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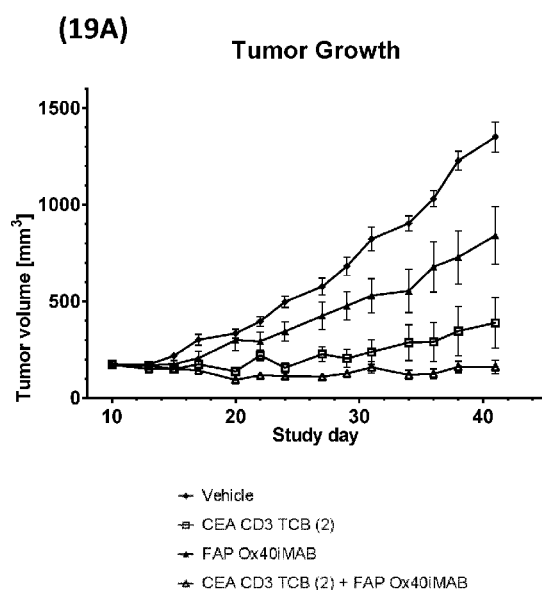
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## (54) Title: COMBINATION THERAPY WITH TARGETED OX40 AGONISTS

Fig. 19



(57) Abstract: The present invention relates to combination therapies employing tumor targeted bispecific OX40 antibodies, in particular anti-FAP/anti-OX40 antibodies in combination with T-cell activating anti-CD3 bispecific antibodies specific for a tumor-associated antigen, the use of these combination therapies for the treatment of cancer and methods of using the combination therapies.

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## Combination therapy with targeted OX40 agonists

### FIELD OF THE INVENTION

The present invention relates to combination therapies employing tumor targeted anti-CD3 bispecific antibodies and targeted OX40 agonists, in particular bispecific OX40 antibodies comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, the use of these combination therapies for the treatment of cancer and methods of using the combination therapies. Included are also combination therapies employing OX40 agonists comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen with a tumor targeted anti-CD3 bispecific antibody and with an agent blocking PD-L1/PD-1 interaction, in particular a PD-L1 antibody.

### BACKGROUND

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

One way to recruit the patient's own immune system to fight cancer is the use of T cell bispecific antibodies (TCBs). 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. For example, an anti-CEA/anti-CD3 bispecific antibody is a molecule that targets CEA expressed on tumor cells and CD3 epsilon chain (CD3ε) present on T cells. 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. In the presence of CEA positive cancer cells, whether circulating or tissue resident, pharmacologically active doses will trigger T-cell activation and associated cytokine release. Parallel to tumor cell depletion anti-CEA/anti-CD3 bispecific antibody leads to a transient decrease of T cells in the peripheral blood within 24 hours after the first administration and to

a peak in cytokine release, followed by rapid T-cell recovery and return of cytokine levels to baseline within 72 hours. Thus, in order to achieve complete elimination of tumor cells, there is a need of an additional agent that conserves T-cell activation and immune response to cancer cells.

5           Triggering of the TCR increases, depending on the strength and duration of this primary stimulus, the expression of costimulatory molecules, e.g. OX40, which is 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 (IFN- $\gamma$ , IL-2, TNF-  
10    $\alpha$ ) while it inhibits suppressive mechanisms, e.g. expression of FoxP3 and secretion of IL-10 (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  
15 first attack allowing for protective memory formation.

          However, the immune suppressive microenvironment in certain tumors is high in coinhibitory signