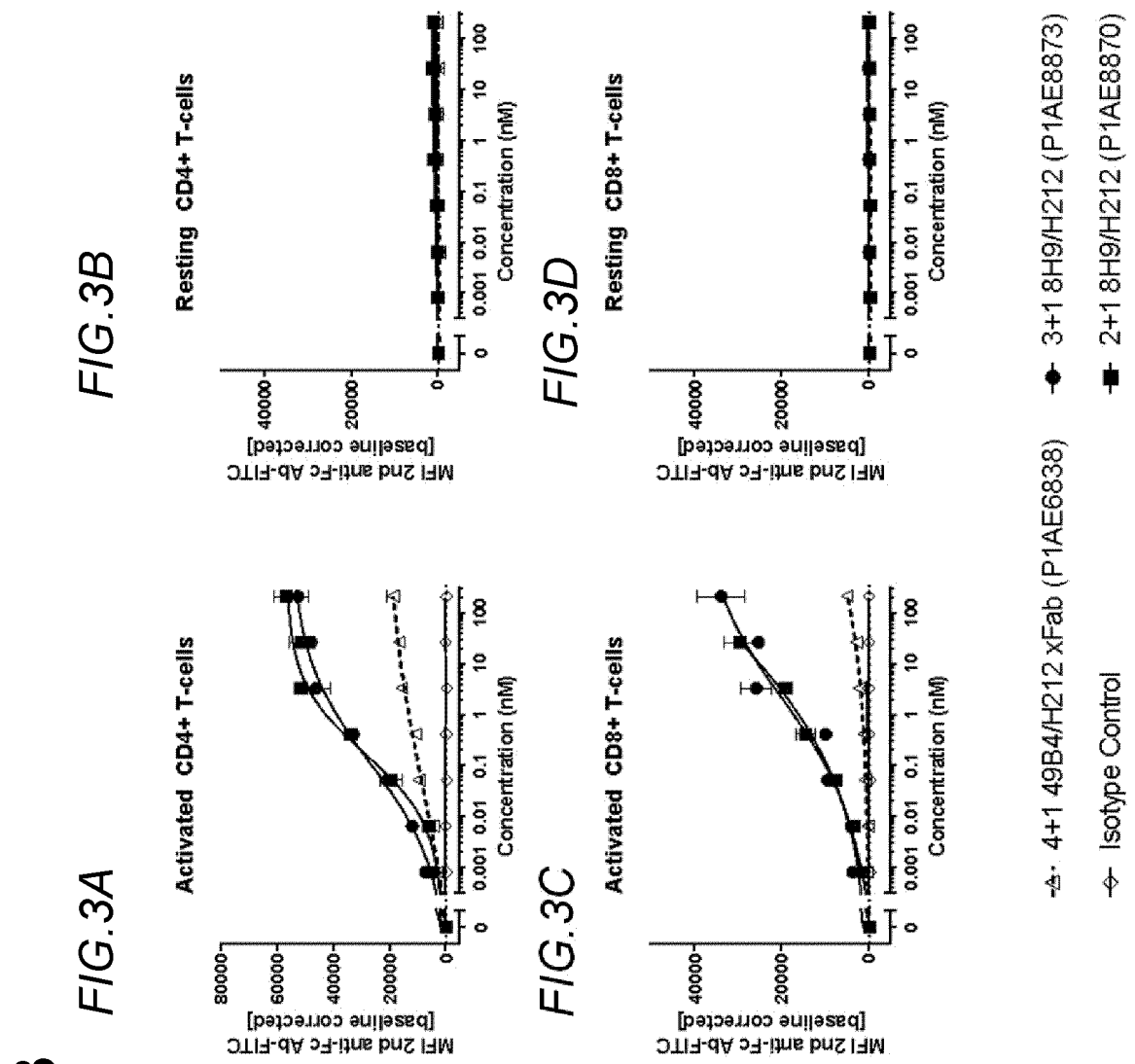

FIG.3A
Activated CD4+ T-cells
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
Concentration (nM)
FIG.3B
Resting CD4+ T-cells
FIG.3C
Activated CD8+ T-cells
FIG.3D
Resting CD8+ T-cells
4+1 49B4/H212 xFab (P1AE6838)
3+1 8H9/H212 (P1AE8873)
Isotype Control
2+1 8H9/H212 (P1AE8870)

Fig.3

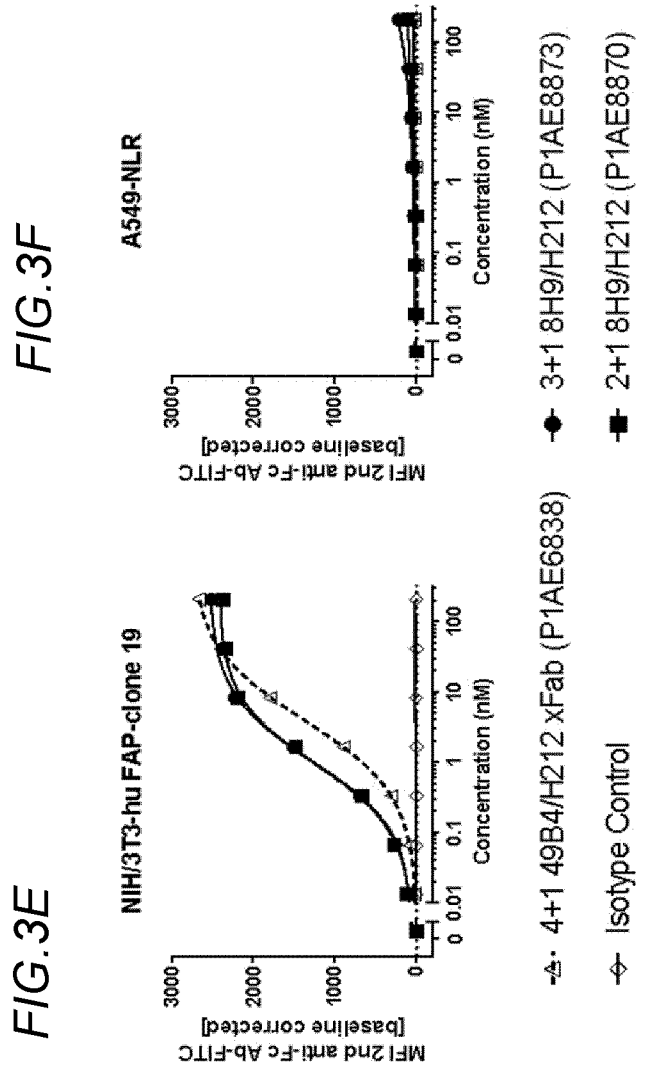

FIG.3E
NIH/3T3-hu FAP-clone 19
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
3000
2000
1000
0
0 0.01 0.1 1 10 100
Concentration (nM)
FIG.3F
A549-NLR
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
3000
2000
1000
0
0 0.01 0.1 1 10 100
Concentration (nM)
4+1 49B4/H212 xFab (P1AE6838)
3+1 8H9/H212 (P1AE8873)
Isotype Control
2+1 8H9/H212 (P1AE8870)

Fig. 4

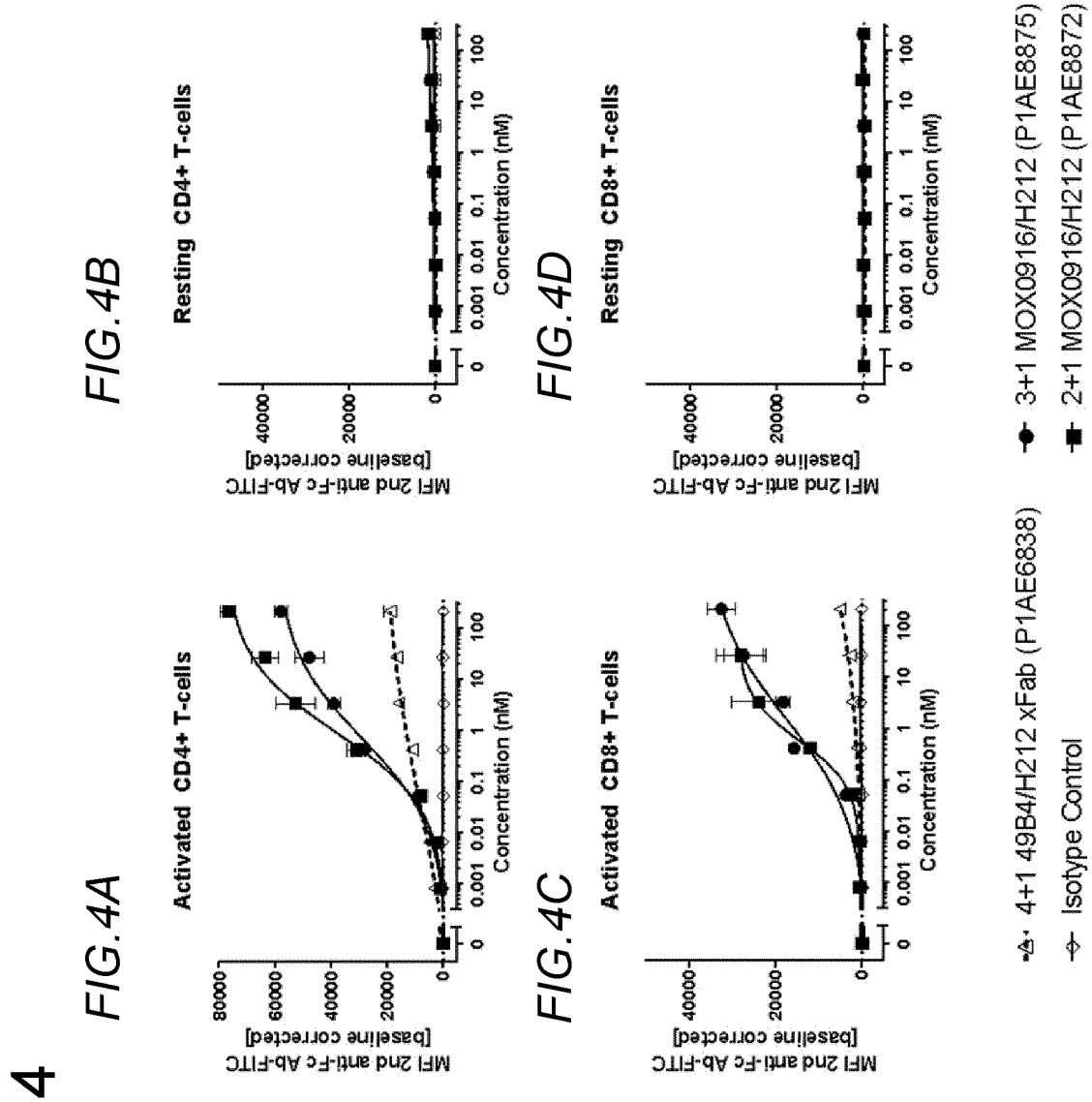

FIG.4A
Activated CD4+ T-cells
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
80000
60000
40000
20000
0
0 0.001 0.01 0.1 1 10 100
Concentration (nM)
FIG.4B
Resting CD4+ T-cells
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
40000
20000
0
0 0.001 0.01 0.1 1 10 100
Concentration (nM)
FIG.4C
Activated CD8+ T-cells
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
40000
20000
0
0 0.001 0.01 0.1 1 10 100
Concentration (nM)
FIG.4D
Resting CD8+ T-cells
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
40000
20000
0
0 0.001 0.01 0.1 1 10 100
Concentration (nM)
4+1 49B4/H212 xFab (P1AE6838)
3+1 MOX0916/H212 (P1AE8875)
Isotype Control
2+1 MOX0916/H212 (P1AE8872)

Fig. 4

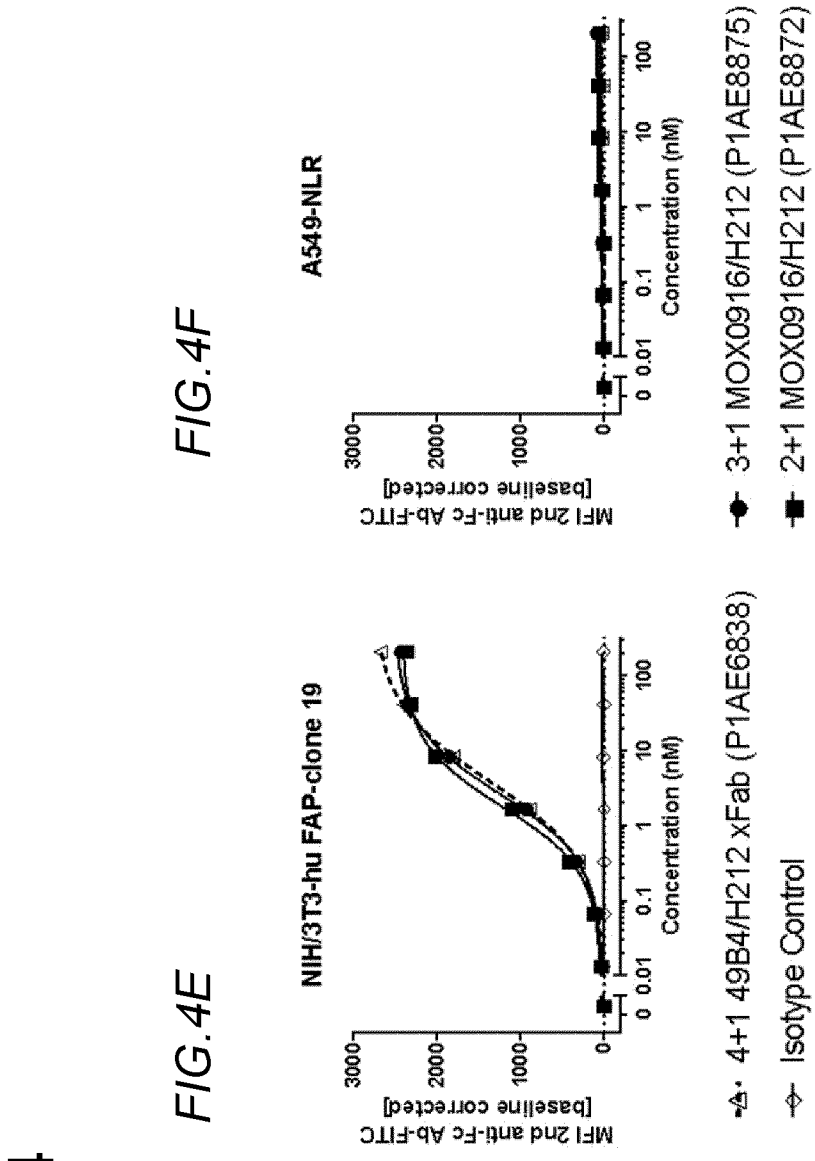

FIG.4E
NIH/3T3-hu FAP-clone 19
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
3000
2000
1000
0
0 0.01 0.1 1 10 100
Concentration (nM)
FIG.4F
A549-NLR
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
3000
2000
1000
0
0 0.01 0.1 1 10 100
Concentration (nM)
4+1 49B4/H212 xFab (P1AE6838)
Isotype Control
3+1 MOX0916/H212 (P1AE8875)
2+1 MOX0916/H212 (P1AE8872)

Fig.5

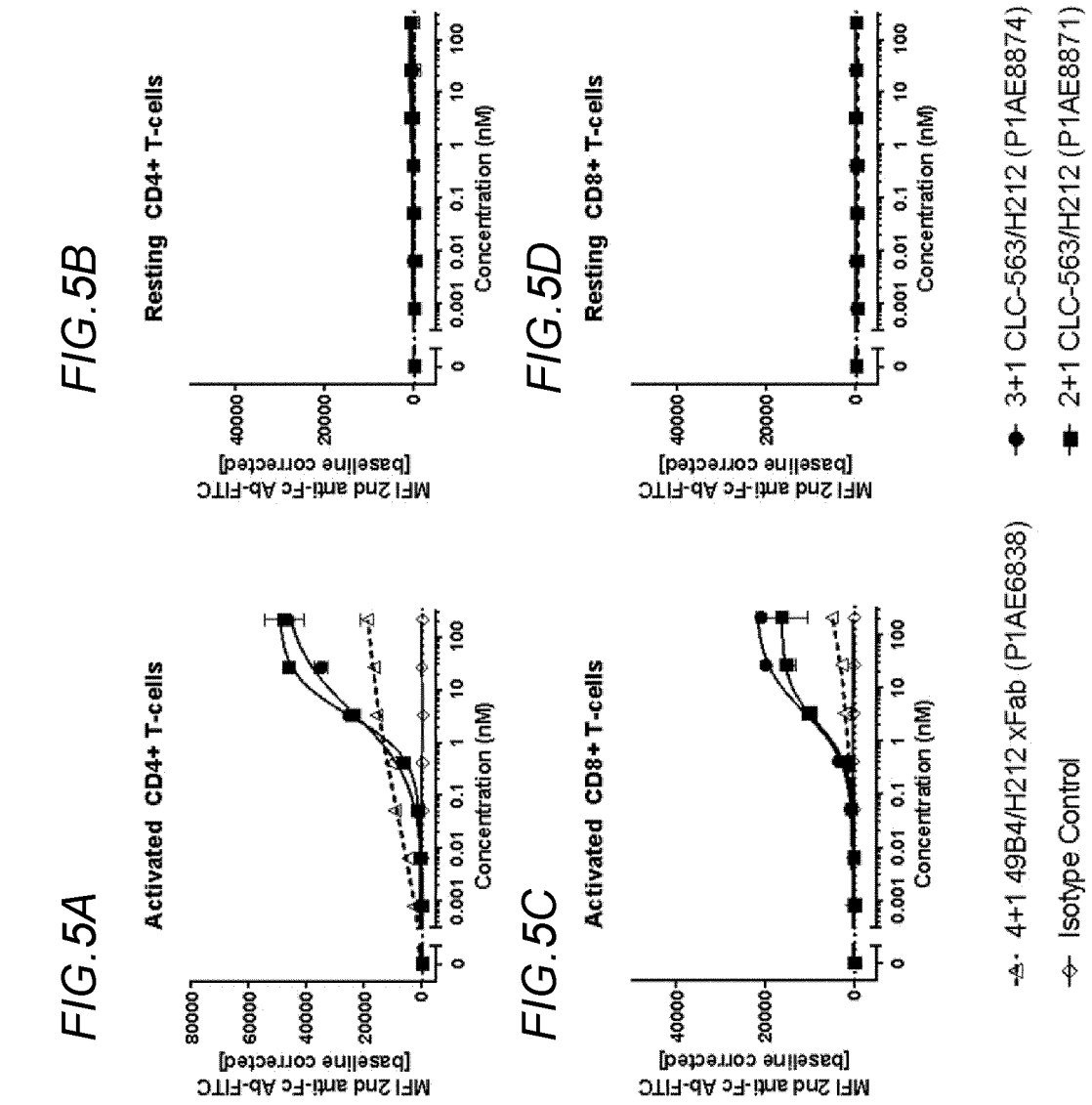

FIG.5A
Activated CD4+ T-cells
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
Concentration (nM)
FIG.5B
Resting CD4+ T-cells
FIG.5C
Activated CD8+ T-cells
FIG.5D
Resting CD8+ T-cells
4+1 49B4/H212 xFab (P1AE6838)
3+1 CLC-563/H212 (P1AE8874)
Isotype Control
2+1 CLC-563/H212 (P1AE8871)

Fig.5

FIG.5E

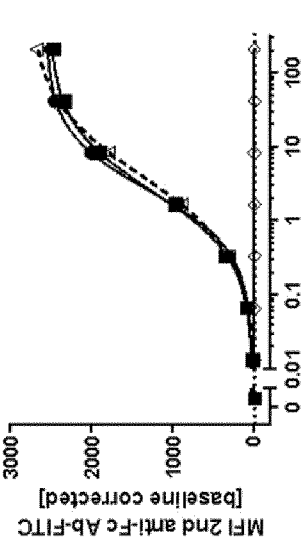


FIG.5F

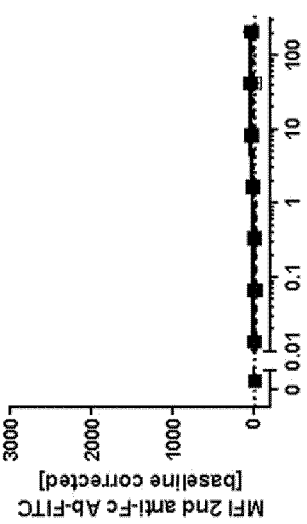


Fig.6

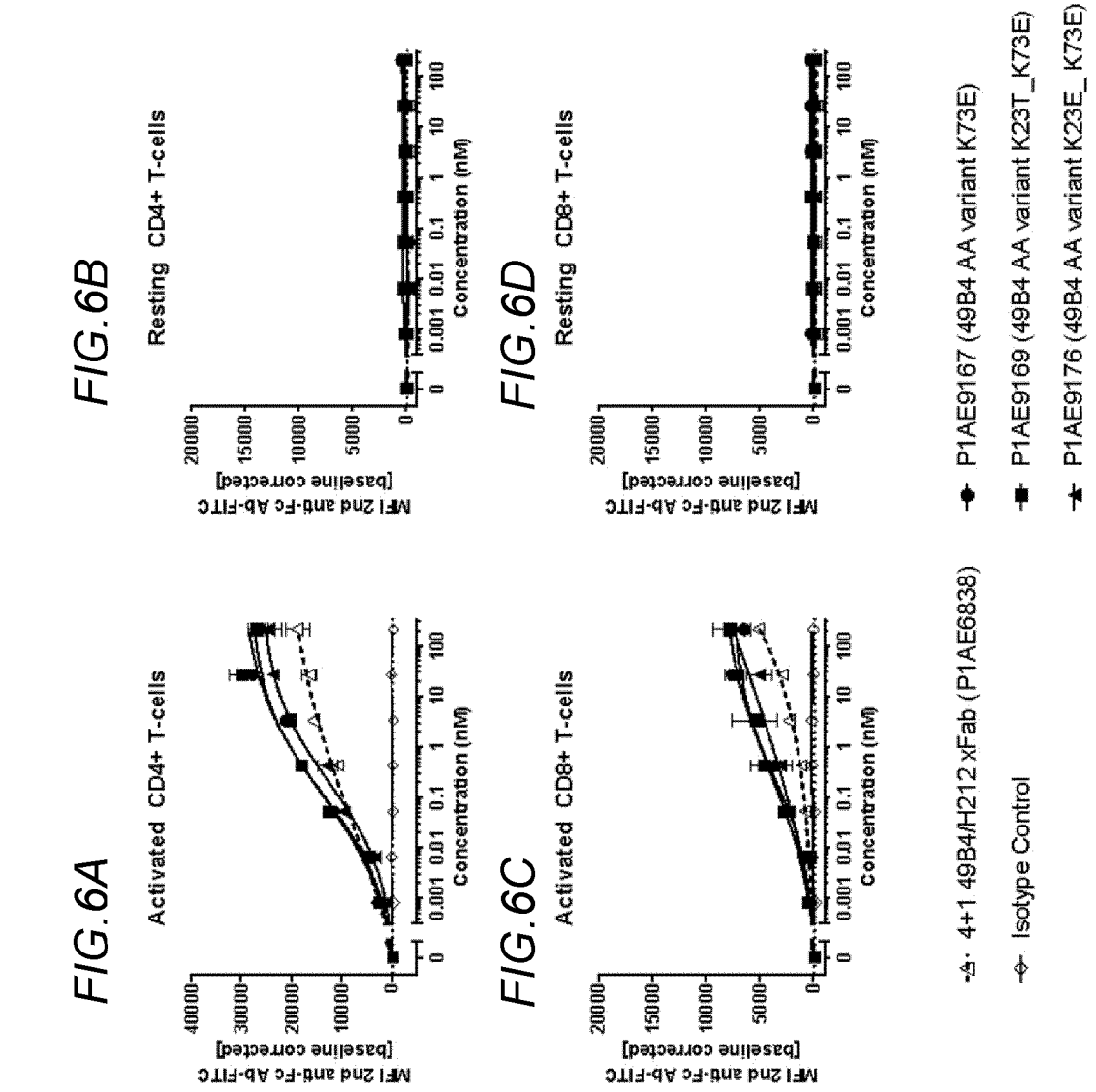

FIG.6A
Activated CD4+ T-cells
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
Concentration (nM)
FIG.6B
Resting CD4+ T-cells
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
Concentration (nM)
FIG.6C
Activated CD8+ T-cells
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
Concentration (nM)
FIG.6D
Resting CD8+ T-cells
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
Concentration (nM)
4+1 49B4/H212 xFab (P1AE6838)
Isotype Control
P1AE9167 (49B4 AA variant K73E)
P1AE9169 (49B4 AA variant K23T_K73E)
P1AE9176 (49B4 AA variant K23E_ K73E)

Fig.6

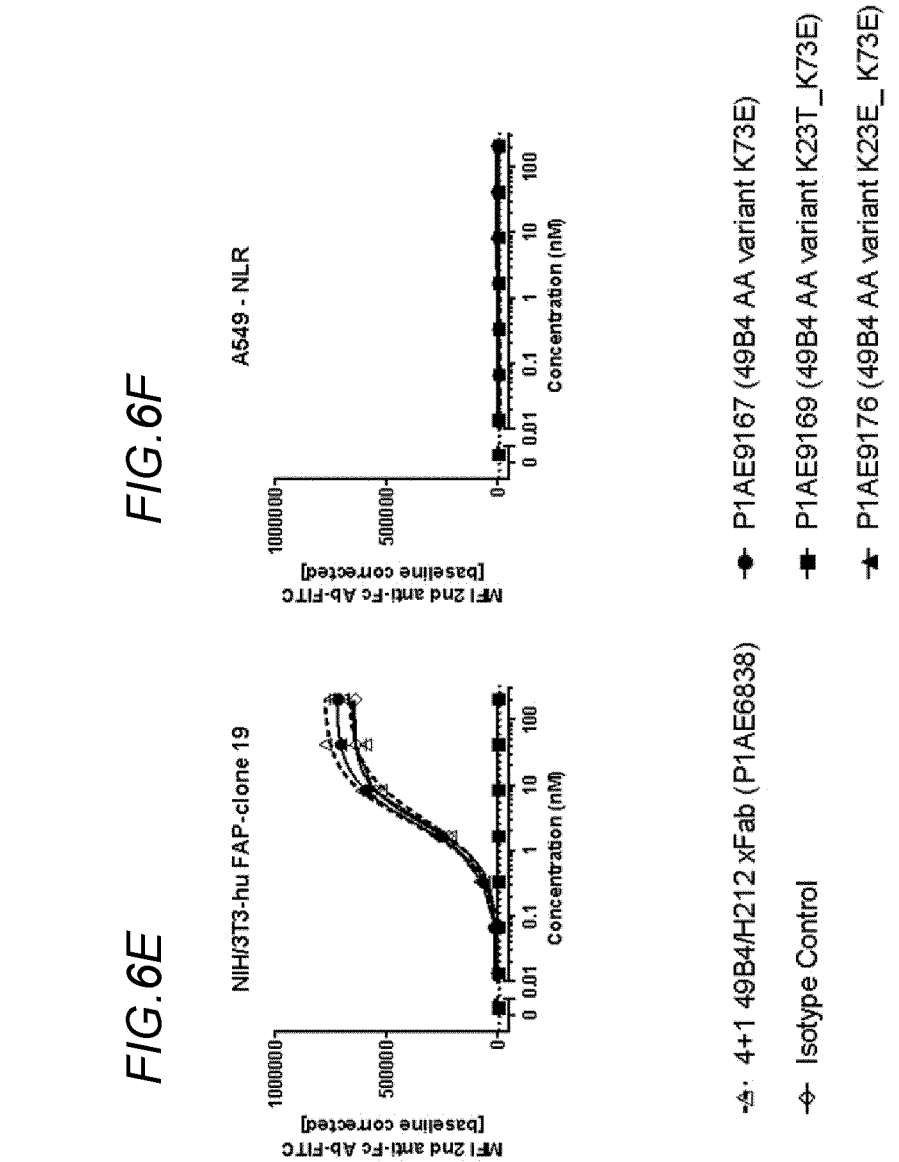

FIG.6E
NIH/3T3-hu FAP-clone 19
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
1000000
500000
0
0 0.01 0.1 1 10 100
Concentration (nM)
FIG.6F
A549 - NLR
MFI 2nd anti-Fc Ab-FITC [baseline corrected]
1000000
500000
0
0 0.01 0.1 1 10 100
Concentration (nM)
4+1 49B4/H212 xFab (P1AE6838)
Isotype Control
P1AE9167 (49B4 AA variant K73E)
P1AE9169 (49B4 AA variant K23T_K73E)
P1AE9176 (49B4 AA variant K23E_ K73E)

Fig.7

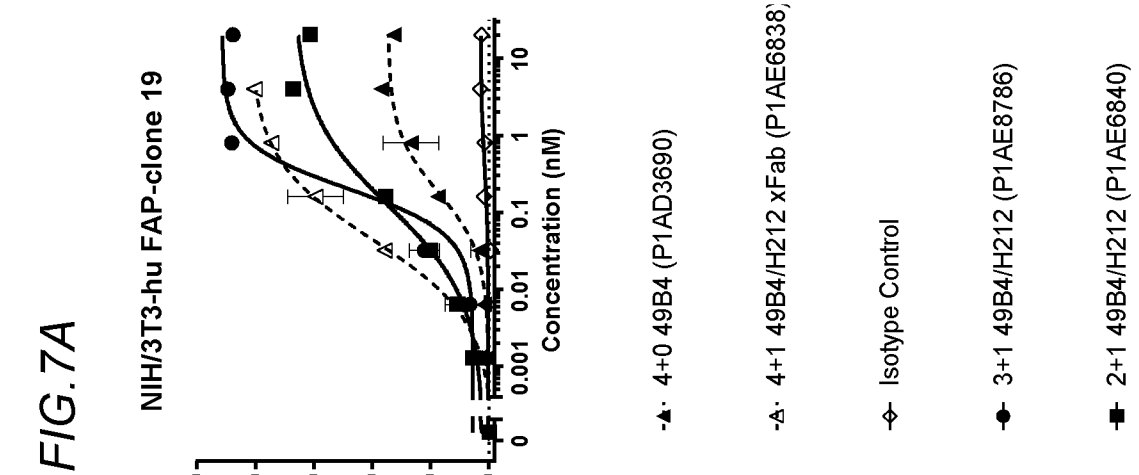

FIG.7A
NIH/3T3-hu FAP-clone 19
URL (0.5 sec/sample) baseline corrected
100000
80000
60000
40000
20000
0
0
0.001
0.01
0.1
1
10
Concentration (nM)
FIG.7B
2nd Antibody anti-human IgG Fc-γ specific
FIG.7C
w/o
4+0 49B4 (P1AD3690)
4+1 49B4/H212 xFab (P1AE6838)
Isotype Control
3+1 49B4/H212 (P1AE8786)
2+1 49B4/H212 (P1AE6840)

Fig.8

FIG.8A

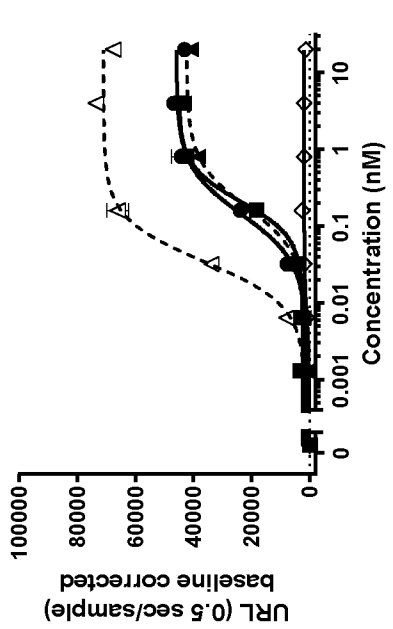


FIG.8B

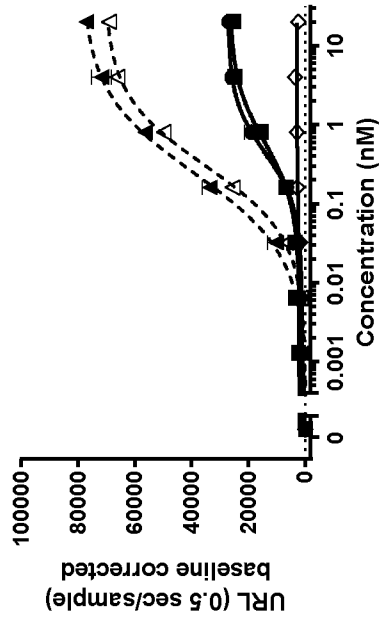


FIG.8C w/o

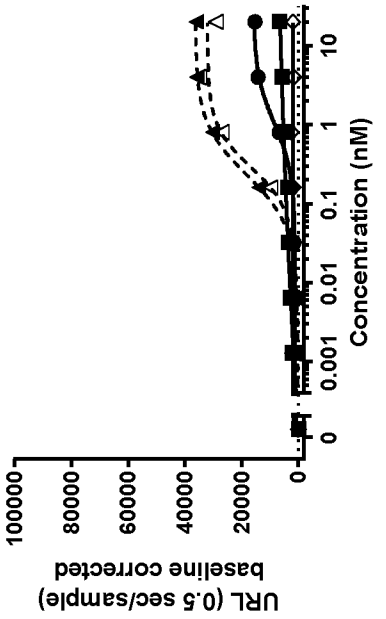


Fig.9

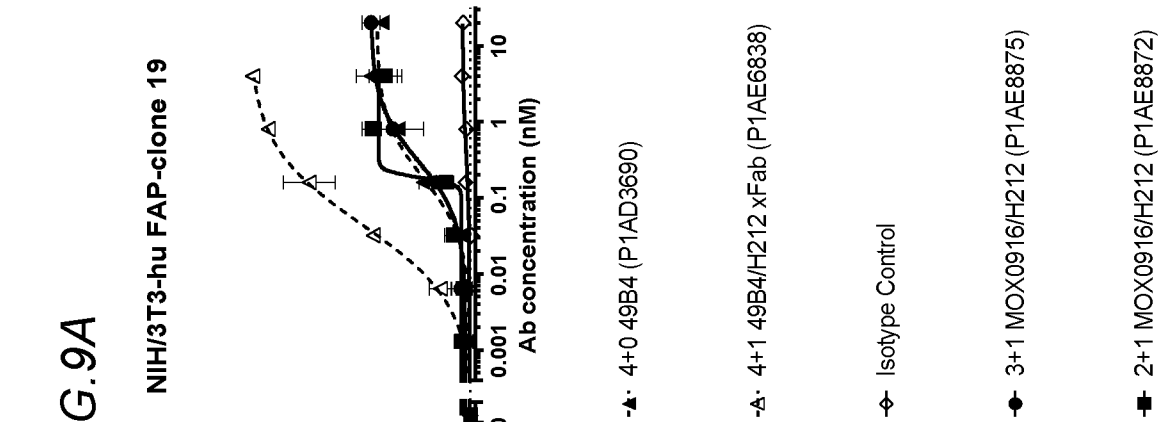

FIG.9A
NIH/3T3-hu FAP-clone 19
URL (0.5 sec/sample) baseline corrected
100000
80000
60000
40000
20000
0
0
0.001
0.01
0.1
1
10
Ab concentration (nM)
4+0 49B4 (P1AD3690)
4+1 49B4/H212 xFab (P1AE6838)
Isotype Control
3+1 MOX0916/H212 (P1AE8875)
2+1 MOX0916/H212 (P1AE8872)
FIG.9B
2nd Antibody
anti-human IgG Fc-γ specific
FIG.9C
w/o

Fig.10

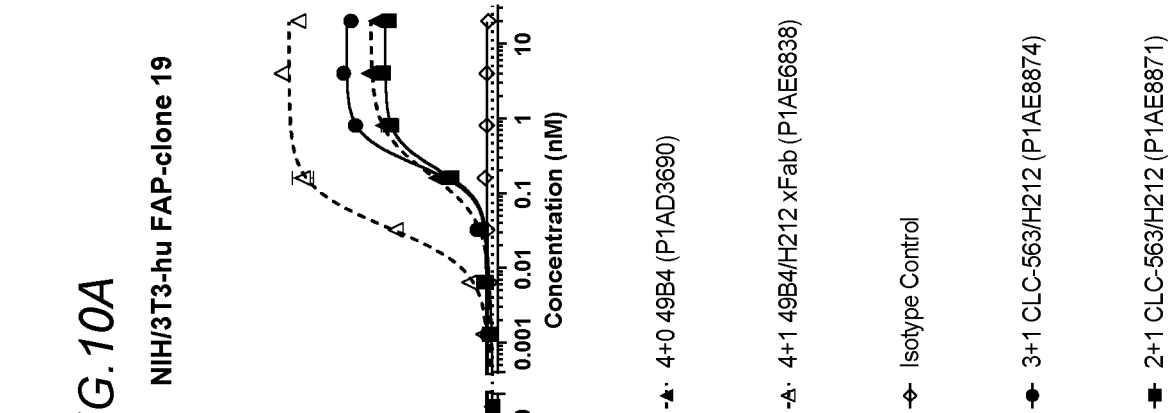

FIG.10A
NIH/3T3-hu FAP-clone 19
URL (0.5 sec/sample) baseline corrected
100000
80000
60000
40000
20000
0
0
0.001
0.01
0.1
1
10
Concentration (nM)
FIG.10B
2nd Antibody anti-human IgG Fc-γ specific
FIG.10C
w/o
4+0 49B4 (P1AD3690)
4+1 49B4/H212 xFab (P1AE6838)
Isotype Control
3+1 CLC-563/H212 (P1AE8874)
2+1 CLC-563/H212 (P1AE8871)

Fig.11

FIG.11A

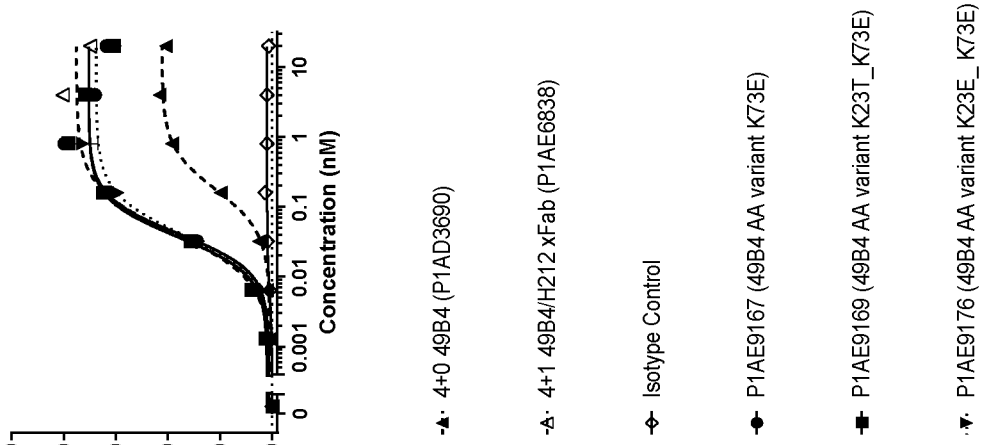


FIG.11B

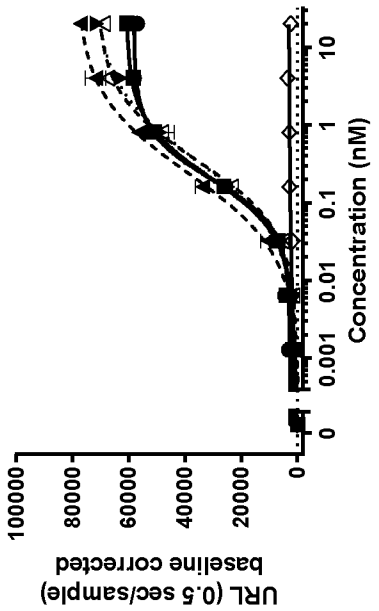


FIG.11C w/o

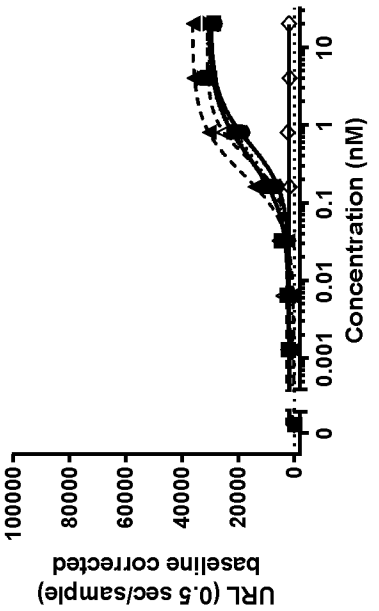


Fig.12

FIG.12A

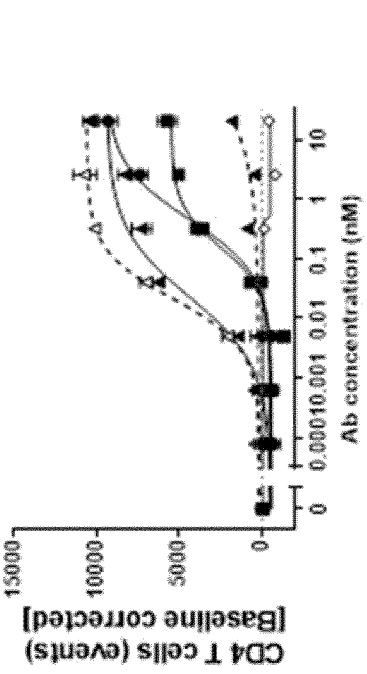


FIG.12B

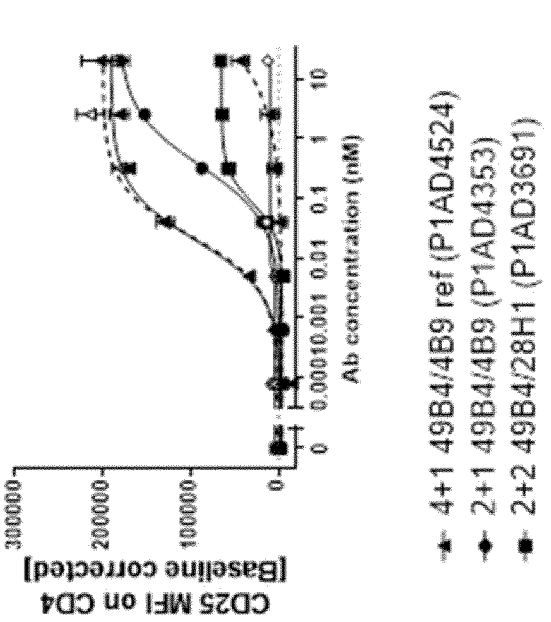


Fig.13

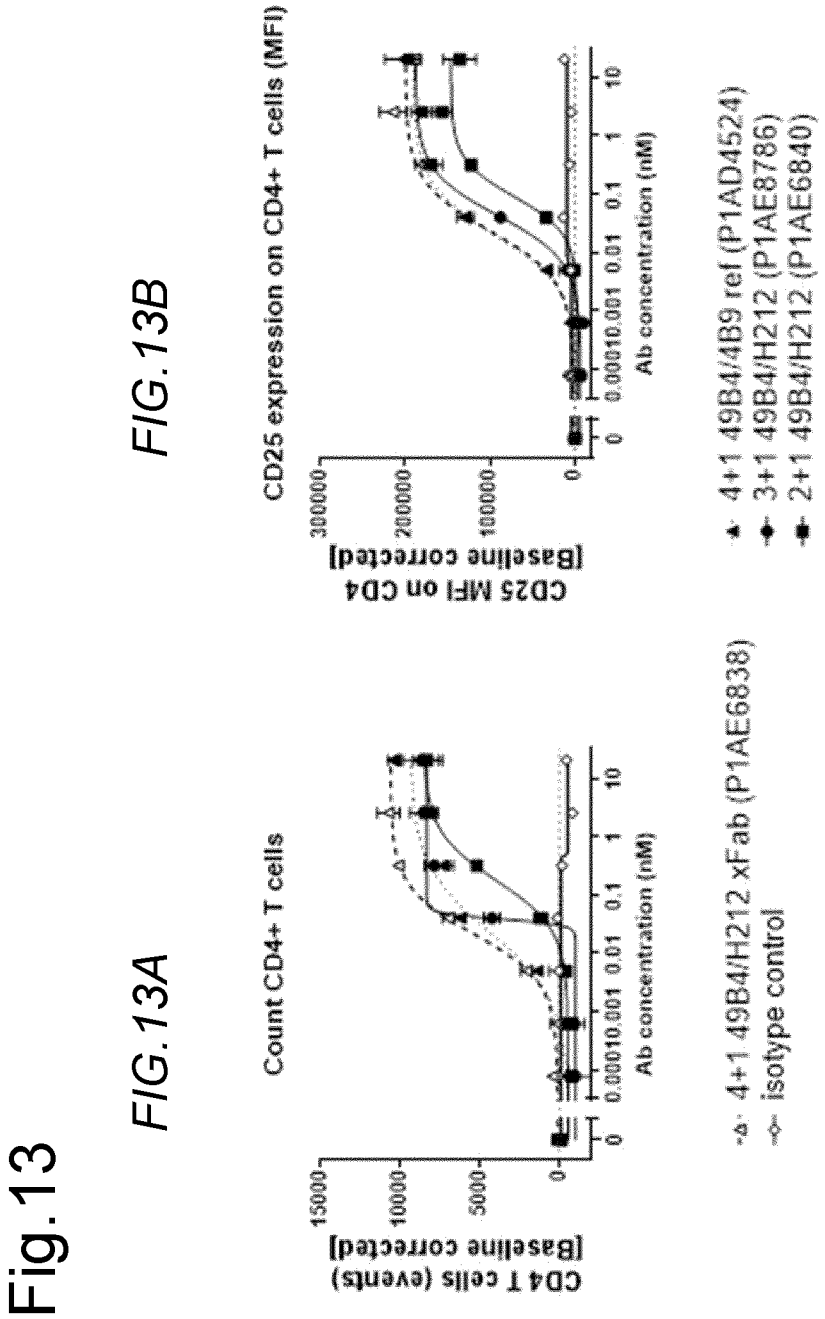

FIG.13A
Count CD4+ T cells
CD4 T cells (events) [Baseline corrected]
15000
10000
5000
0
0
0.00010.001 0.01 0.1 1 10
Ab concentration (nM)
4+1 49B4/H212 xFab (P1AE6838)
isotype control
FIG.13B
CD25 expression on CD4+ T cells (MFI)
CD25 MFI on CD4 [Baseline corrected]
300000
200000
100000
0
0
0.00010.001 0.01 0.1 1 10
Ab concentration (nM)
4+1 49B4/4B9 ref (P1AD4524)
3+1 49B4/H212 (P1AE8786)
2+1 49B4/H212 (P1AE6840)

Fig.14

FIG.14A

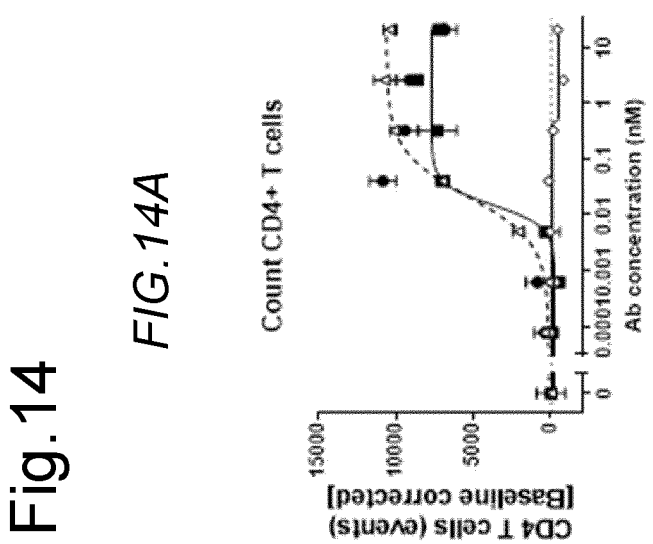


FIG.14B

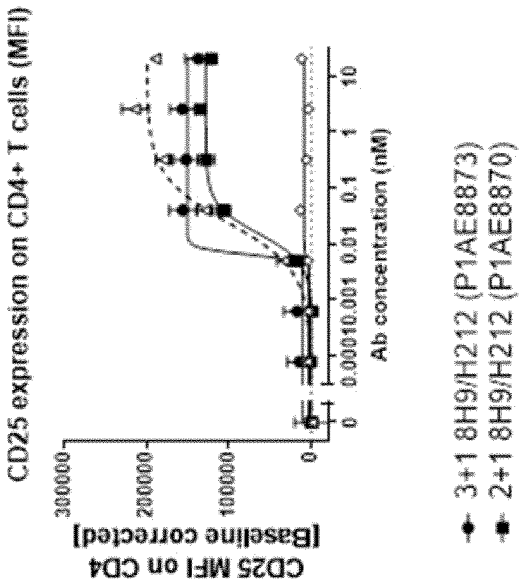


Fig.15

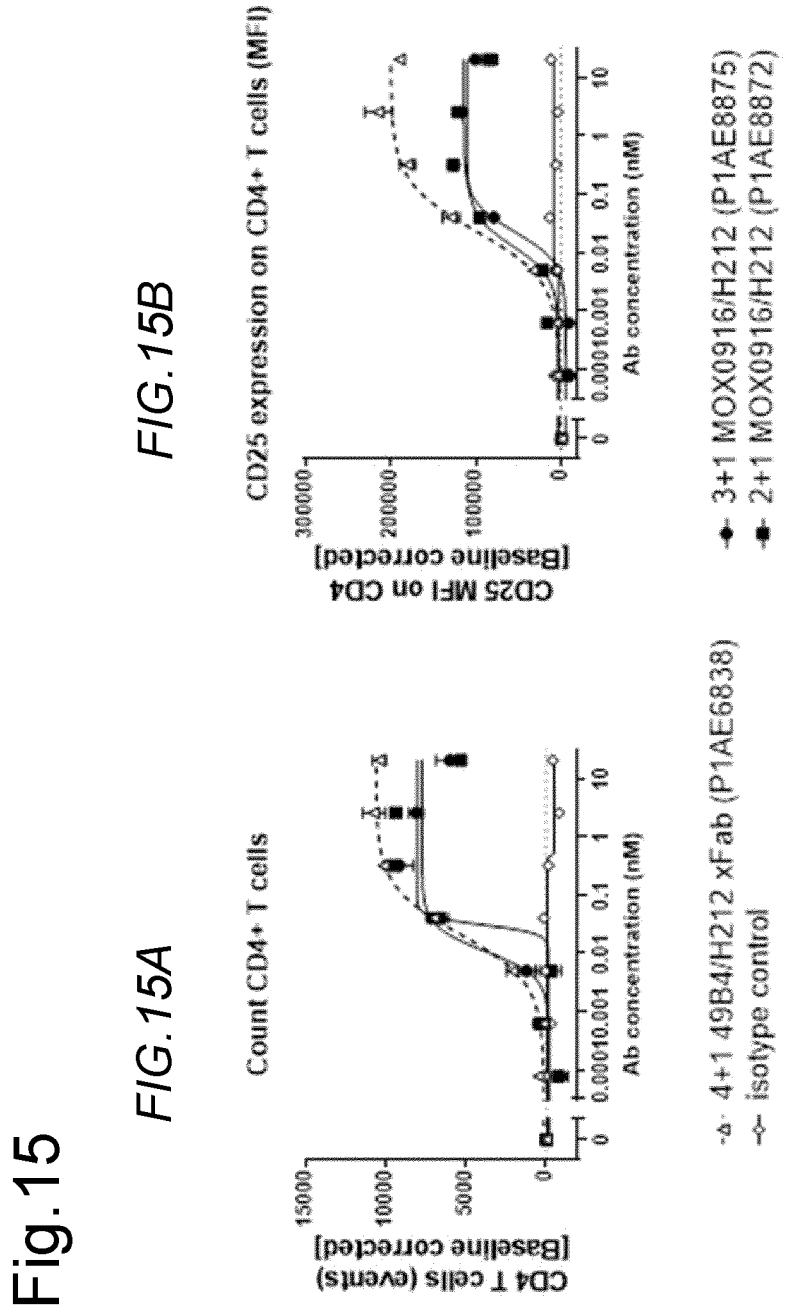

FIG.15A
Count CD4+ T cells
CD4 T cells (events) [Baseline corrected]
15000
10000
5000
0
0
0.00010.001
0.01
0.1
1
10
Ab concentration (nM)
FIG.15B
CD25 expression on CD4+ T cells (MFI)
CD25 MFI on CD4 [Baseline corrected]
300000
200000
100000
0
4+1 49B4/H212 xFab (P1AE6838)
isotype control
3+1 MOX0916/H212 (P1AE8875)
2+1 MOX0916/H212 (P1AE8872)

Fig.16

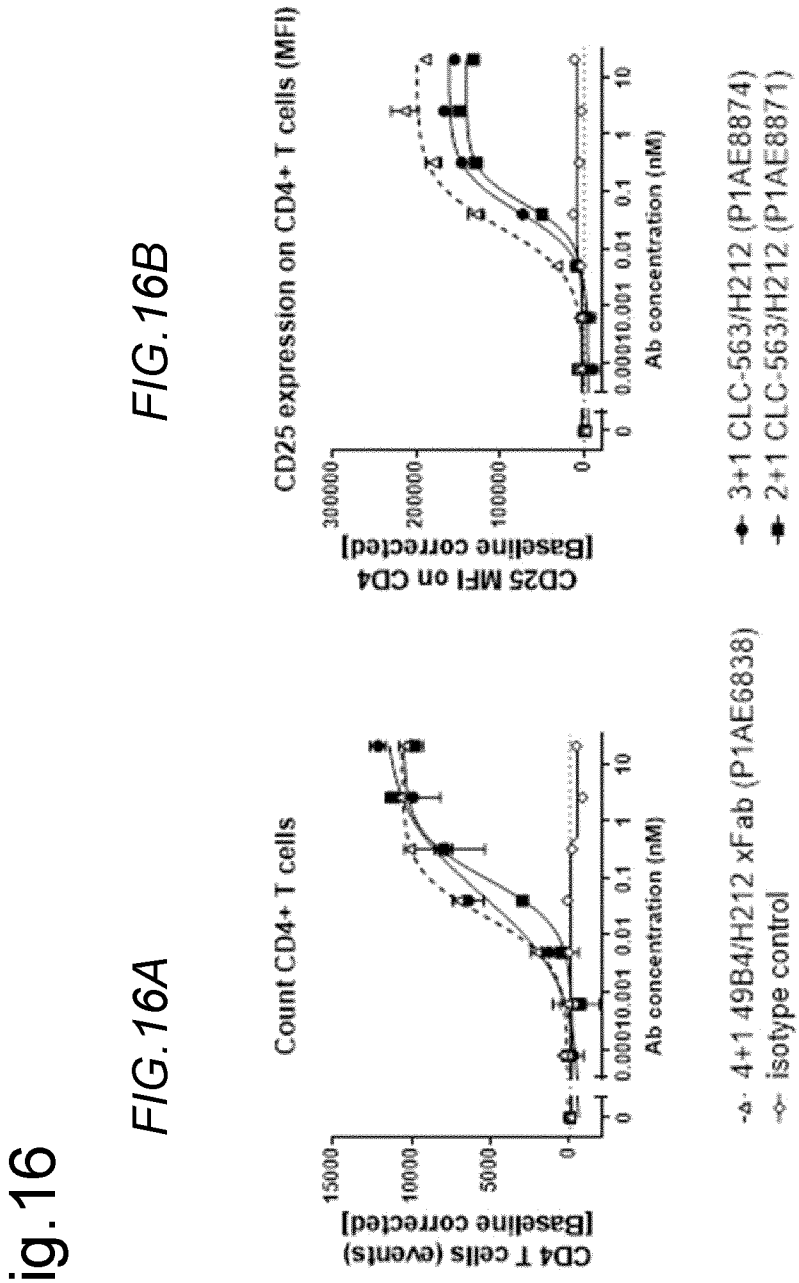

FIG.16A
Count CD4+ T cells
CD4 T cells (events) [Baseline corrected]
15000
10000
5000
0
0 0.00010.001 0.01 0.1 1 10
Ab concentration (nM)
FIG.16B
CD25 expression on CD4+ T cells (MFI)
CD25 MFI on CD4 [Baseline corrected]
300000
200000
100000
0
0 0.00010.001 0.01 0.1 1 10
Ab concentration (nM)
4+1 49B4/H212 xFab (P1AE6838)
isotype control
3+1 CLC-563/H212 (P1AE8874)
2+1 CLC-563/H212 (P1AE8871)

Fig.17

FIG.17A

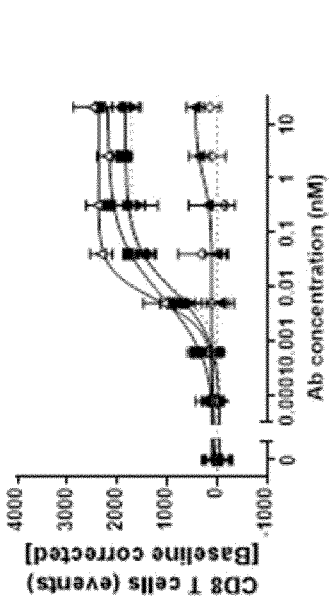


FIG.17B

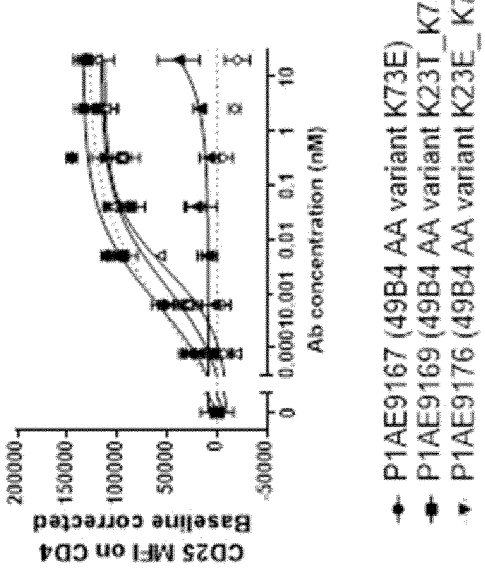


Fig.18

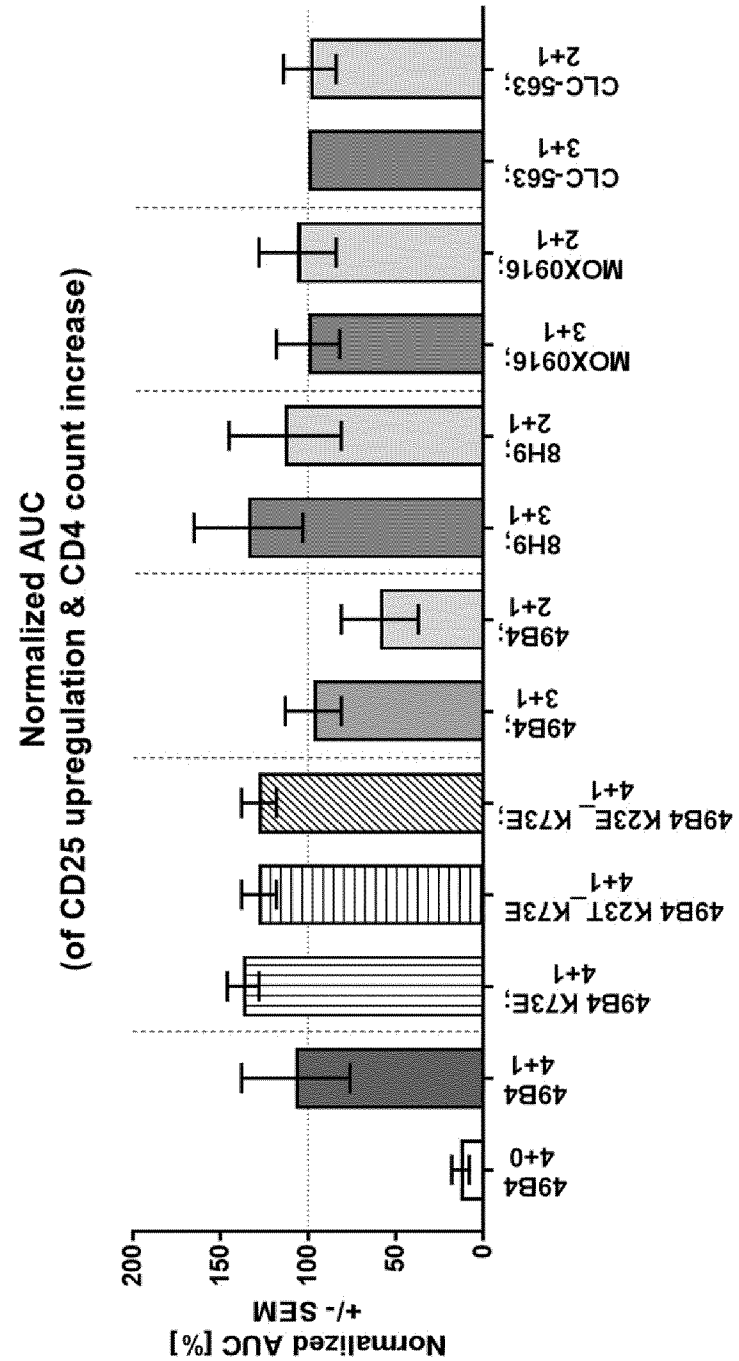

Normalized AUC
(of CD25 upregulation & CD4 count increase)
Normalized AUC [%]
+/- SEM
200
150
100
50
0
49B4 4+0
49B4 4+1
49B4 K73E; 4+1
49B4 K23T_K73E 4+1
49B4 K23E_K73E; 4+1
49B4; 3+1
49B4; 2+1
8H9; 3+1
8H9; 2+1
MOX0916; 3+1
MOX0916; 2+1
CLC-563; 3+1
CLC-563; 2+1

Fig.19

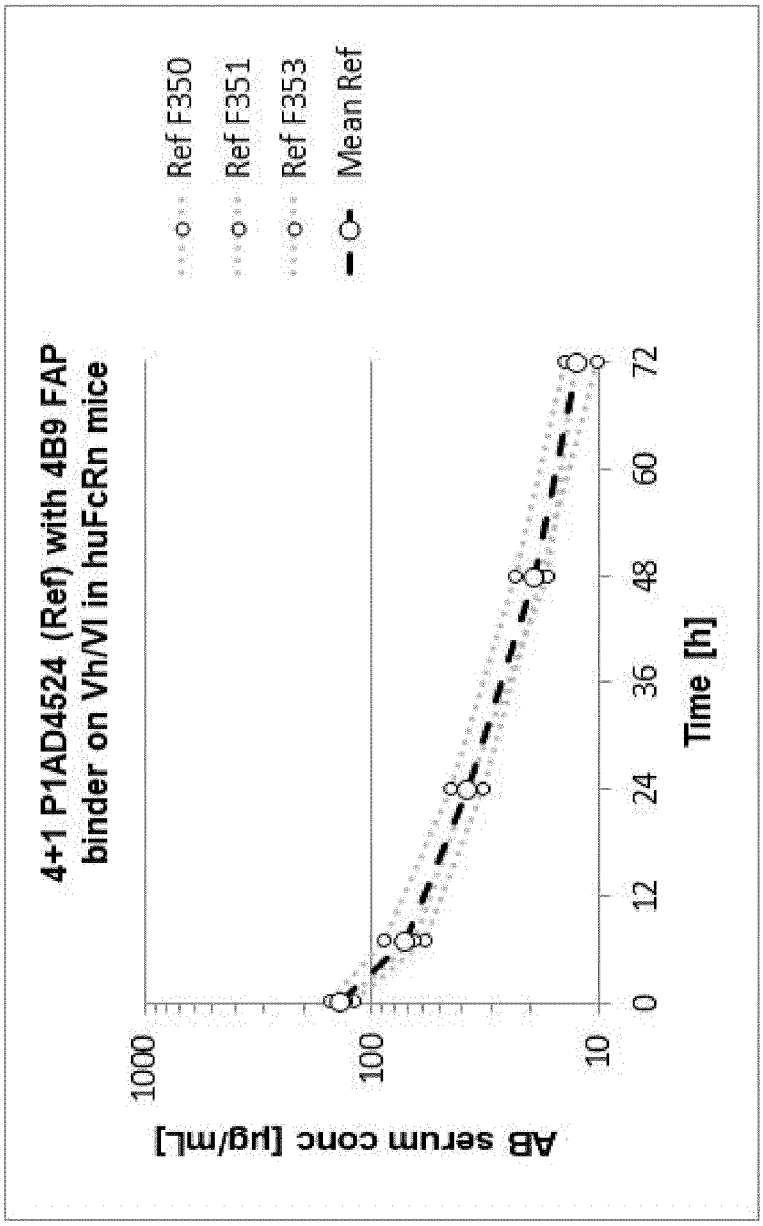

4+1 P1AD4524 (Ref) with 4B9 FAP
binder on Vh/Vl in huFcRn mice
Ref F350
Ref F351
Ref F353
Mean Ref
AB serum conc [µg/mL]
1000
100
10
0
12
24
36
48
60
72
Time [h]

Fig. 20

FIG.20A

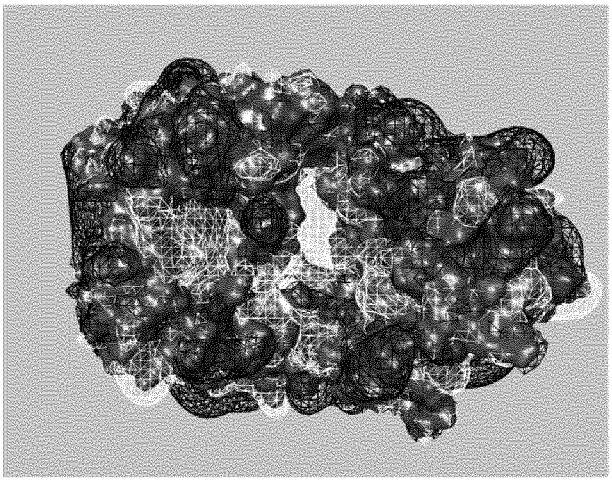


FIG.20B

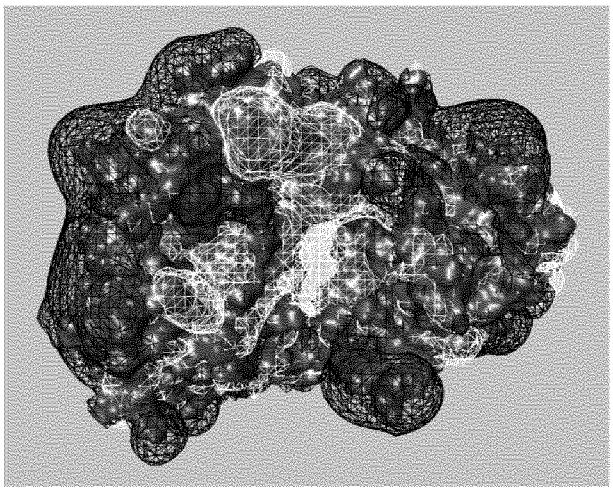
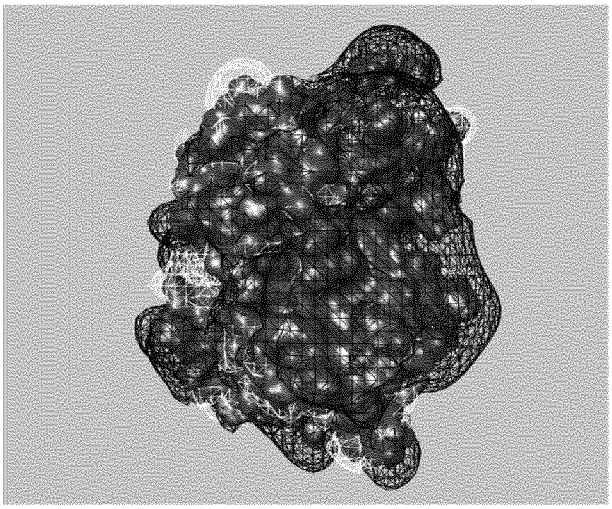


FIG.20C



4B9 FAP clone

Fig. 20

FIG.20D

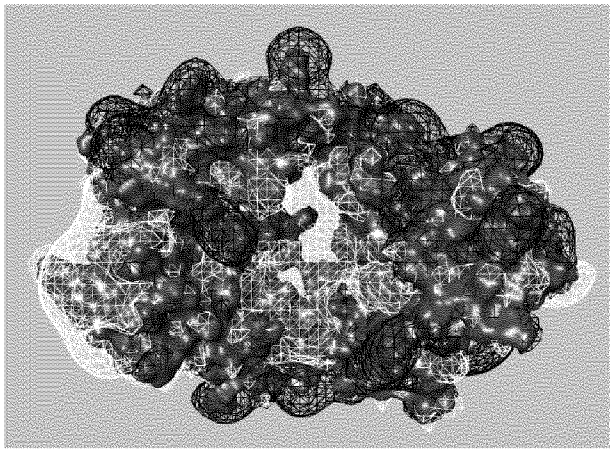


FIG.20E

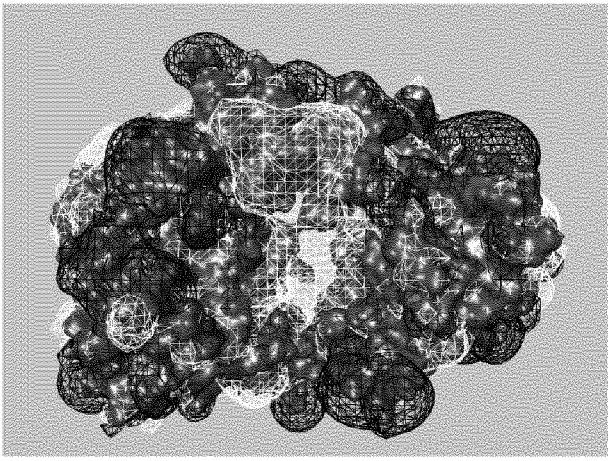
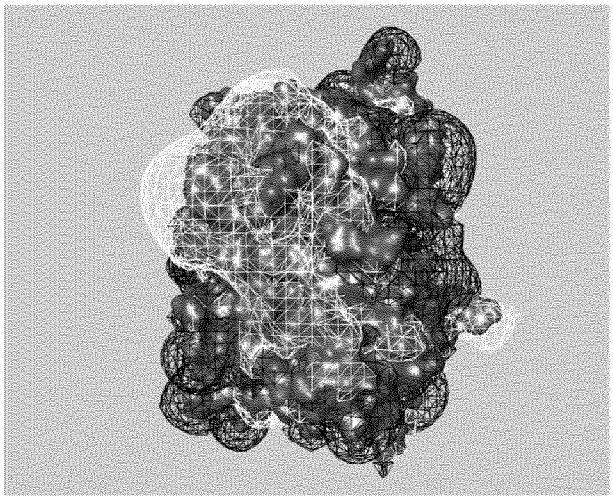


FIG.20F



1G1a (h212) FAP clone

Fig. 21

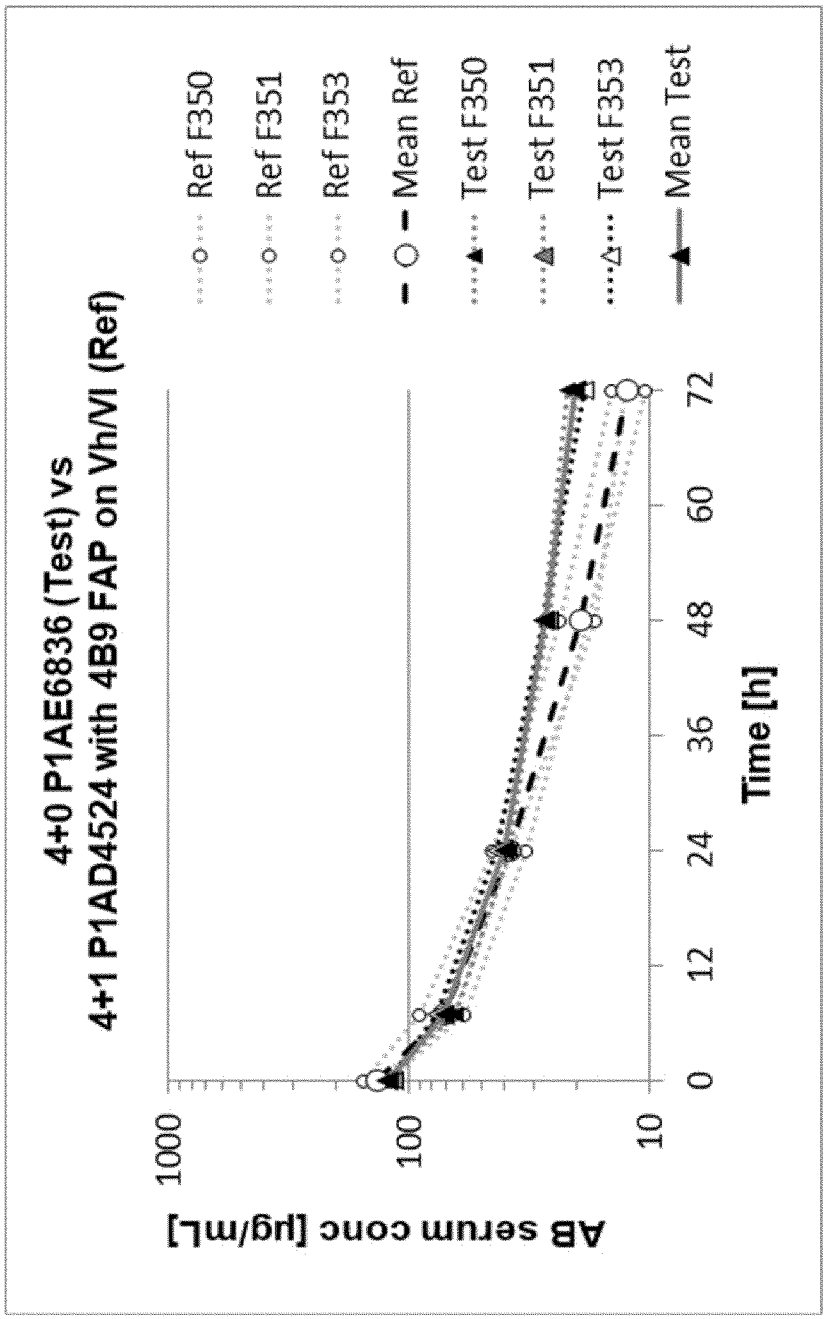

4+0 P1AE6836 (Test) vs
4+1 P1AD4524 with 4B9 FAP on Vh/Vl (Ref)
Ref F350
Ref F351
Ref F353
Mean Ref
Test F350
Test F351
Test F353
Mean Test
AB serum conc [µg/mL]
1000
100
10
0
12
24
36
48
60
72
Time [h]

Fig. 22

FIG.22A

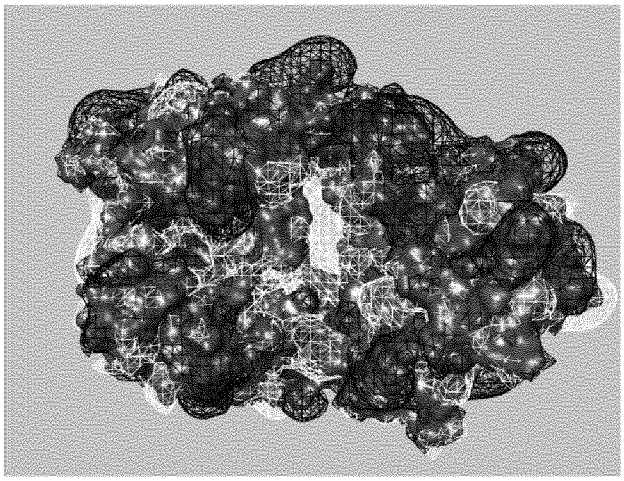


FIG.22B

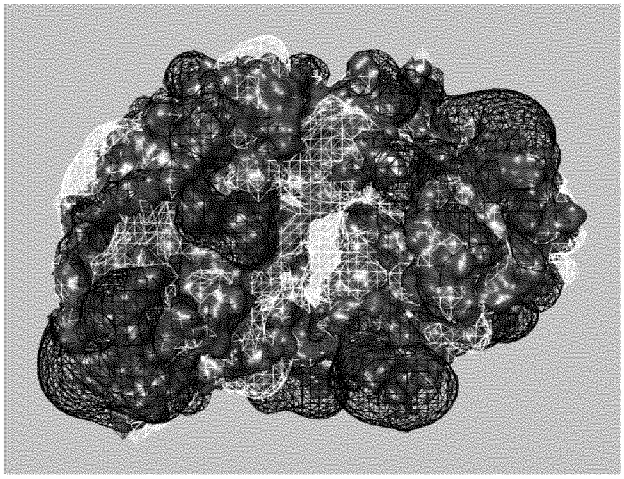
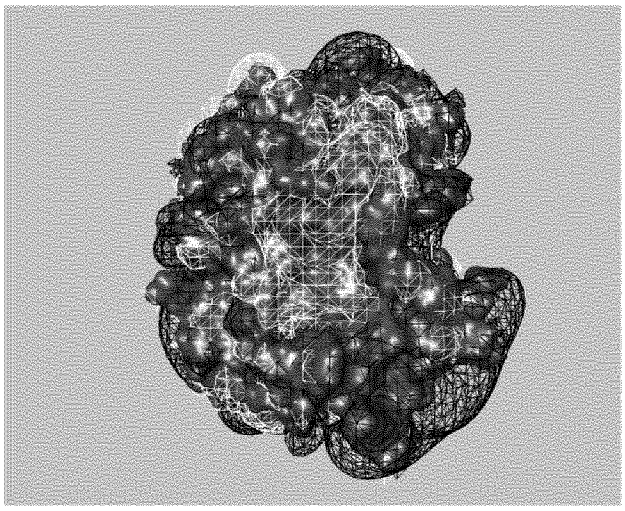


FIG.22C



49B4 OX40 clone

Fig. 23

FIG.23A

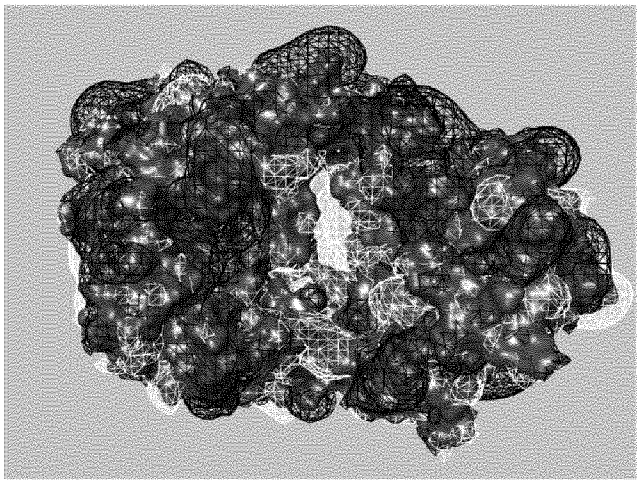


FIG.23B

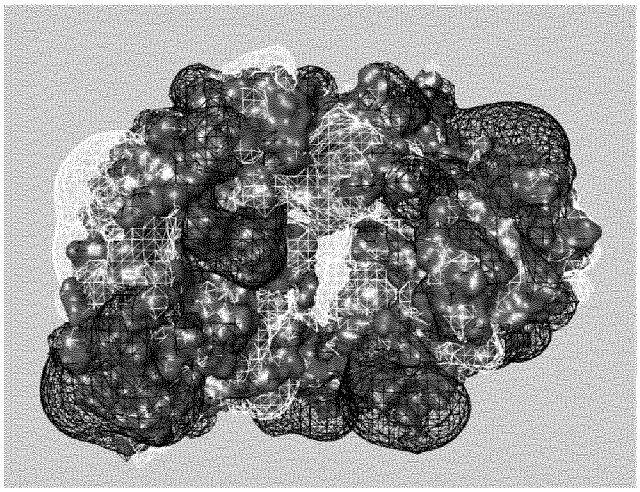
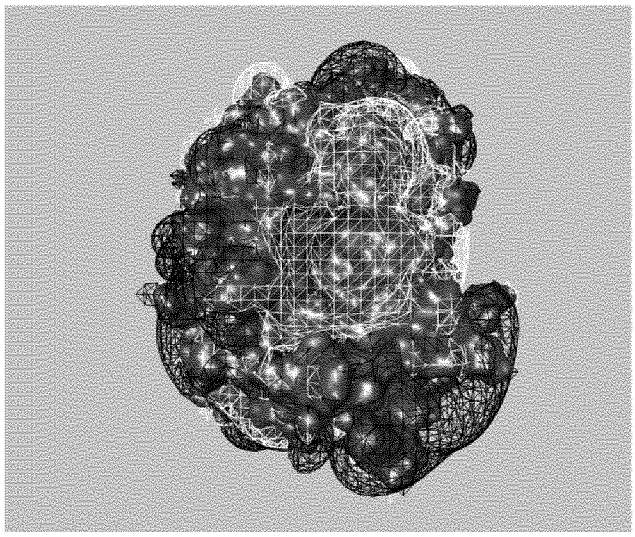


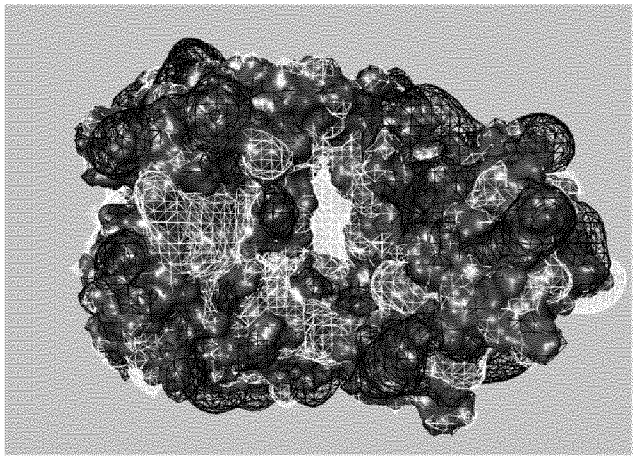
FIG.23C



8H9 OX40 clone

Fig. 24

FIG.24A



CLC563 OX40 clone

FIG.24B

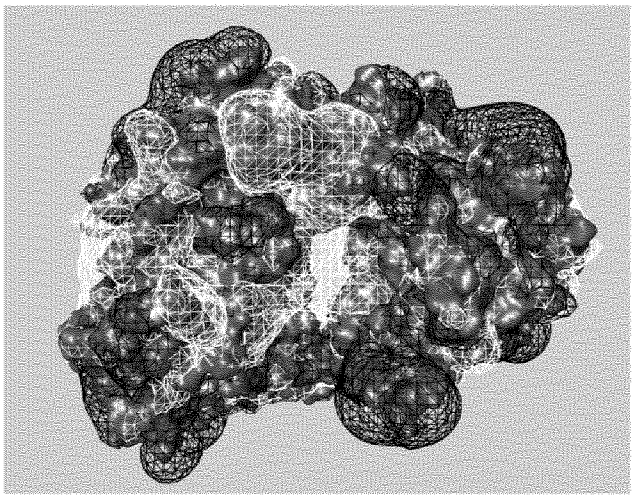


FIG.24C

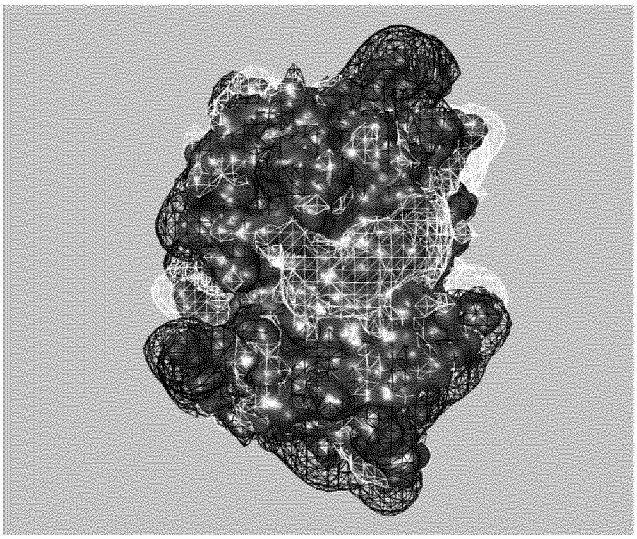


Fig. 25

FIG.25A

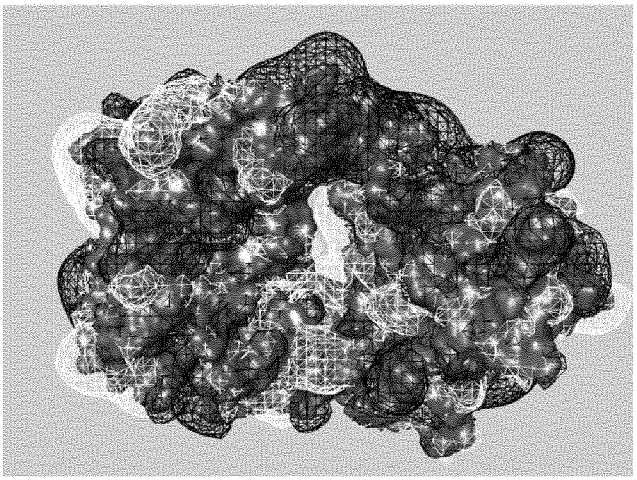


FIG.25B

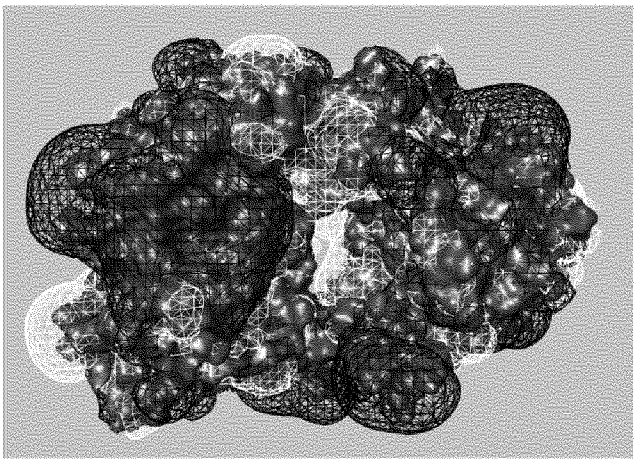
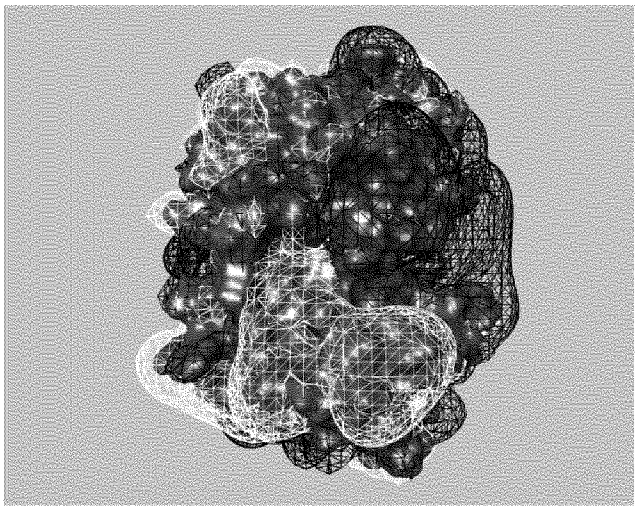


FIG.25C



MOXR0916 OX40 clone

Fig. 26

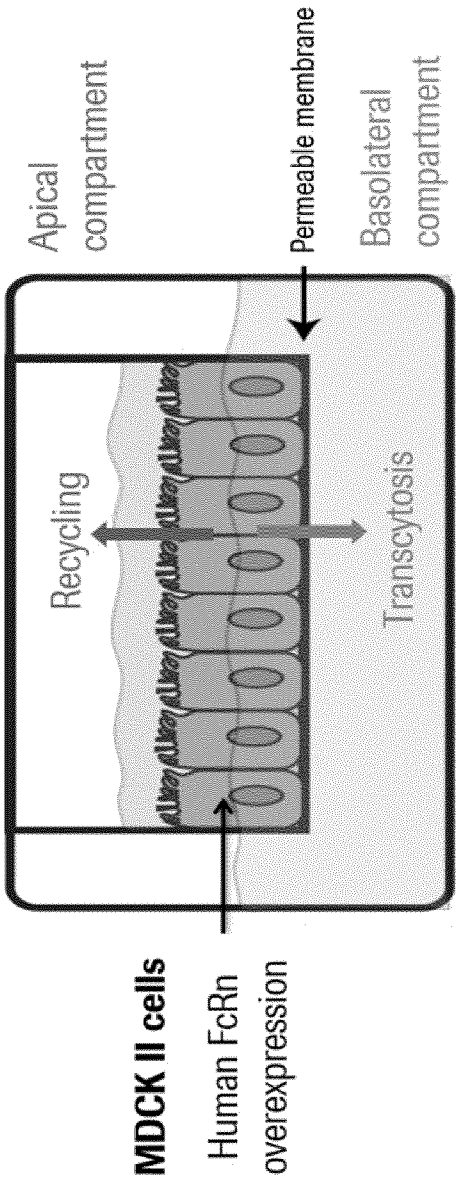

Apical compartment
Permeable membrane
Basolateral compartment
Recycling
Transcytosis
MDCK II cells
Human FcRn overexpression

Fig. 27

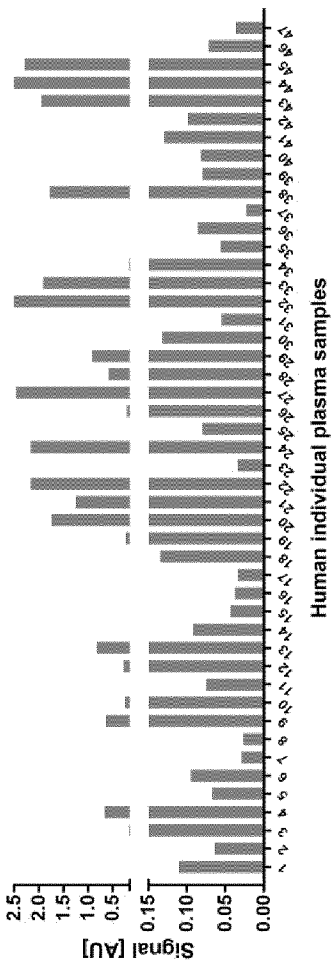


FIG.27A

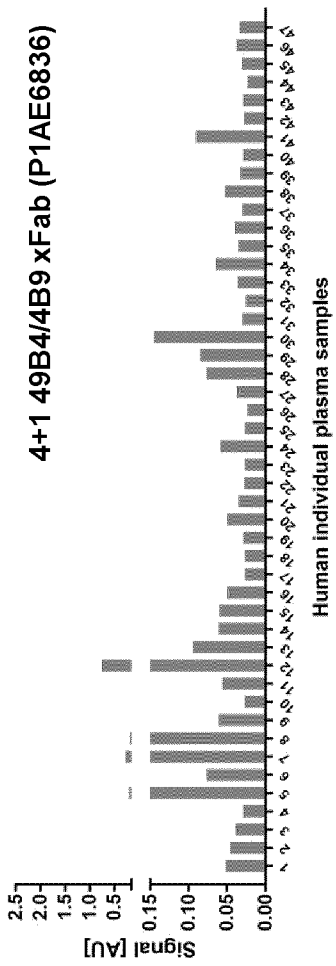


FIG.27B

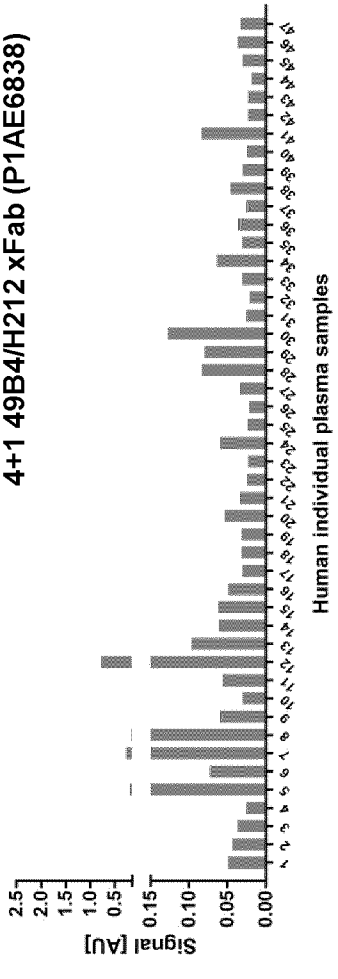


FIG.27C

FAPOX40 4+1 constructs

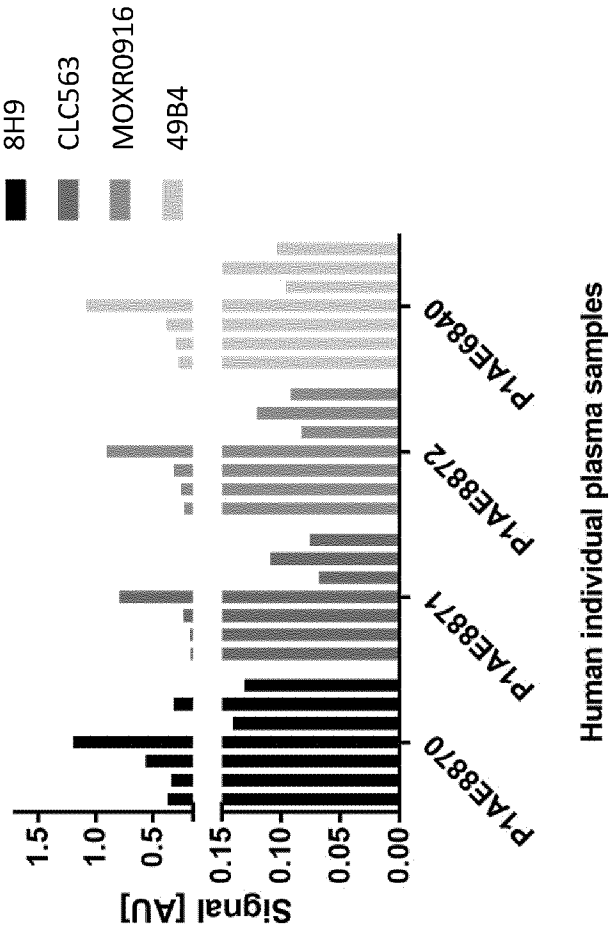

8H9
CLC563
MOXR0916
49B4
Signal [AU]
1.5
1.0
0.5
0.15
0.10
0.05
0.00
P1AE6840
P1AE8872
P1AE8871
P1AE8870
Human individual plasma samples

Fig. 28

Fig. 29

FIG.29A

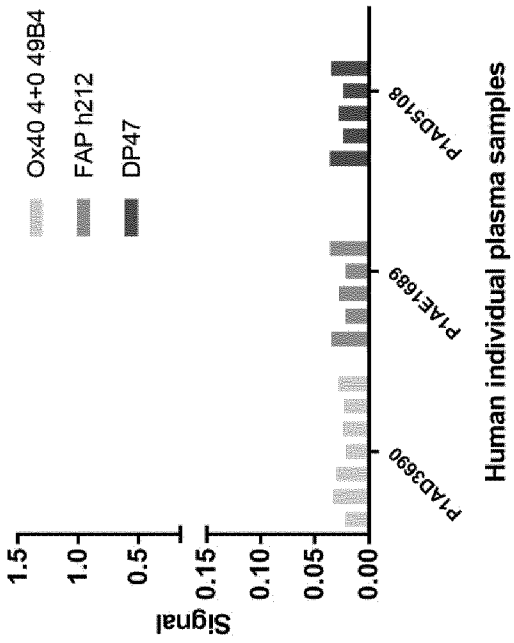


FIG.29B

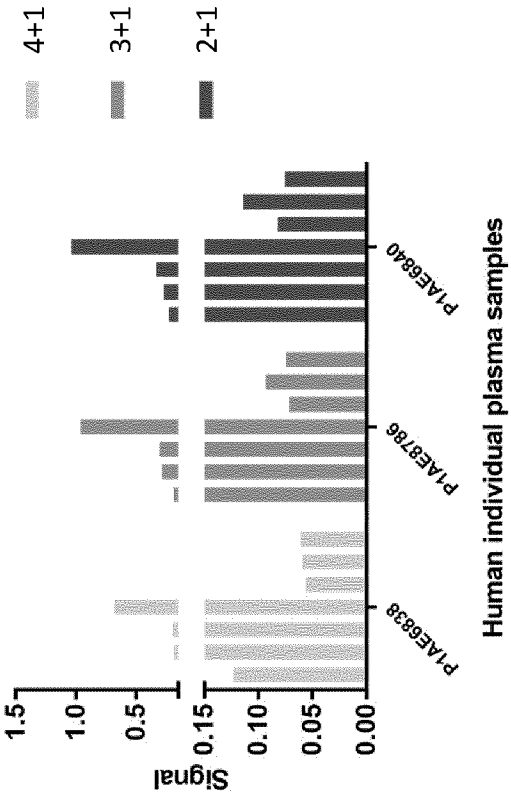


Fig. 30

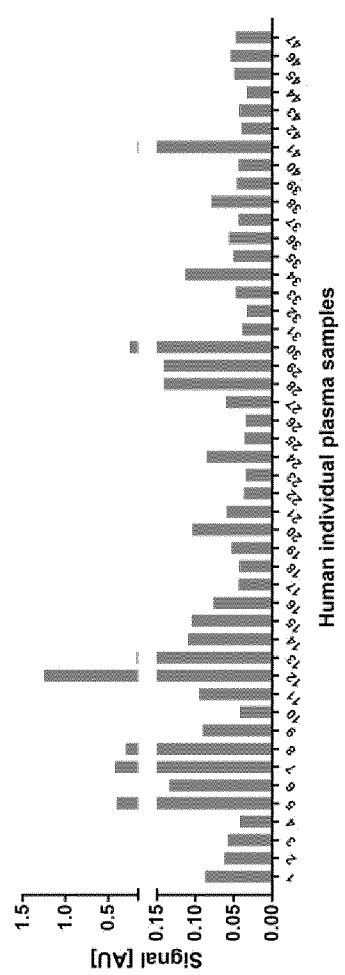


FIG.30A

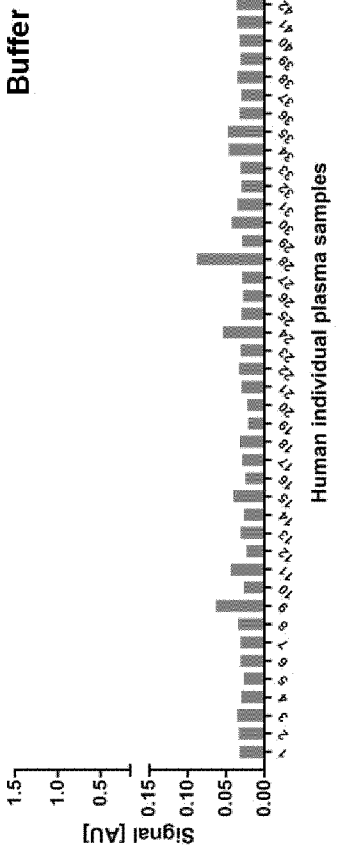


FIG.30B

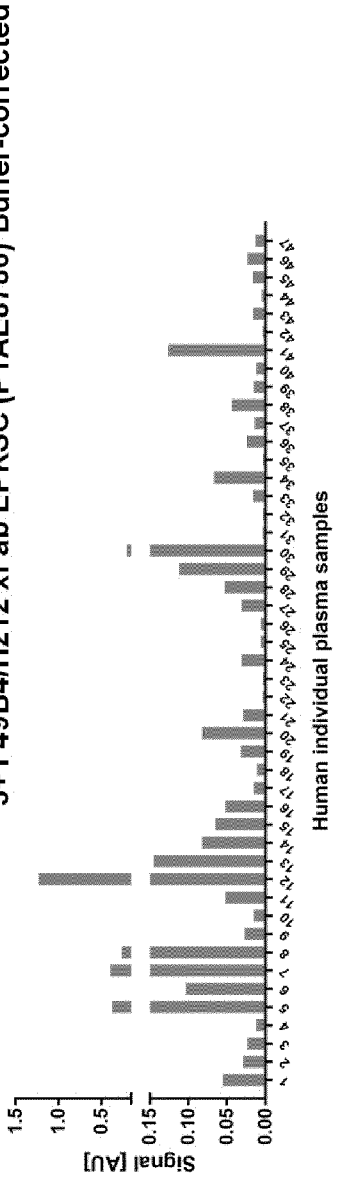


FIG.30C

Fig. 31

FIG.31A

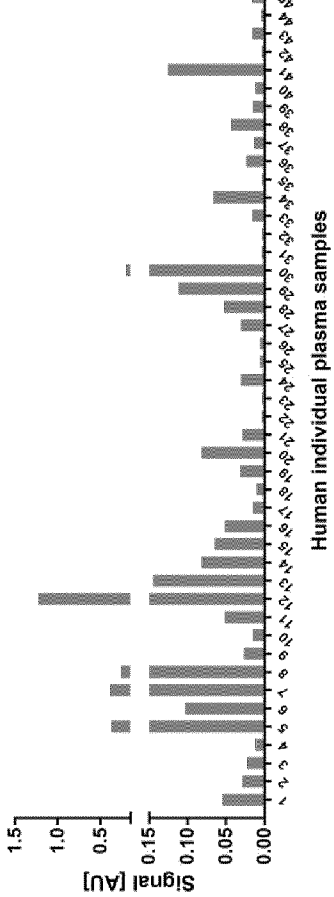


FIG.31B

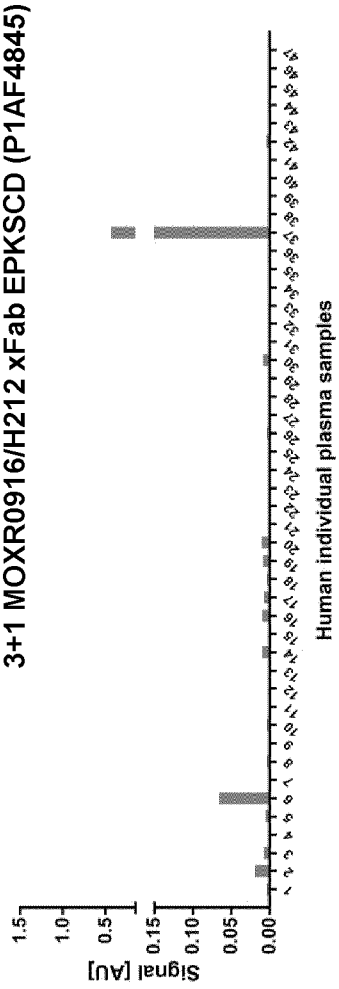


FIG.31C

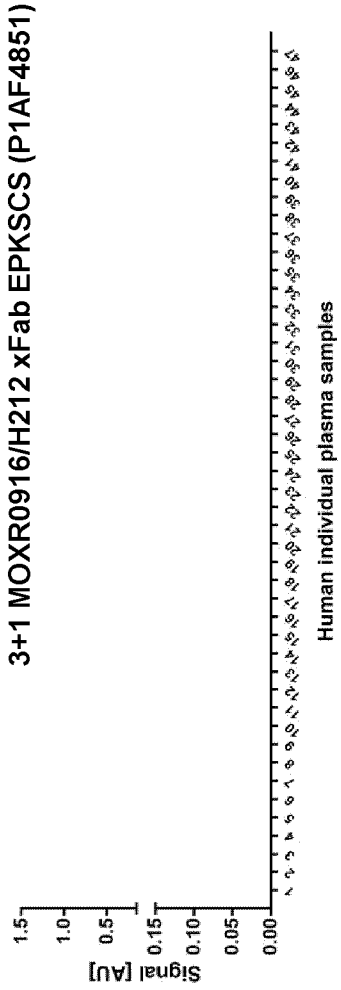


Fig. 32

FIG.32A

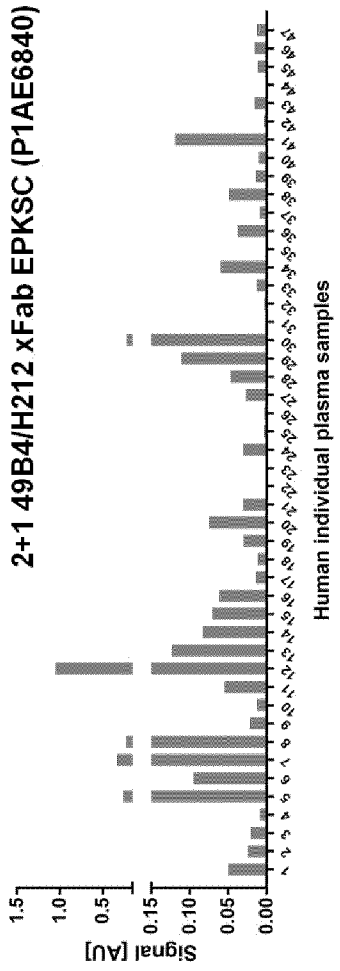


FIG.32B

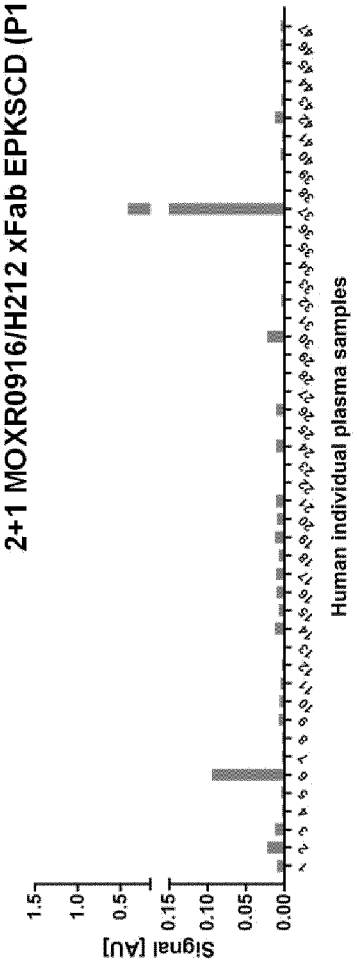


FIG.32C

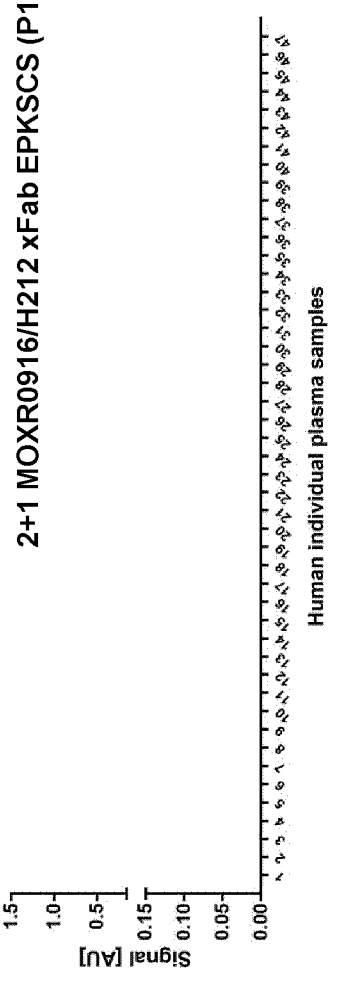


Fig. 33

FIG.33A

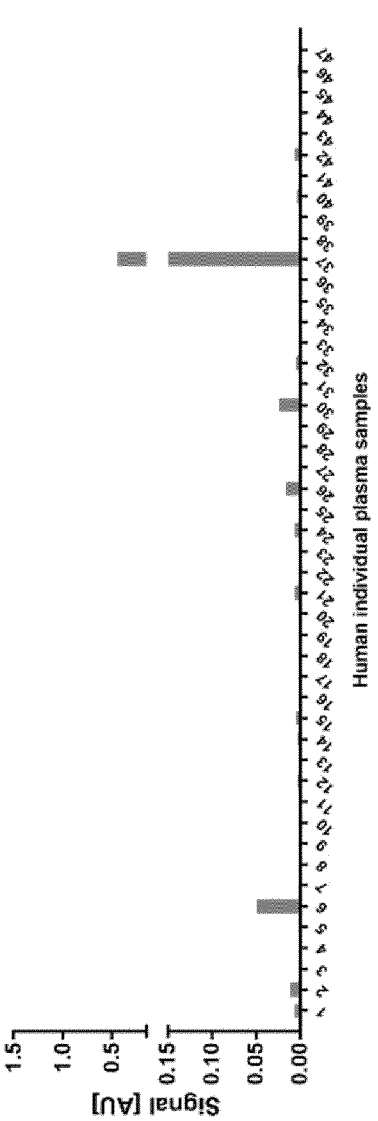


FIG.33B

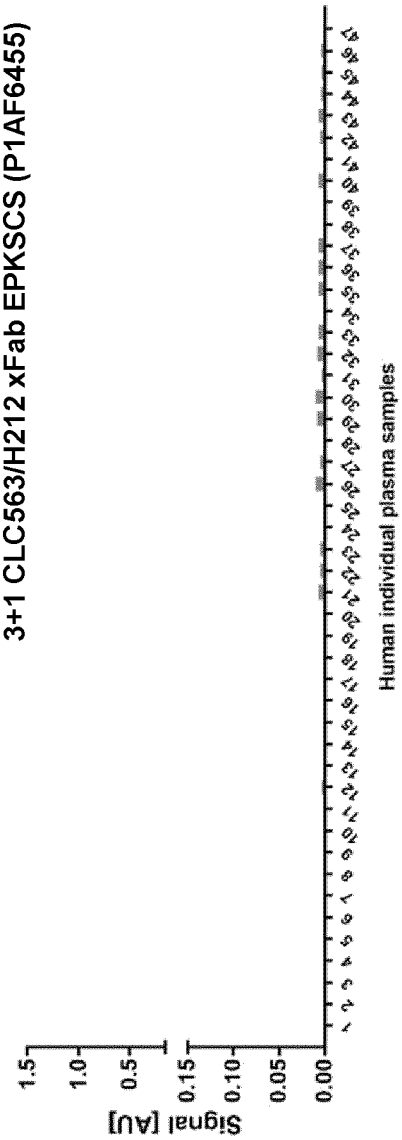


Fig. 33

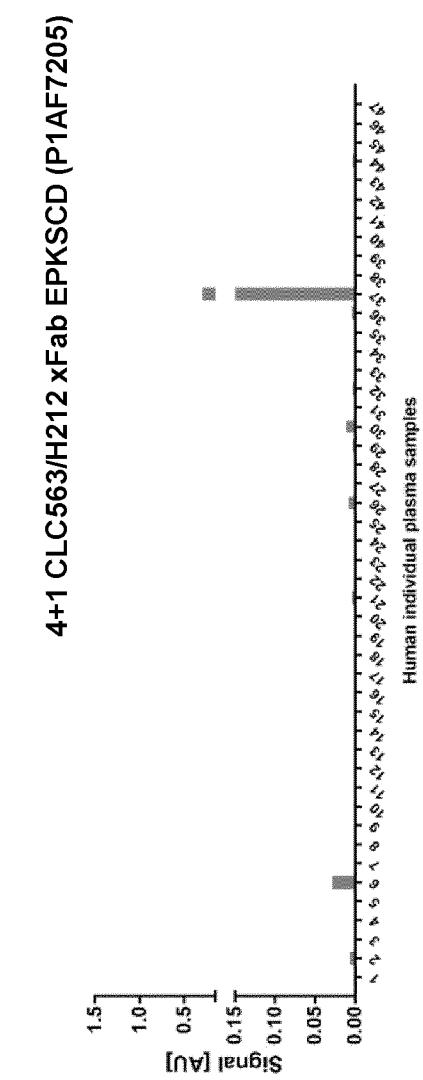


FIG.33C

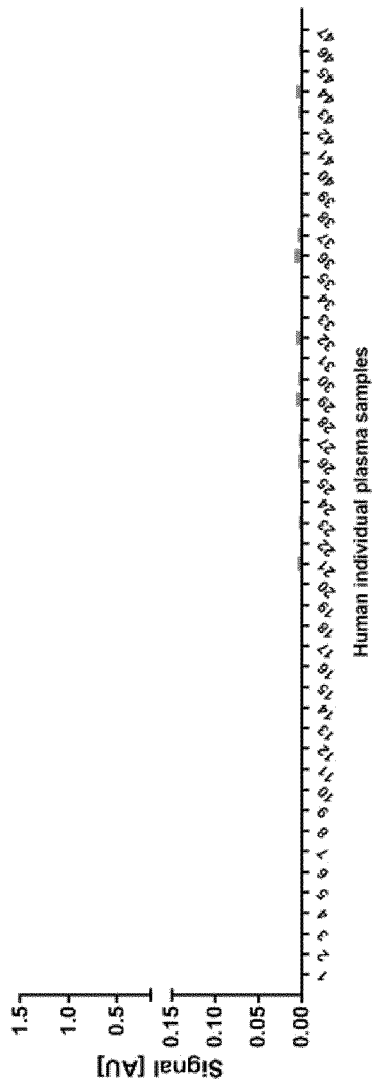


FIG.33D

Fig. 33

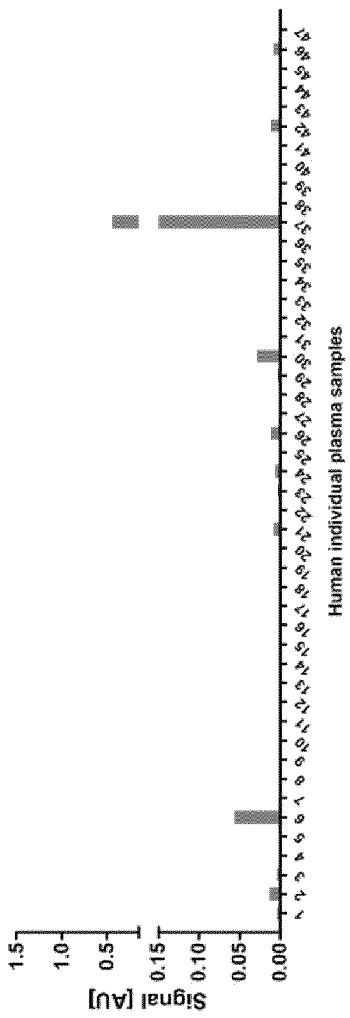


FIG.33E

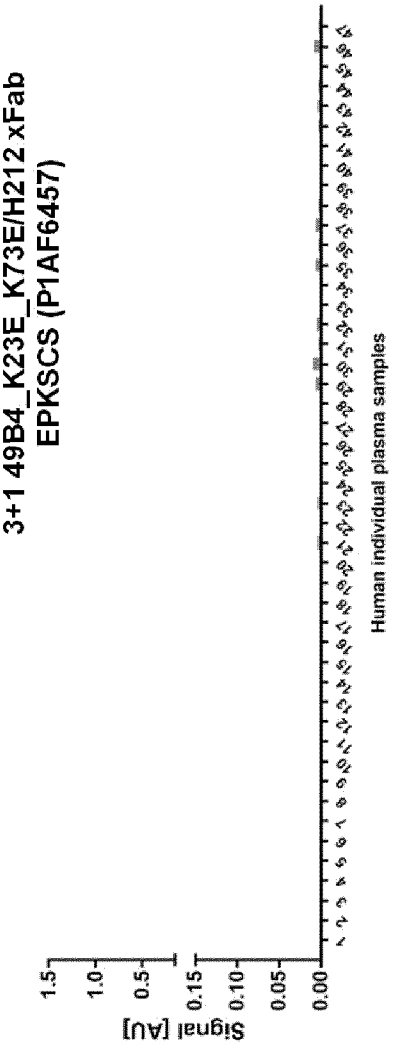


FIG.33F

Fig. 34

FIG.34A

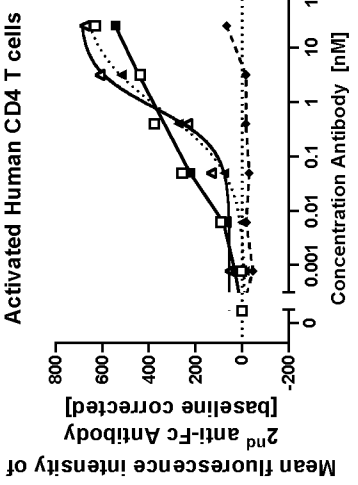


FIG.34B

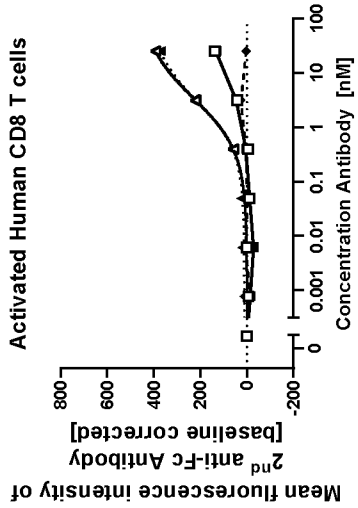
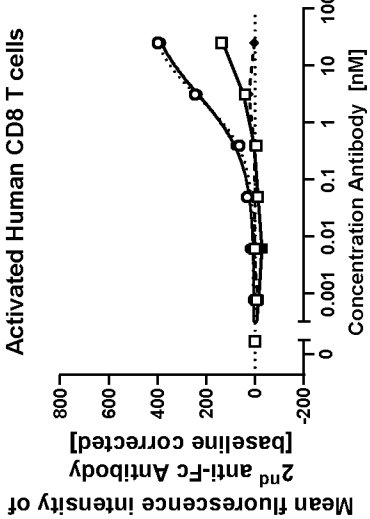


FIG.34C

FIG.34D



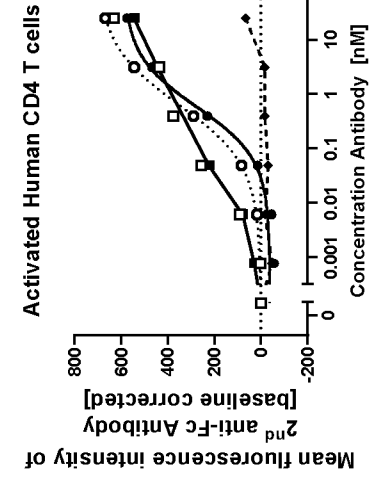


Fig. 34

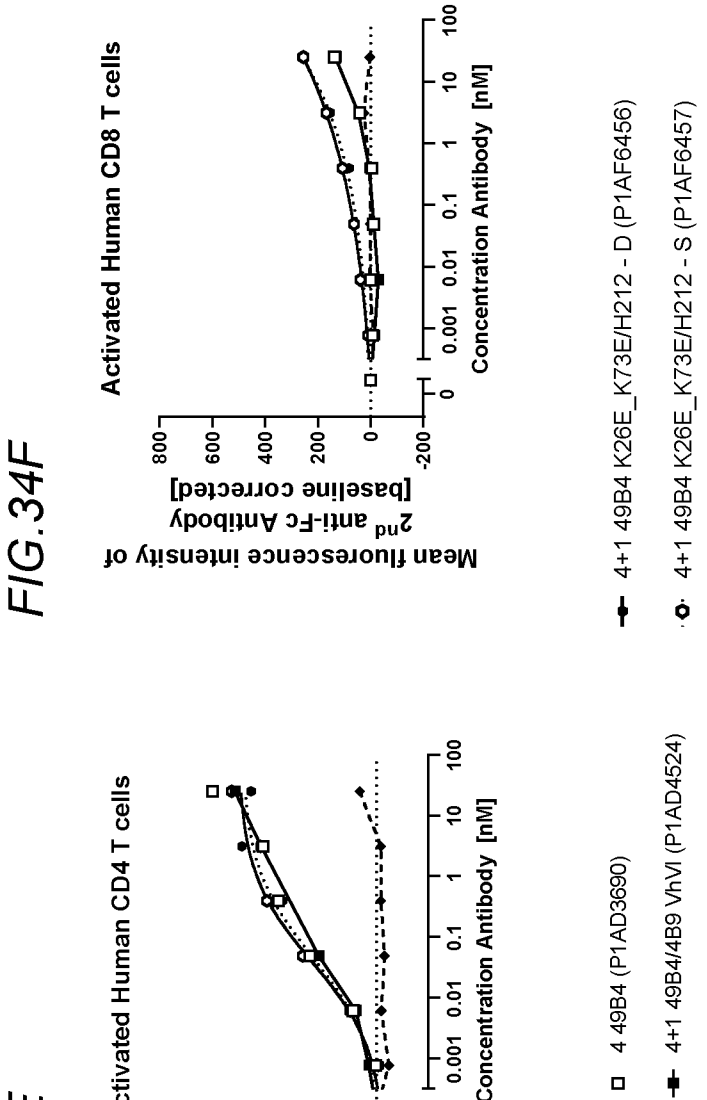

FIG.34E
Activated Human CD4 T cells
Mean fluorescence intensity of 2nd anti-Fc Antibody [baseline corrected]
800
600
400
200
0
-200
0
0.001
0.01
0.1
1
10
100
Concentration Antibody [nM]
FIG.34F
Activated Human CD8 T cells
4 49B4 (P1AD3690)
4+1 49B4/4B9 VhVl (P1AD4524)
Isotype Control
4+1 49B4 K26E_K73E/H212 - D (P1AF6456)
4+1 49B4 K26E_K73E/H212 - S (P1AF6457)

Fig. 35

FIG.35A

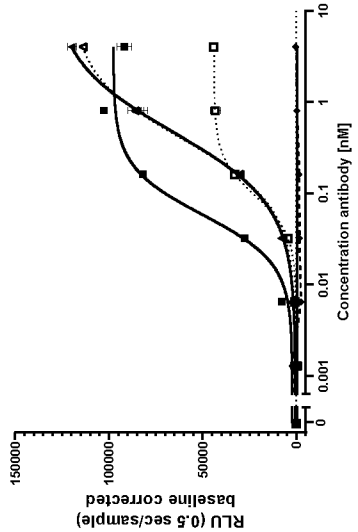


FIG.35B

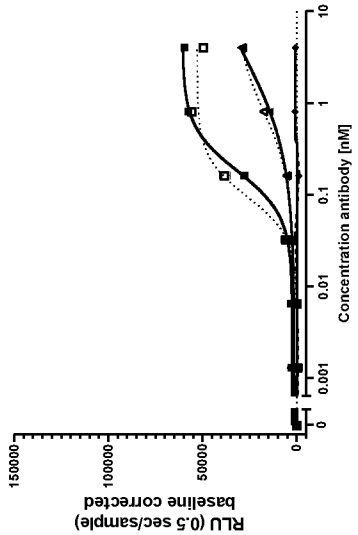
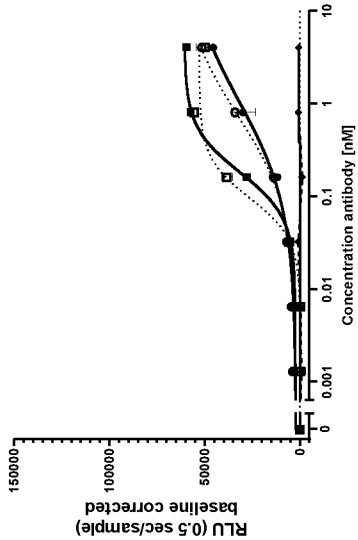


FIG.35C

FIG.35D



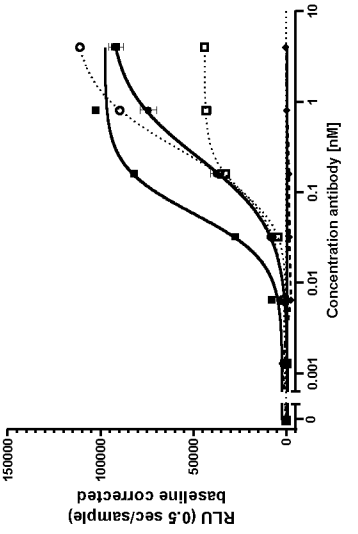


Fig. 35

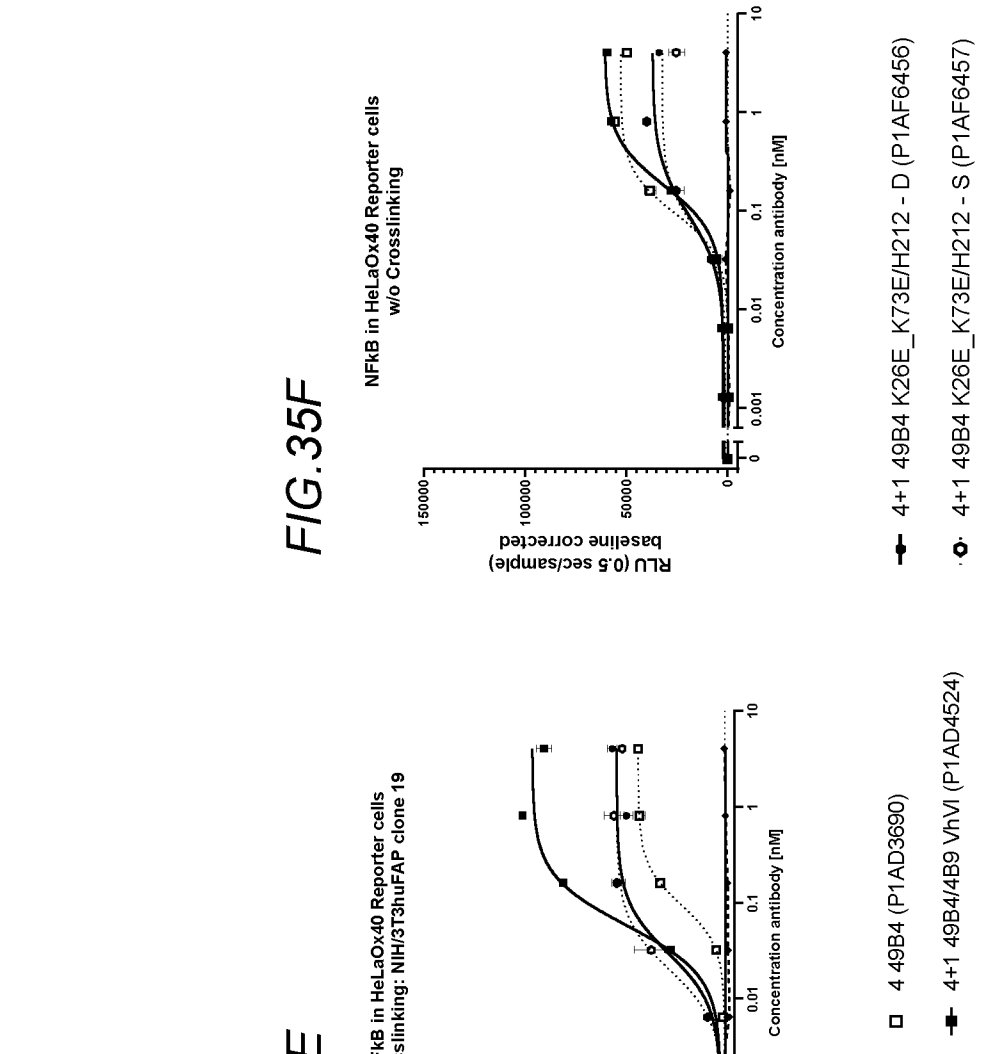

FIG.35E
NFkB in HeLaOx40 Reporter cells
Crosslinking: NIH/3T3huFAP clone 19
RLU (0.5 sec/sample)
baseline corrected
150000
100000
50000
0
0
0.001
0.01
0.1
1
10
Concentration antibody [nM]
FIG.35F
NFkB in HeLaOx40 Reporter cells
w/o Crosslinking
RLU (0.5 sec/sample)
baseline corrected
150000
100000
50000
0
0
0.001
0.01
0.1
1
10
Concentration antibody [nM]
4 49B4 (P1AD3690)
4+1 49B4/4B9 VhVl (P1AD4524)
Isotype Control
4+1 49B4 K26E_K73E/H212 - D (P1AF6456)
4+1 49B4 K26E_K73E/H212 - S (P1AF6457)

Fig. 36

FIG.36A

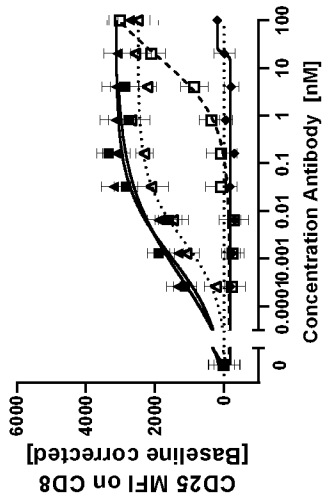


FIG.36B

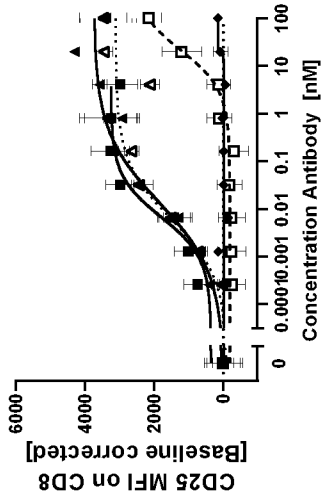


FIG.36C

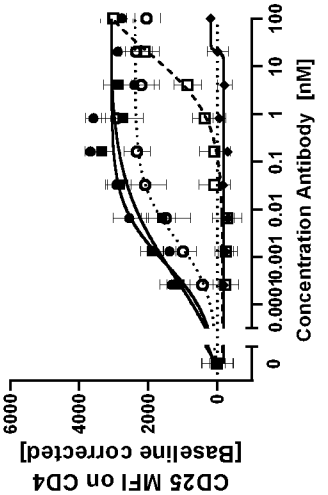


FIG.36D

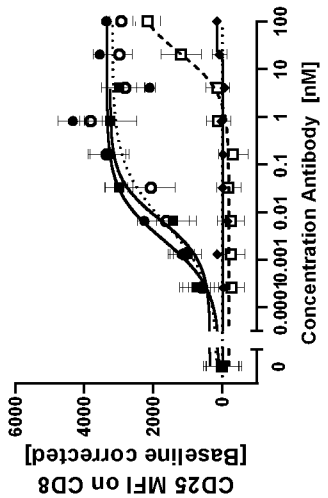


Fig. 36

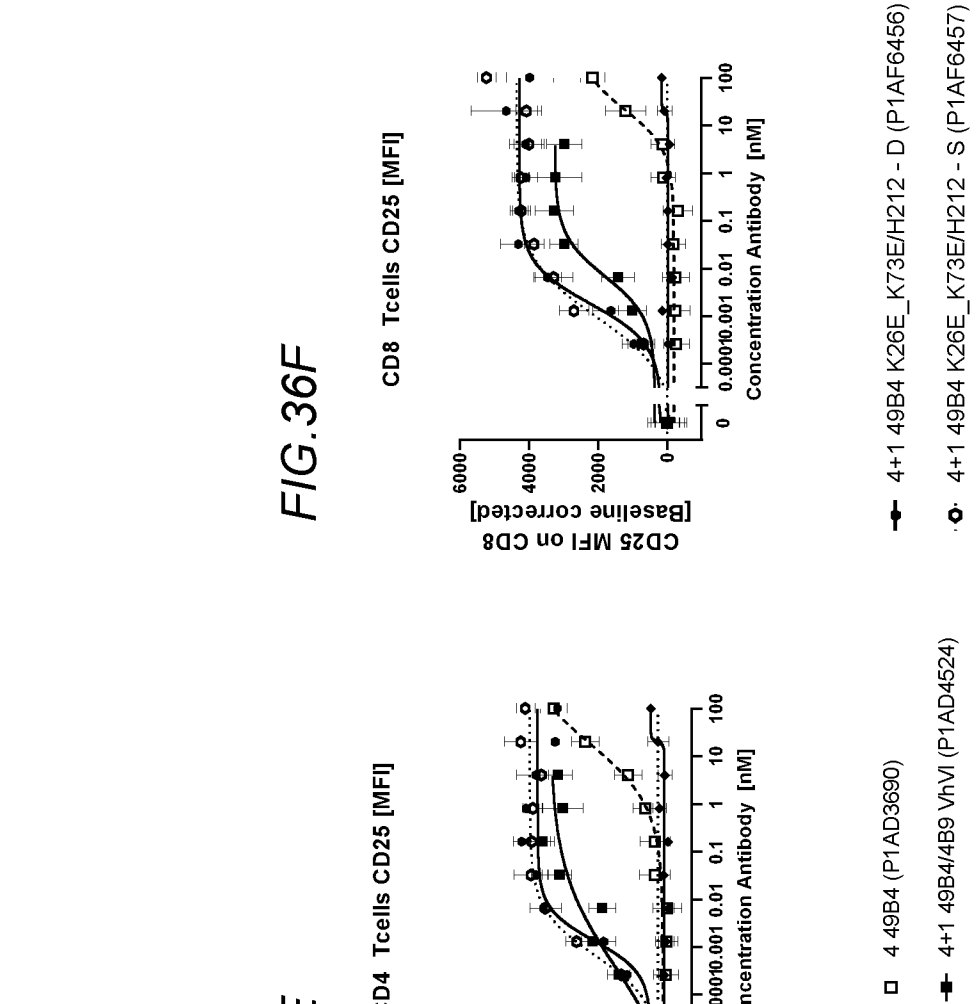

FIG.36E
CD4 Tcells CD25 [MFI]
CD25 MFI on CD4 [Baseline corrected]
6000
4000
2000
0
0
0.0001
0.001
0.01
0.1
1
10
100
Concentration Antibody [nM]
FIG.36F
CD8 Tcells CD25 [MFI]
CD25 MFI on CD8 [Baseline corrected]
4 49B4 (P1AD3690)
4+1 49B4/4B9 VhVl (P1AD4524)
Isotype Control
4+1 49B4 K26E_K73E/H212 - D (P1AF6456)
4+1 49B4 K26E_K73E/H212 - S (P1AF6457)

Fig. 37

FIG.37A

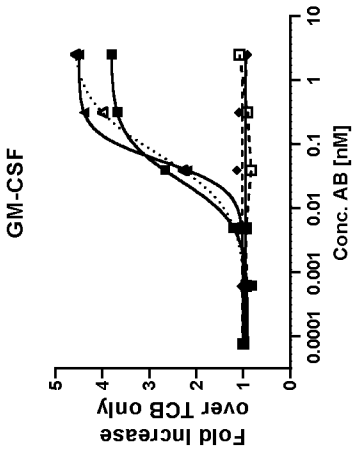


FIG.37B

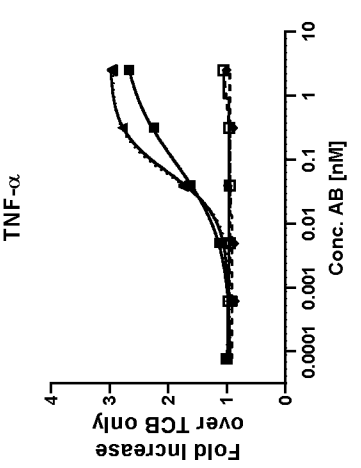


FIG.37C

FIG.37D

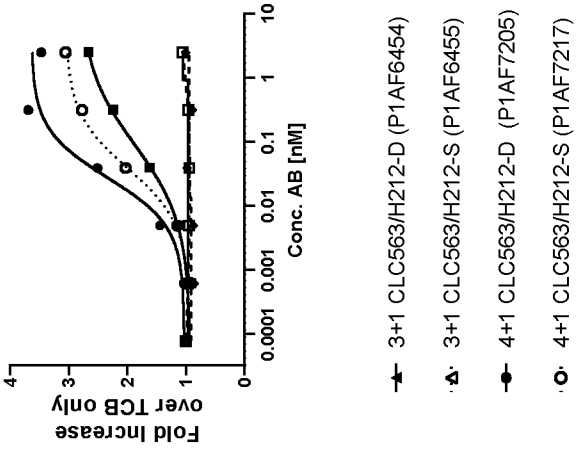


Fig. 37

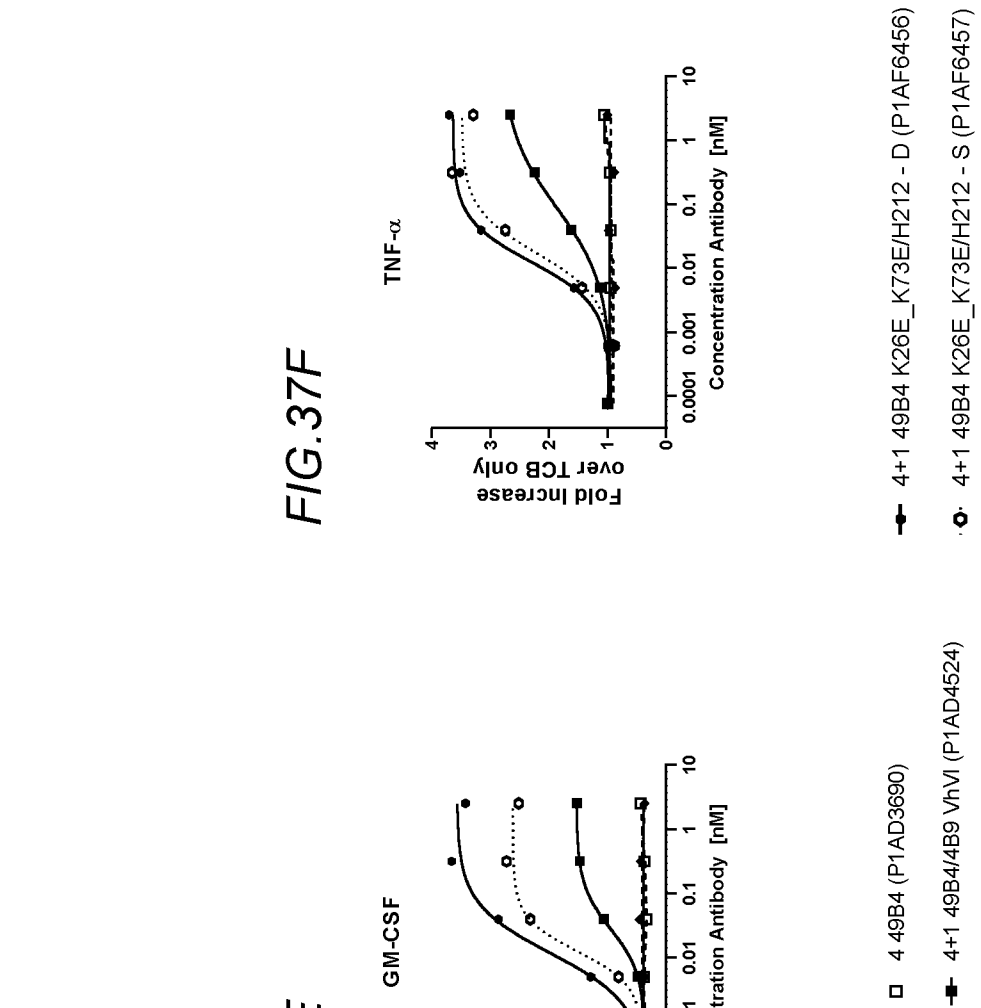

FIG.37E
GM-CSF
Fold Increase over TCB only
Concentration Antibody [nM]
0.0001 0.001 0.01 0.1 1 10
0 2 4 6 8 10
FIG.37F
TNF-α
Fold Increase over TCB only
Concentration Antibody [nM]
0.0001 0.001 0.01 0.1 1 10
0 1 2 3 4
4 49B4 (P1AD3690)
4+1 49B4/4B9 VhVl (P1AD4524)
Isotype Control
4+1 49B4 K26E_K73E/H212 - D (P1AF6456)
4+1 49B4 K26E_K73E/H212 - S (P1AF6457)

Fig. 38

FIG.38A

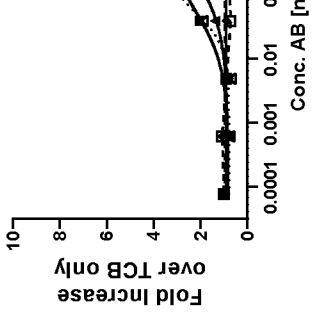


FIG.38B

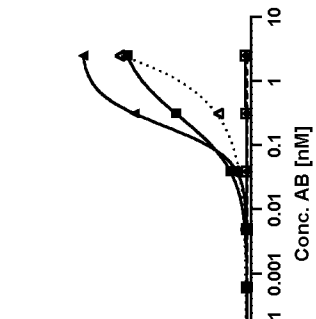


FIG.38C

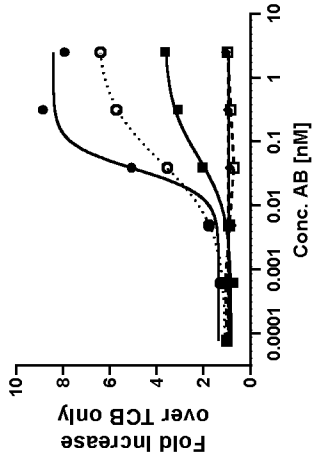


FIG.38D

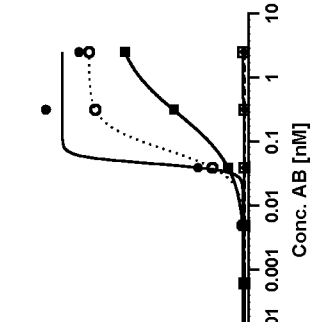


Fig. 38

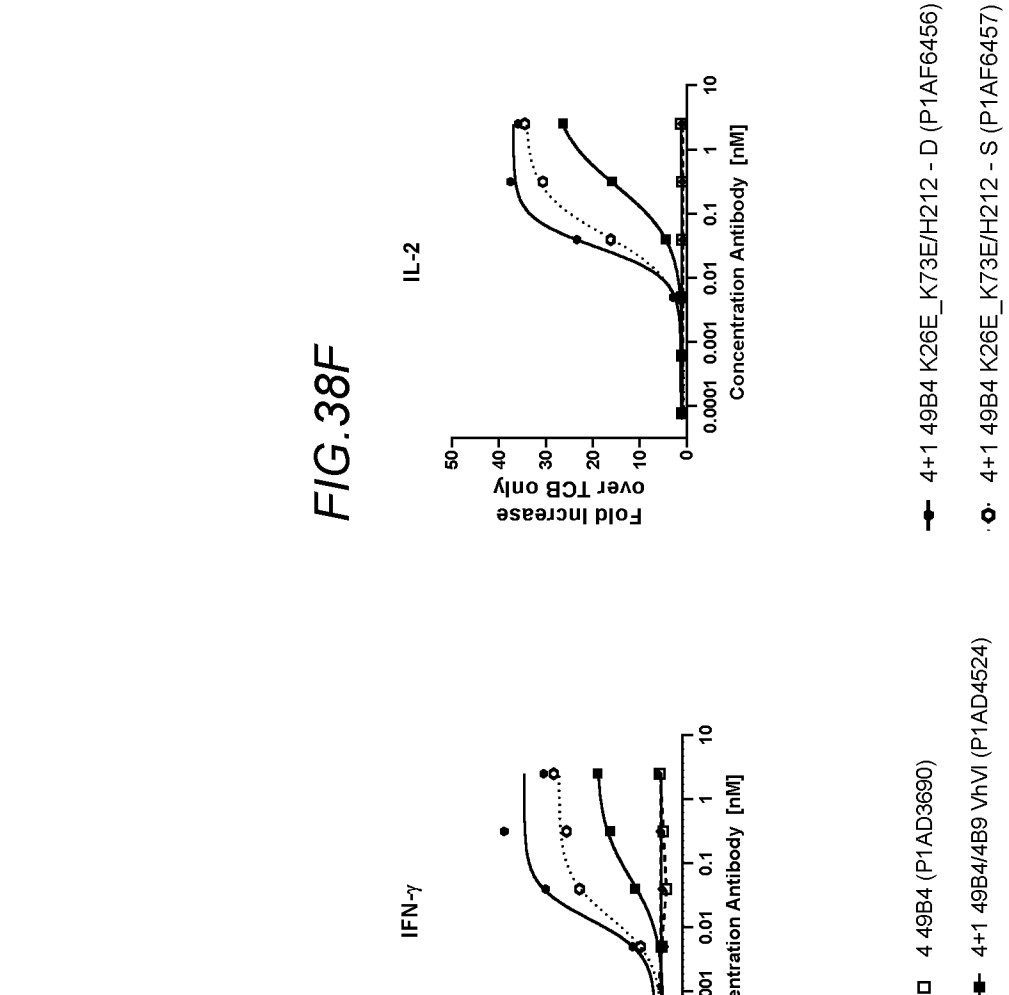

FIG.38E
IFN-γ
Fold Increase over TCB only
Concentration Antibody [nM]
FIG.38F
IL-2
Fold Increase over TCB only
Concentration Antibody [nM]
4 49B4 (P1AD3690)
4+1 49B4/4B9 VhVl (P1AD4524)
Isotype Control
4+1 49B4 K26E_K73E/H212 - D (P1AF6456)
4+1 49B4 K26E_K73E/H212 - S (P1AF6457)

Fig. 39

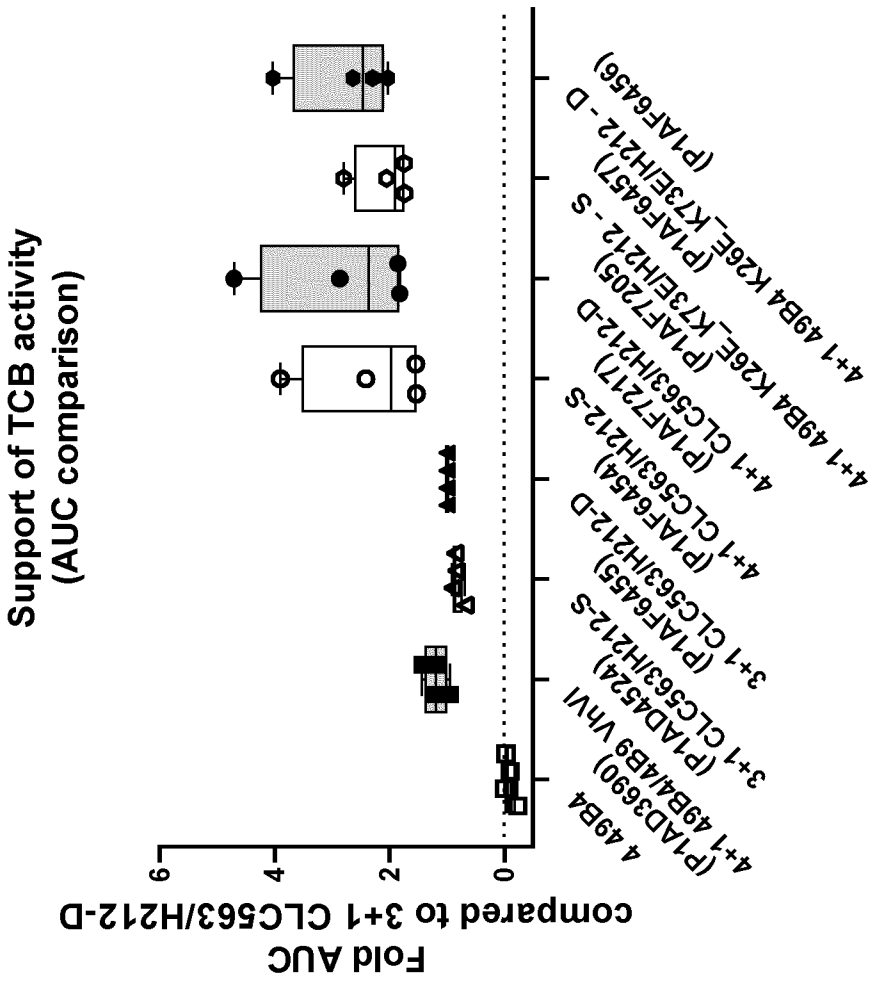

Support of TCB activity
(AUC comparison)
Fold AUC
compared to 3+1 CLC563/H212-D
0
2
4
6
4 49B4
(P1AD3690)
4+1 49B4/4B9 VhVl
(P1AD4524)
3+1 CLC563/H212-S
(P1AF6455)
3+1 CLC563/H212-D
(P1AF6454)
4+1 CLC563/H212-S
(P1AF7217)
4+1 CLC563/H212-D
(P1AF7205)
4+1 49B4 K26E_K73E/H212 - S
(P1AF6457)
4+1 49B4 K26E_K73E/H212 - D
(P1AF6456)

Fig. 40

FIG.40A

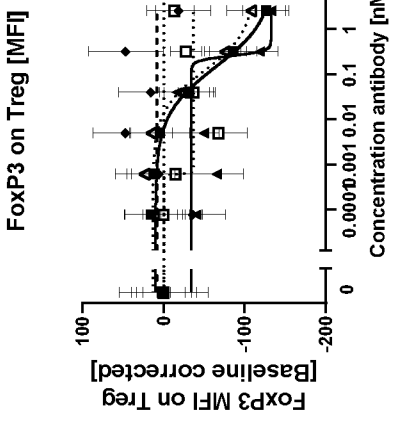


FIG.40B

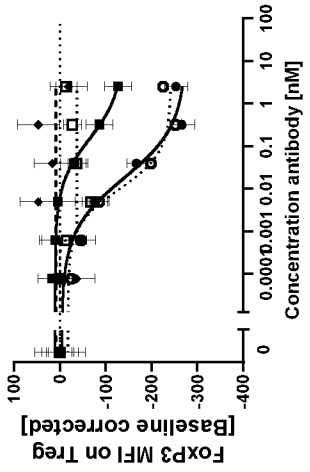


FIG.40C

Fig. 41

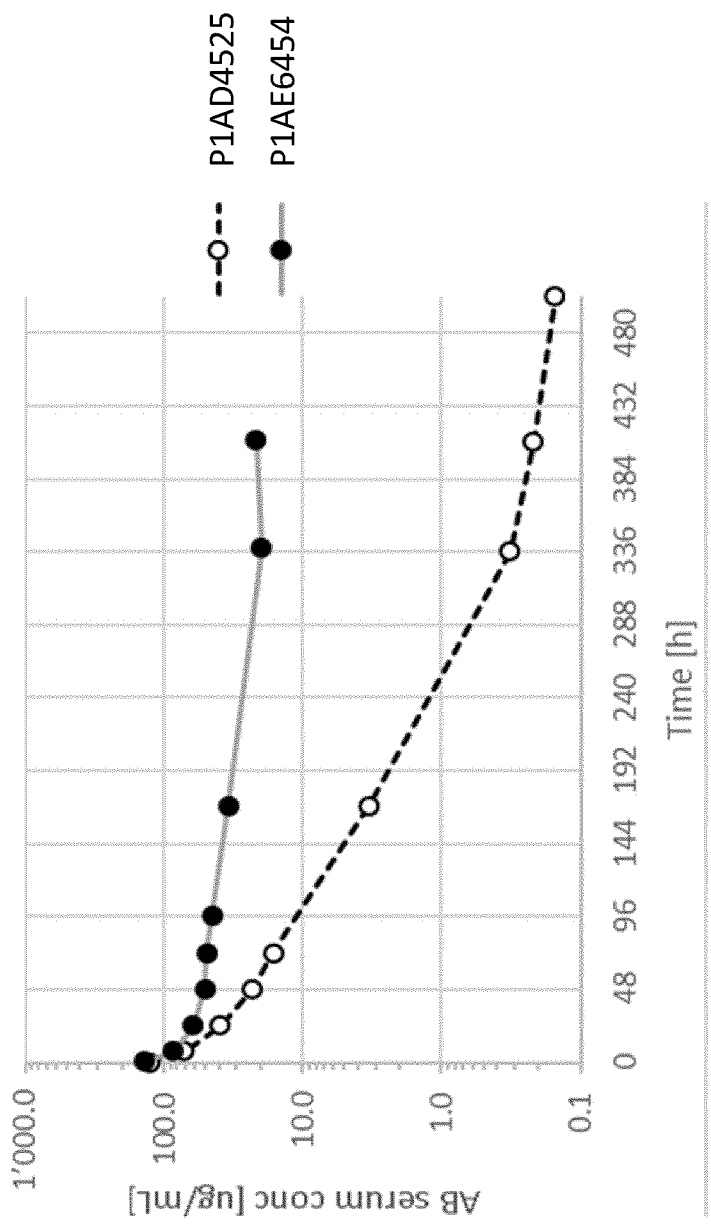

P1AD4525
P1AE6454
AB serum conc [ug/mL]
1'000.0
100.0
10.0
1.0
0.1
0
48
96
144
192
240
288
336
384
432
480
Time [h]

Fig. 42A

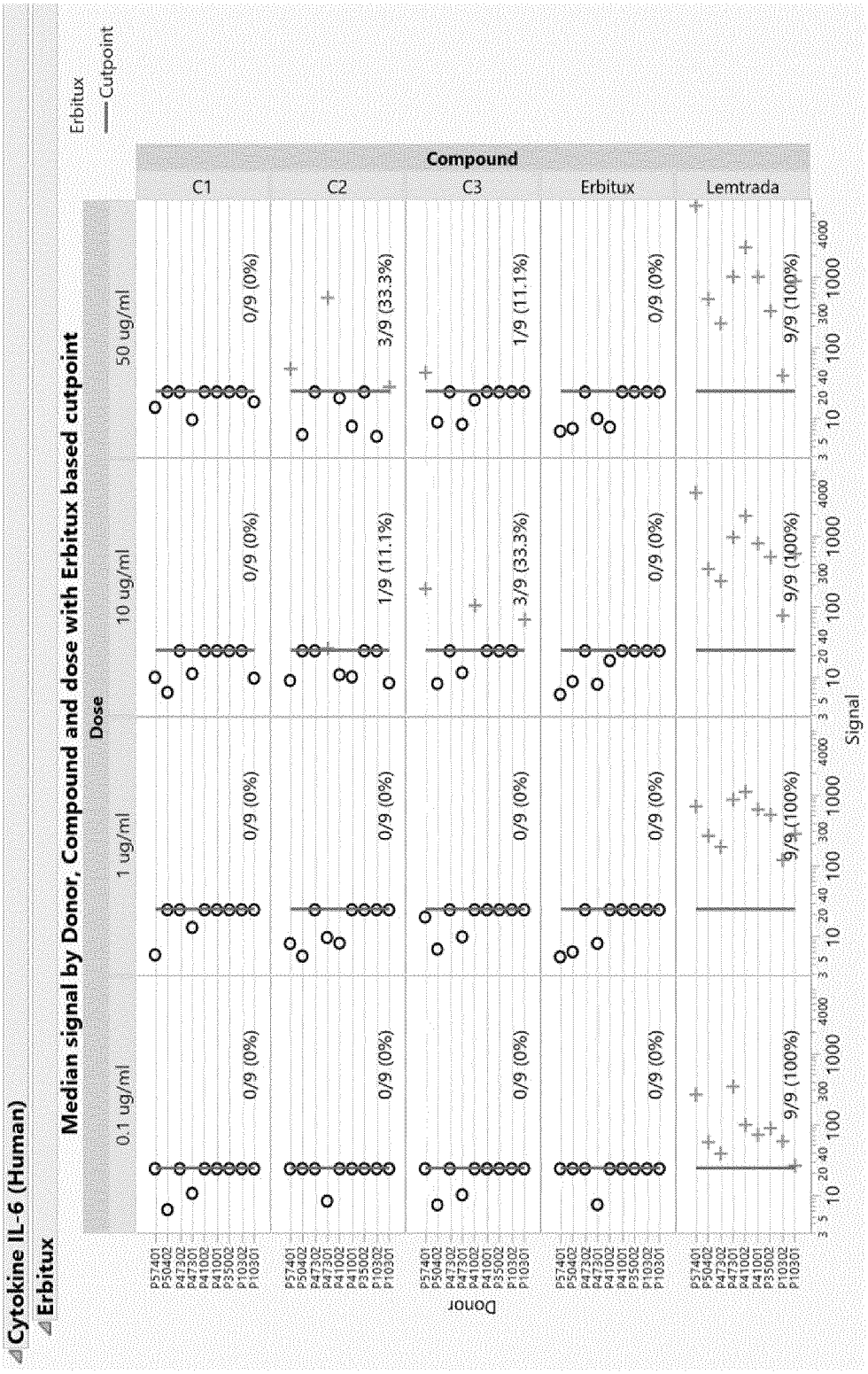

Cytokine IL-6 (Human)
Erbitux
Median signal by Donor, Compound and dose with Erbitux based cutpoint
Dose
0.1 ug/ml
1 ug/ml
10 ug/ml
50 ug/ml
Compound
C1
C2
C3
Erbitux
Lemtrada
Erbitux
Cutpoint
Donor
P57401
P50402
P47302
P47301
P41002
P41001
P35002
P10302
P10301
0/9 (0%)
1/9 (11.1%)
3/9 (33.3%)
9/9 (100%)
Signal
3 5 10 20 40 100 300 1000 4000

Fig. 42B

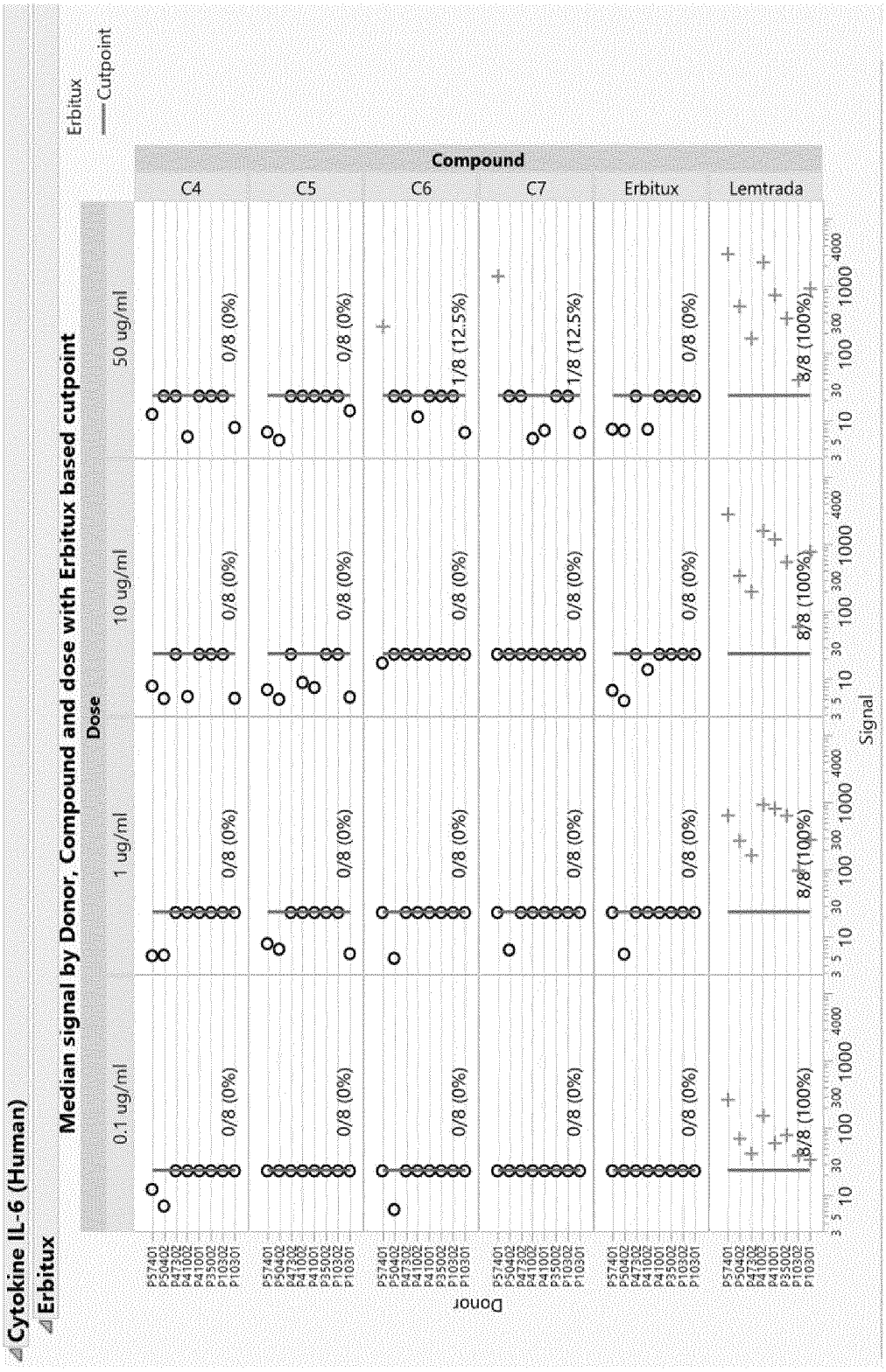

Cytokine IL-6 (Human)
Erbitux
Median signal by Donor, Compound and dose with Erbitux based cutpoint
Erbitux
Cutpoint
Dose
0.1 ug/ml
1 ug/ml
10 ug/ml
50 ug/ml
Compound
C4
C5
C6
C7
Erbitux
Lemtrada
0/8 (0%)
1/8 (12.5%)
8/8 (100%)
Donor
P57401
P50402
P47302
P41002
P41001
P35002
P10302
P10301
Signal
3
5
10
30
100
300
1000
4000

Fig. 43A

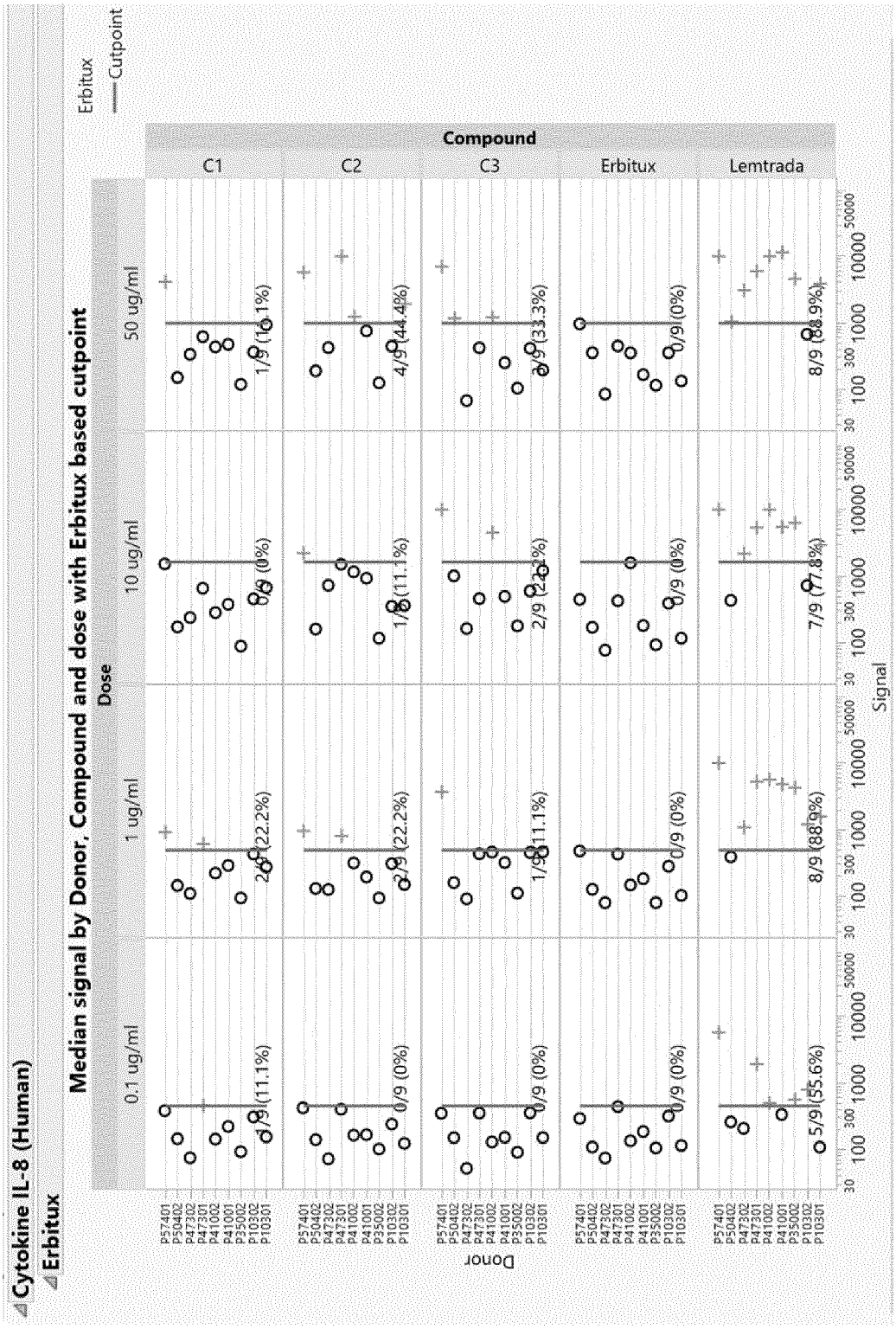

Cytokine IL-8 (Human)
Erbitux
Median signal by Donor, Compound and dose with Erbitux based cutpoint
Erbitux
Cutpoint
Dose
0.1 ug/ml
1 ug/ml
10 ug/ml
50 ug/ml
Compound
C1
C2
C3
Erbitux
Lemtrada
Donor
P57401
P50402
P47302
P47301
P41002
P41001
P35002
P10302
P10301
0/9 (0%)
2/9 (22.2%)
1/9 (11.1%)
1/9 (11.1%)
4/9 (44.4%)
3/9 (33.3%)
5/9 (55.6%)
8/9 (88.9%)
7/9 (77.8%)
Signal
30
100
300
1000
10000
50000
100000
300000
500000

Fig. 43B

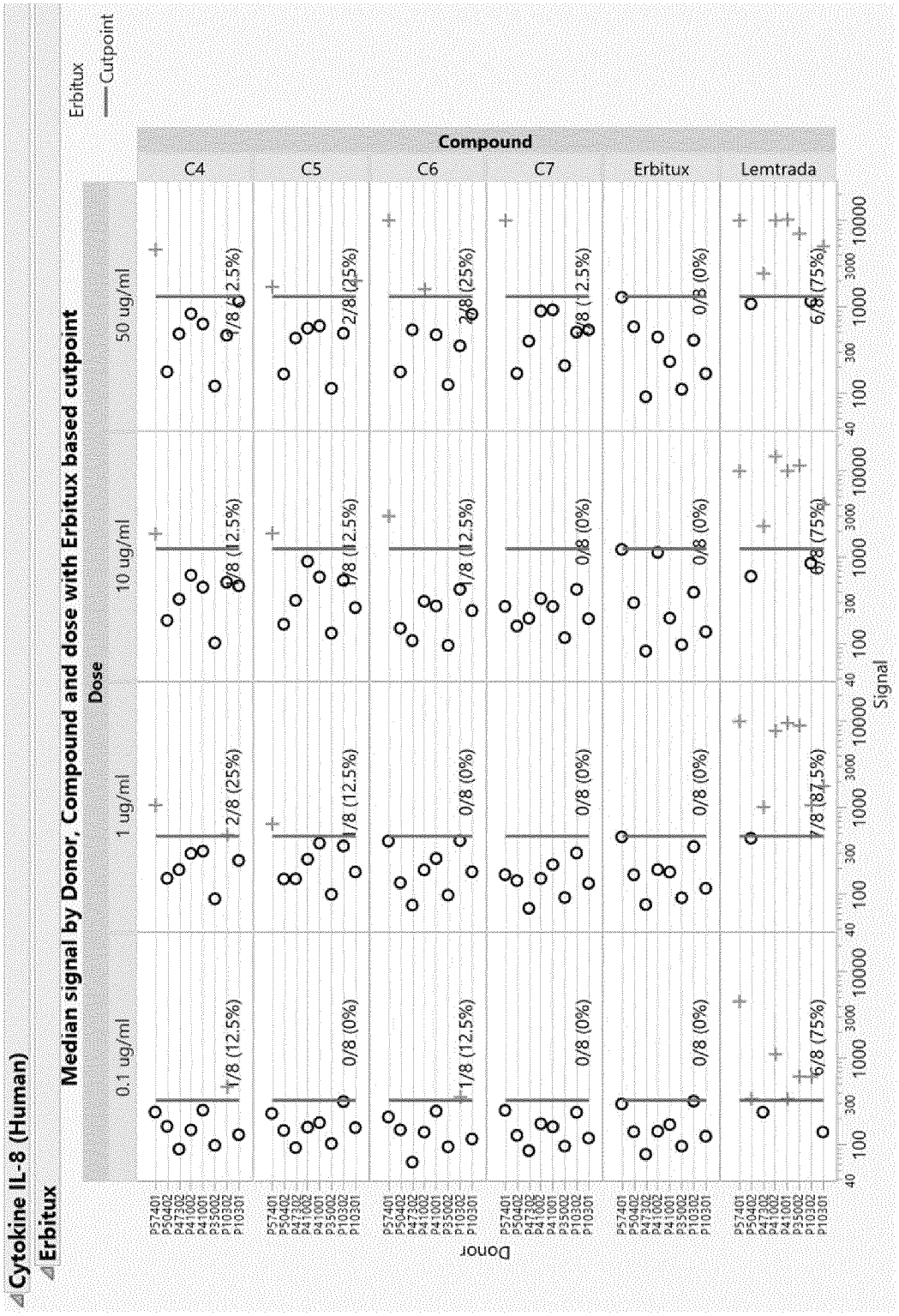

Cytokine IL-8 (Human)
Erbitux
Median signal by Donor, Compound and dose with Erbitux based cutpoint
Erbitux
Cutpoint
Dose
0.1 ug/ml
1 ug/ml
10 ug/ml
50 ug/ml
Compound
C4
C5
C6
C7
Erbitux
Lemtrada
Donor
P57401
P50402
P47302
P41002
P41001
P35002
P10302
P10301
1/8 (12.5%)
2/8 (25%)
0/8 (0%)
6/8 (75%)
7/8 (87.5%)
Signal
40
100
300
1000
3000
10000

Fig. 44A

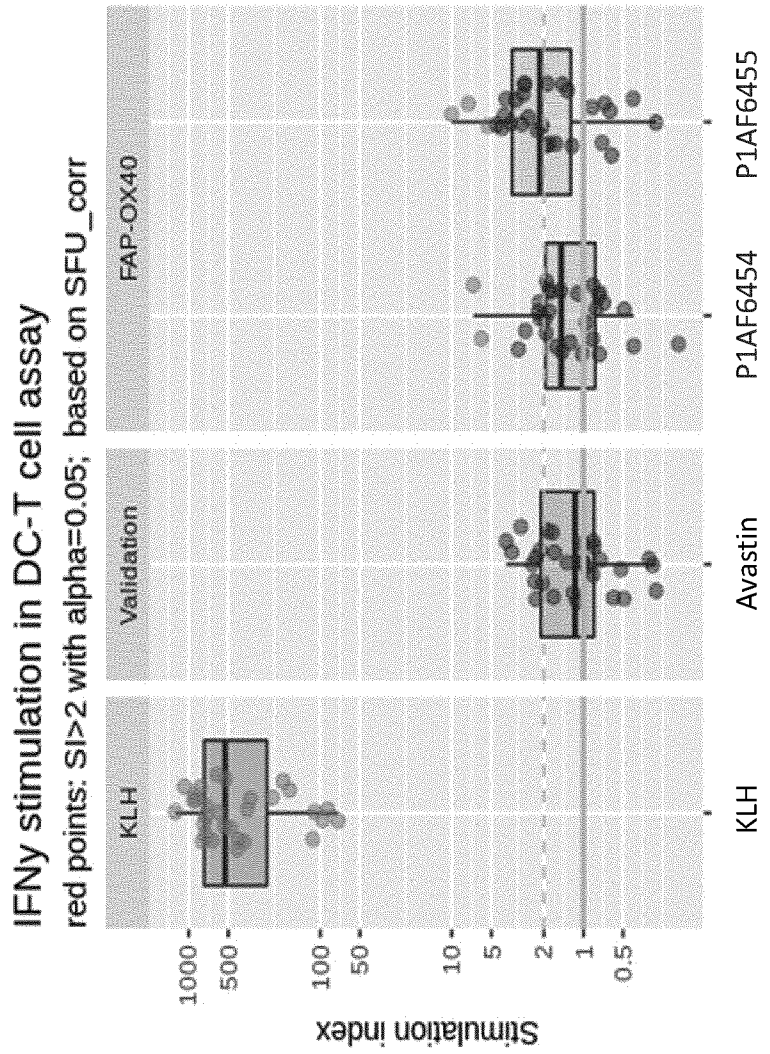

IFNy stimulation in DC-T cell assay
red points: SI>2 with alpha=0.05; based on SFU_corr
KLH
Validation
FAP-OX40
Stimulation index
1000
500
100
50
10
5
2
1
0.5
KLH
Avastin
P1AF6454
P1AF6455

Fig. 44B

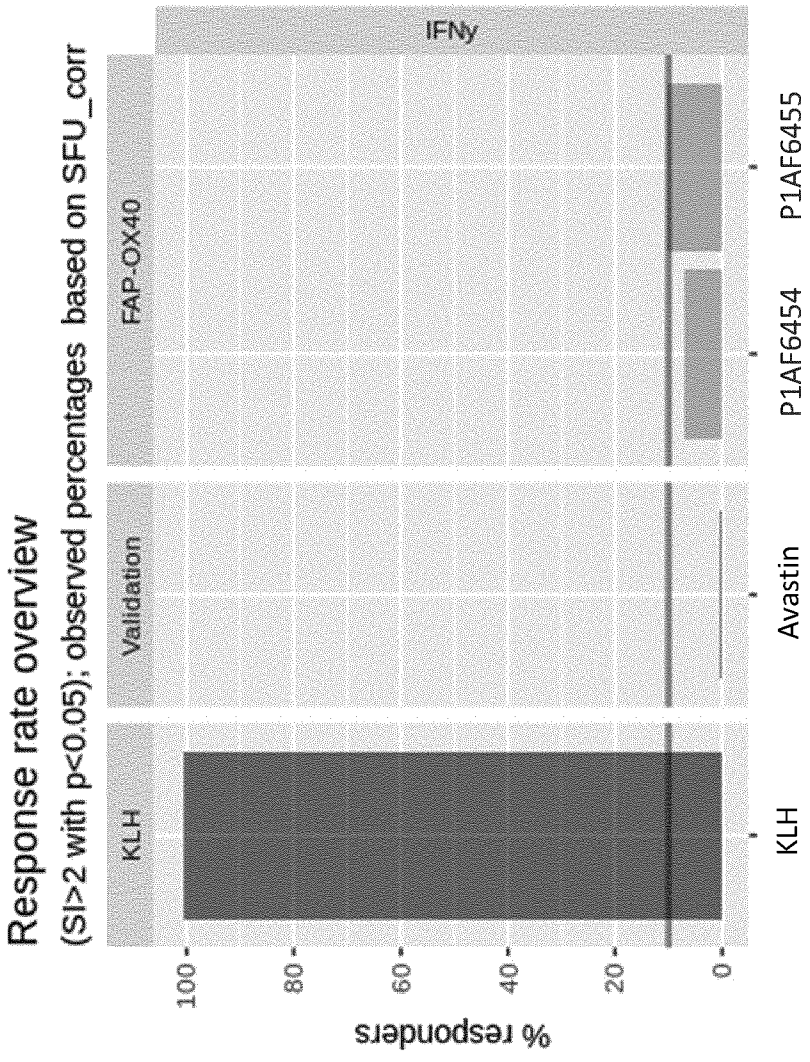

Response rate overview
(SI>2 with p<0.05); observed percentages based on SFU_corr
KLH
Validation
FAP-OX40
IFNγ
% responders
100
80
60
40
20
0
KLH
Avastin
P1AF6454
P1AF6455

Fig. 45A

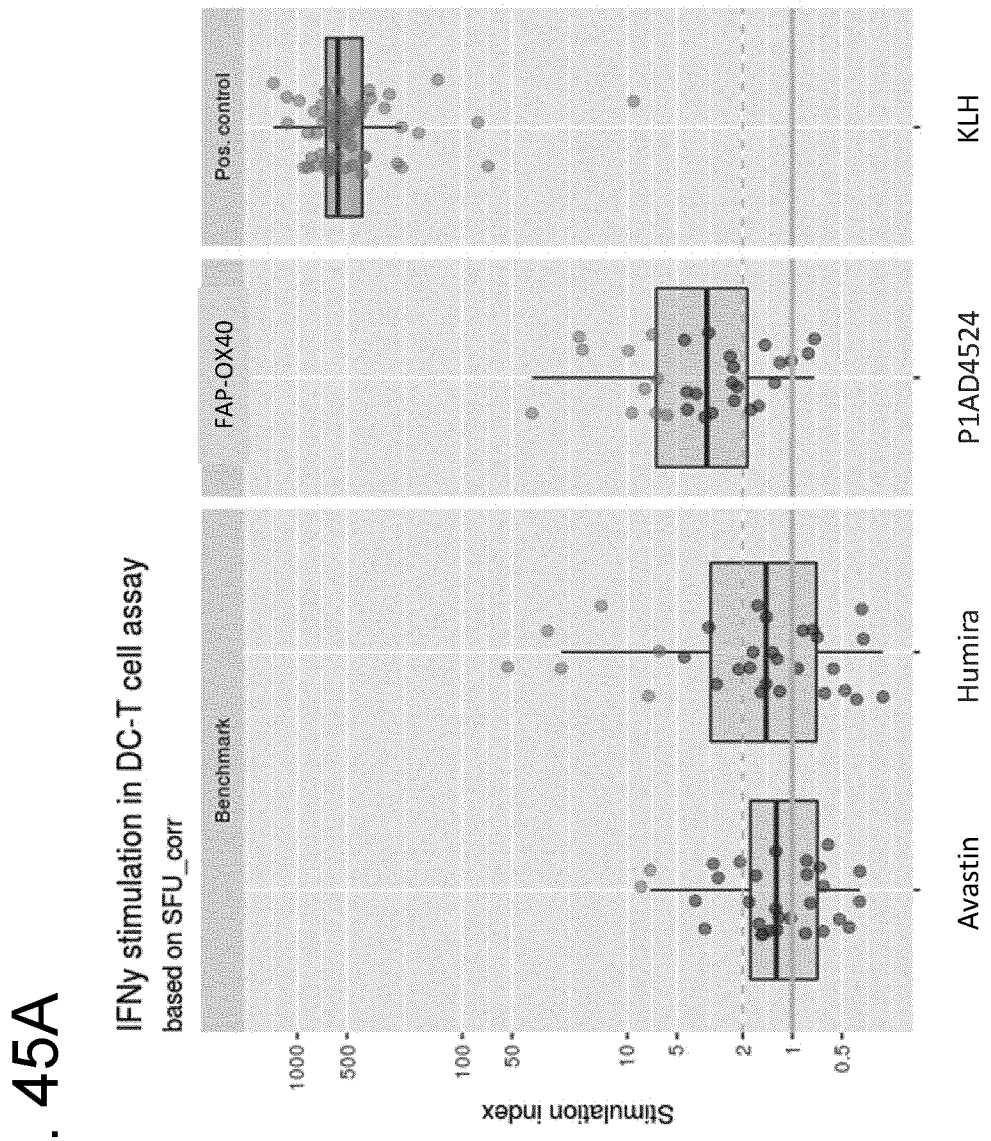

IFNy stimulation in DC-T cell assay
based on SFU_corr
Benchmark
FAP-OX40
Pos. control
Stimulation index
1000
500
100
50
10
5
2
1
0.5
Avastin
Humira
P1AD4524
KLH

Fig. 45B

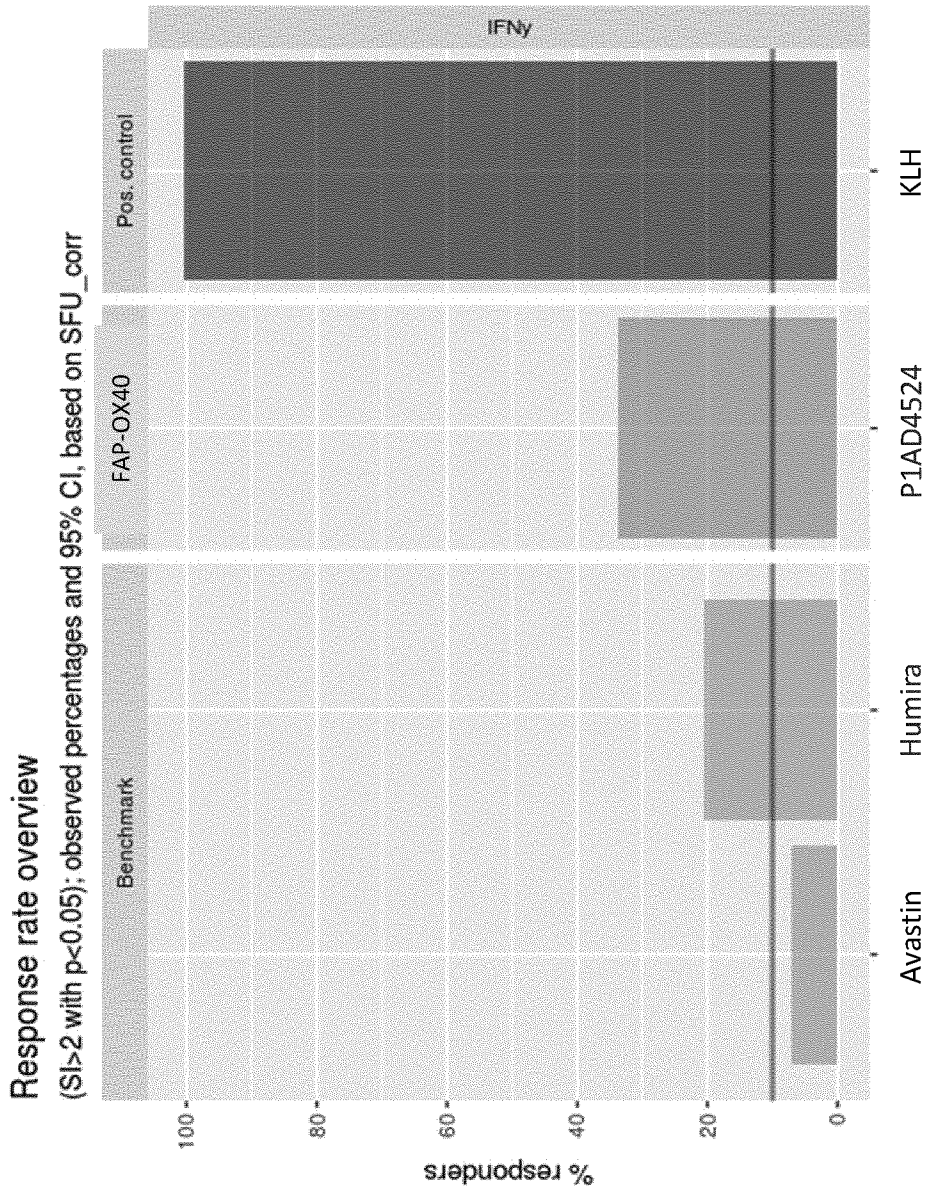

Response rate overview
(SI>2 with p<0.05); observed percentages and 95% CI, based on SFU_corr
Benchmark
FAP-OX40
Pos. control
IFNγ
% responders
100
80
60
40
20
0
Avastin
Humira
P1AD4524
KLH

Fig. 46

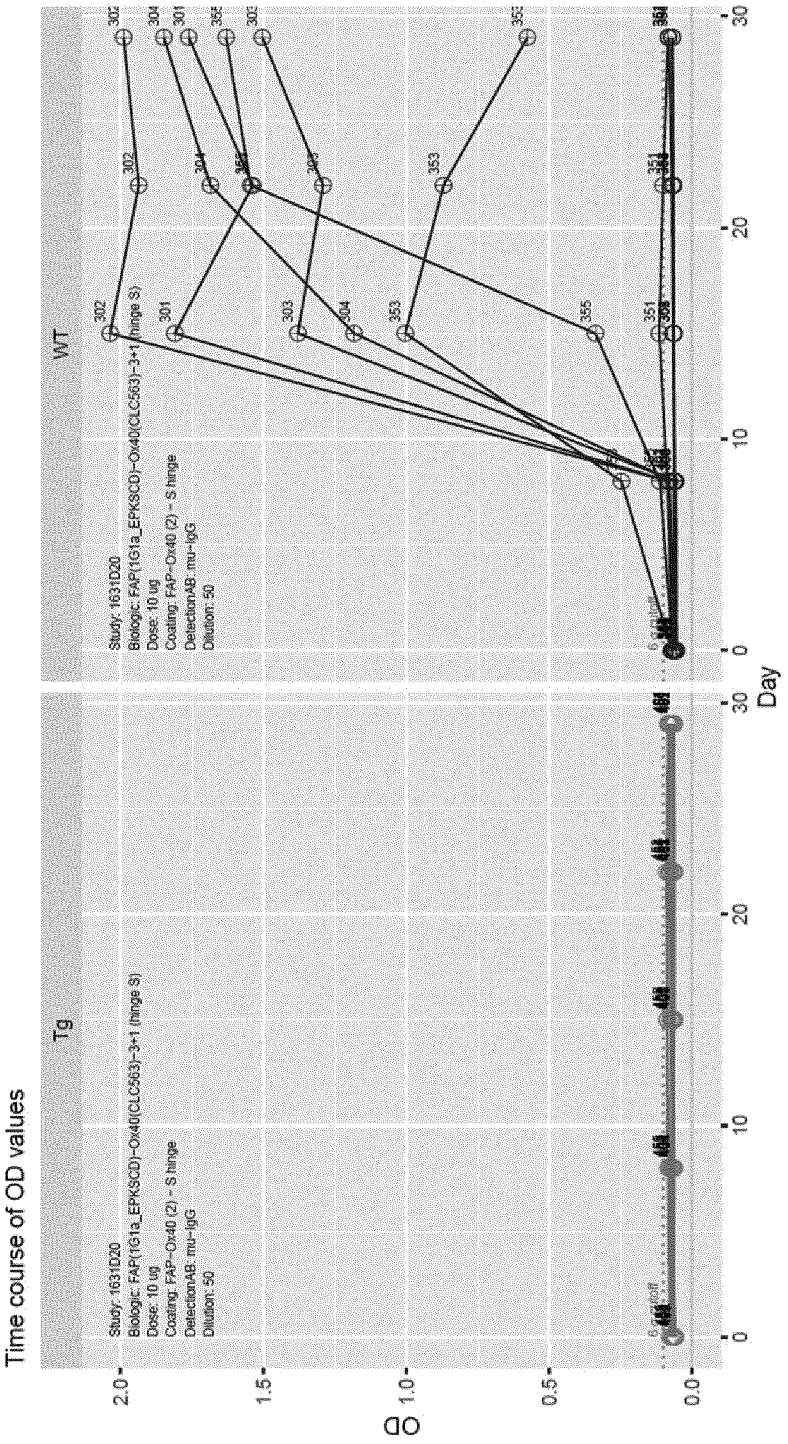

Time course of OD values
Tg
WT
Study: 1631D20
Biologic: FAP(1G1a_EPKSCD)–Ox40(CLC563)–3+1 (hinge S)
Dose: 10 ug
Coating: FAP–Ox40 (2) – S hinge
DetectionAB: mu–IgG
Dilution: 50
OD
Day
Genotype
Tg
WT
Responder
Neg
Pos

Fig. 47

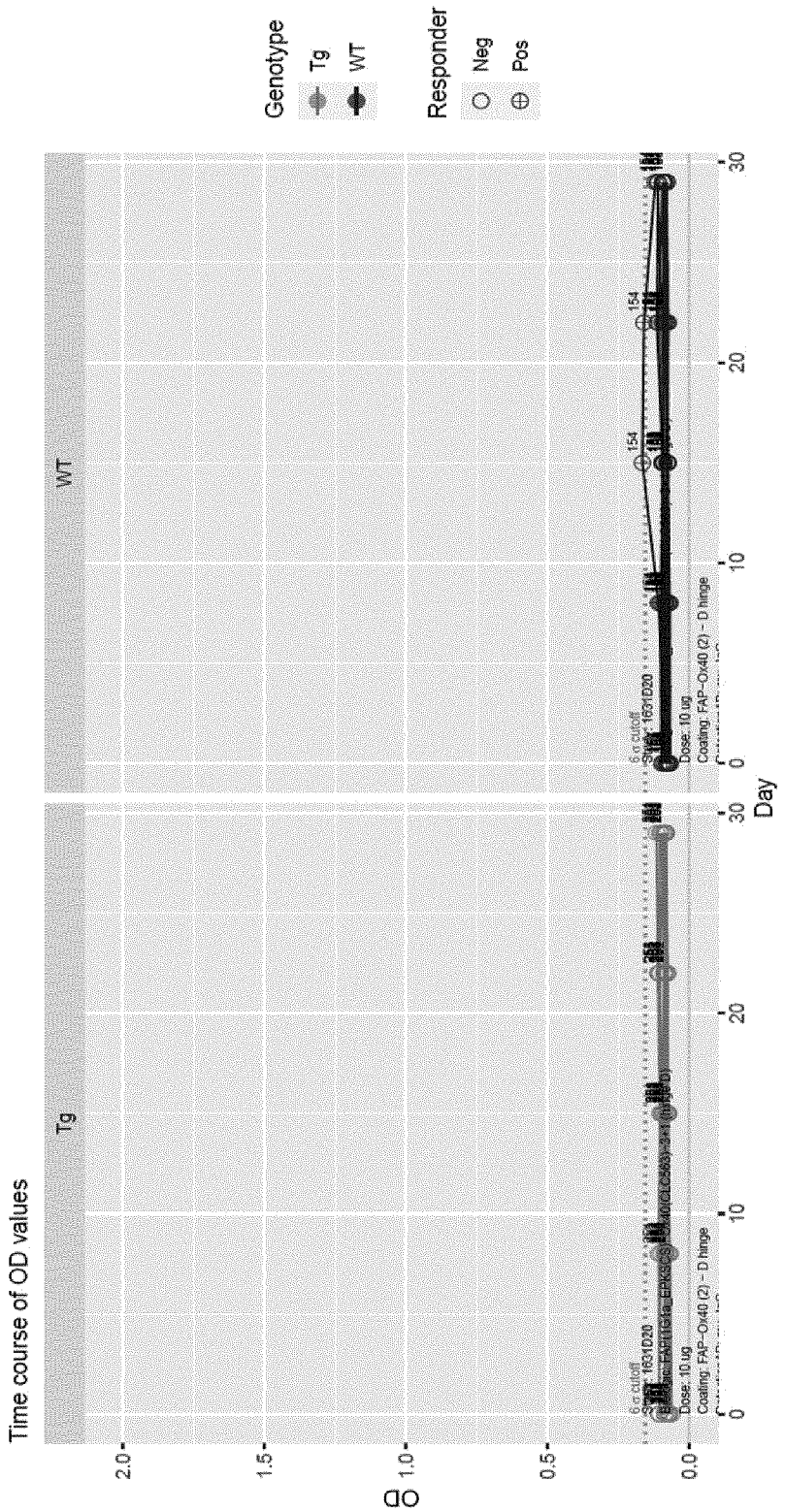

Time course of OD values
Tg
WT
Genotype
Tg
WT
Responder
Neg
Pos
OD
Day
0.0
0.5
1.0
1.5
2.0
0
10
20
30
6 x cutoff
Dose: 10 ug
Coating: FAP-Ox40 (2) - D hinge

Fig. 48

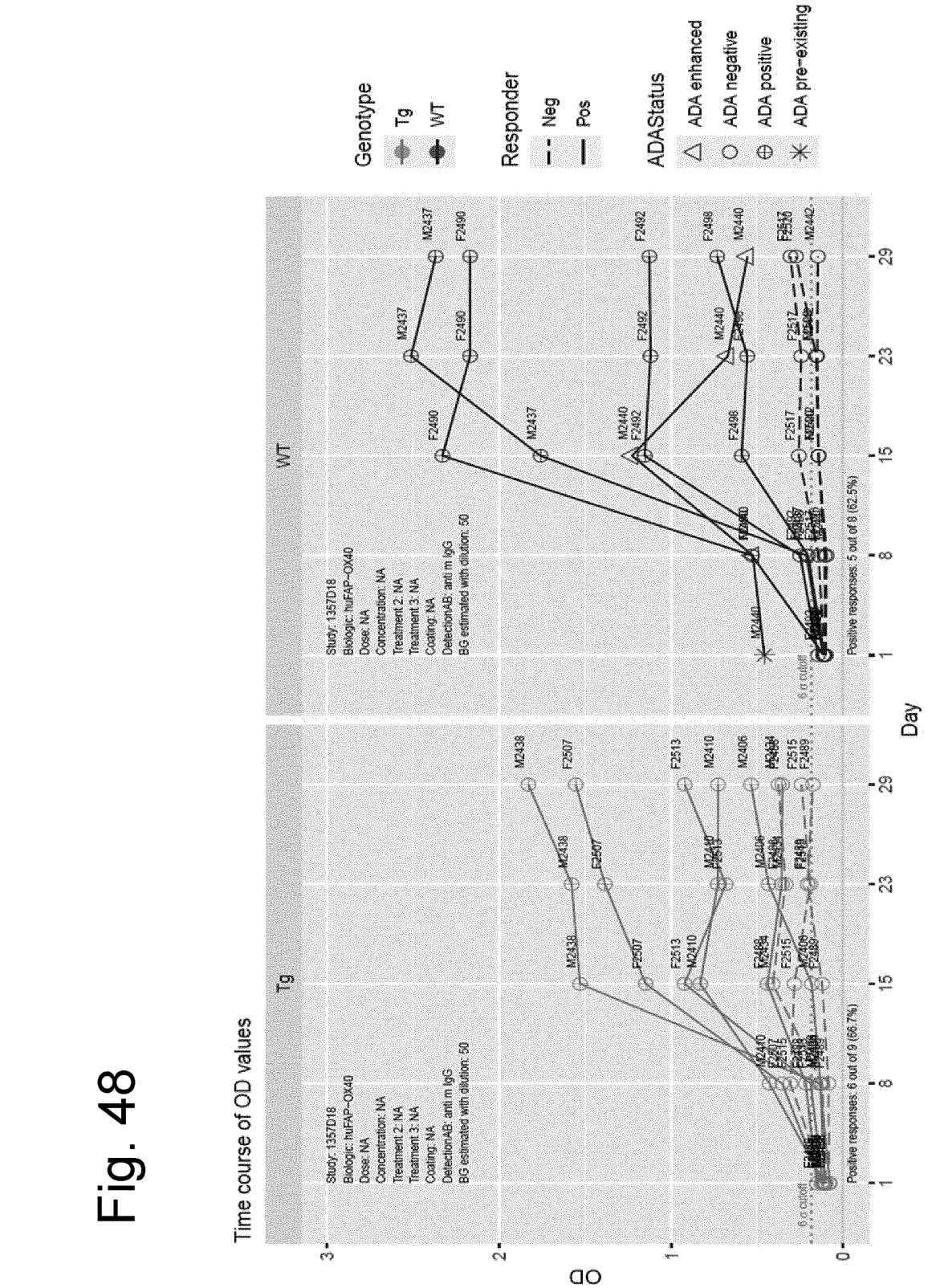
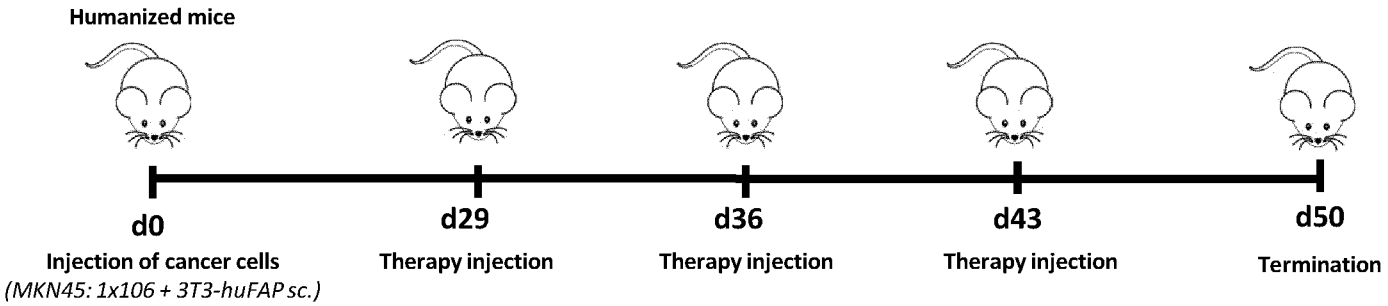
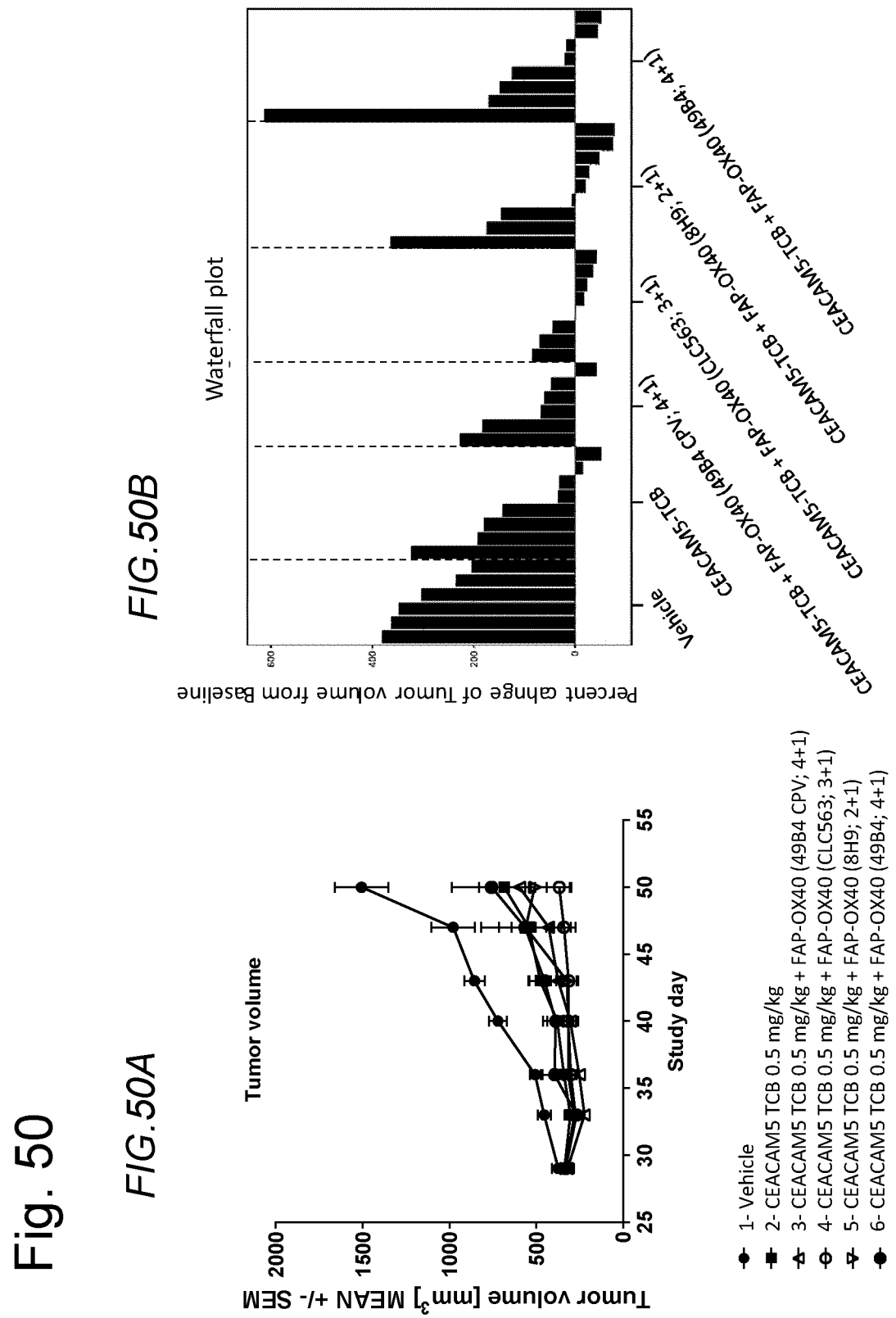

Time course of OD values
Tg
WT
Study: 1357D18
Biologic: huFAP-OX40
Dose: NA
Concentration: NA
Treatment 2: NA
Treatment 3: NA
Coating: NA
DetectionAB: anti m IgG
BG estimated with dilution: 50
6 σ cutoff
Positive responses: 6 out of 9 (66.7%)
Positive responses: 5 out of 8 (62.5%)
OD
Day
Genotype
Tg
WT
Responder
Neg
Pos
ADAStatus
ADA enhanced
ADA negative
ADA positive
ADA pre-existing

Fig. 49



Group	No. of animals	Compound	Dose (mg/kg)	Therapy	No. of treatments
1	9	Vehicle	–	i.v.	3 (once weekly)
2	9	CEACAM5-TCB	0.5	i.v.	3 (once weekly)
3	9	CEACAM5-TCB + FAP-OX40 (49B4 CPV; 4+1)	0.5 13.3	i.v.	3 (once weekly)
4	9	CEACAM5-TCB + FAP-OX40 (CLC563; 3+1)	0.44 12	i.v.	3 (once weekly)
5	9	CEACAM5-TCB + FAP-OX40 (8H9; 2+1)	0.5 9	i.v.	3 (once weekly)
6	9	CEACAM5-TCB + FAP-OX40 (49B4)	0.5 12	i.v.	3 (once weekly)

Fig. 50


FIG.50A
Tumor volume
Tumor volume [mm3] MEAN +/- SEM
Study day
1- Vehicle
2- CEACAM5 TCB 0.5 mg/kg
3- CEACAM5 TCB 0.5 mg/kg + FAP-OX40 (49B4 CPV; 4+1)
4- CEACAM5 TCB 0.5 mg/kg + FAP-OX40 (CLC563; 3+1)
5- CEACAM5 TCB 0.5 mg/kg + FAP-OX40 (8H9; 2+1)
6- CEACAM5 TCB 0.5 mg/kg + FAP-OX40 (49B4; 4+1)
FIG.50B
Waterfall plot
Percent change of Tumor volume from Baseline
Vehicle
CEACAM5-TCB
CEACAM5-TCB + FAP-OX40 (49B4 CPV; 4+1)
CEACAM5-TCB + FAP-OX40 (CLC563; 3+1)
CEACAM5-TCB + FAP-OX40 (8H9; 2+1)
CEACAM5-TCB + FAP-OX40 (49B4; 4+1)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference P36026-WO	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/EP2021/058435	International filing date *(day/month/year)* 31 March 2021 (31-03-2021)	(Earliest) Priority Date *(day/month/year)* 1 April 2020 (01-04-2020)
Applicant F. HOFFMANN-LA ROCHE AG		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of:
      [X] the international application in the language in which it was filed
      [ ] a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. [X] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box No. II)

3. [ ] **Unity of invention is lacking** (see Box No III)

4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      [ ] as suggested by the applicant
      [ ] as selected by this Authority, because the applicant failed to suggest a figure
      [ ] as selected by this Authority, because this figure better characterizes the invention
   b. [X] none of the figures is to be published with the abstract

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2021/058435

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2021/058435

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 C07K16/28 C07K16/40 A61K38/17
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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2019/086497 A2 (HOFFMANN LA ROCHE [CH]; HOFFMANN LA ROCHE [US]) 9 May 2019 (2019-05-09) SeqIdNo.48 and 49; page 66, line 32 - page 67, line 20; claims 6,10,11,31-38; figure 1a page 72, line 3 - line 8	1-30
X	WO 2019/086500 A2 (HOFFMANN LA ROCHE [CH]; HOFFMANN LA ROCHE [US]) 9 May 2019 (2019-05-09) SeqIdNo.48 and 49; claims 9,12,13,25-33; figures 1E-1P	1-30

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 June 2021	21/06/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Lonnoy, Olivier

1

Form PCT/ISA/210 (second sheet) (April 2005)

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 2017/060144 A1 (F HOFFMANN-LA ROCHE AG [CH]; HOFFMANN-LA ROCHE INC [US]) 13 April 2017 (2017-04-13) cited in the application SeqIdNo.33 and 34; page 13, line 6 - line 9; claims 30-41; figure 12A	1-30
A,P	WO 2020/070041 A1 (HOFFMANN LA ROCHE [CH]; HOFFMANN LA ROCHE [US]) 9 April 2020 (2020-04-09) the whole document	1-30
A,P	WO 2020/070035 A1 (HOFFMANN LA ROCHE [CH]; HOFFMANN LA ROCHE [US]) 9 April 2020 (2020-04-09) the whole document	1-30

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2021/058435

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2019086497 A2	09-05-2019	AU 2018359506 A1	23-04-2020
		BR 112020007630 A2	17-11-2020
		CA 3079036 A1	09-05-2019
		CN 111315781 A	19-06-2020
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		JP 2021501162 A	14-01-2021
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		WO 2019086497 A2	09-05-2019
WO 2019086500 A2	09-05-2019	AU 2018357923 A1	05-03-2020
		BR 112020006443 A2	29-09-2020
		CA 3076027 A1	09-05-2019
		CL 2020000662 A1	02-10-2020
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		CR 20200171 A	14-06-2020
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		TW 201922791 A	16-06-2019
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		ZA 202001139 B	31-03-2021
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		AU 2016334623 A1	15-02-2018
		BR 112018002128 A2	11-09-2018
		CA 2992900 A1	13-04-2017
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		CN 108271377 A	10-07-2018
		CO 2018000967 A2	19-04-2018
		CR 20180171 A	03-05-2018
		EP 3359568 A1	15-08-2018
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		KR 20180054877 A	24-05-2018
		PE 20181349 A1	22-08-2018
		PH 12018500766 A1	01-10-2018
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		US 2017247467 A1	31-08-2017
		US 2019211113 A1	11-07-2019
		WO 2017060144 A1	13-04-2017
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		CA 3113548 A1	09-04-2020
		CO 2021003900 A2	19-04-2021
		CR 20210154 A	21-04-2021
		TW 202028240 A	01-08-2020
		US 2020190207 A1	18-06-2020
		WO 2020070041 A1	09-04-2020
WO 2020070035 A1	09-04-2020	CN 112654641 A	13-04-2021
		TW 202028241 A	01-08-2020
		WO 2020070035 A1	09-04-2020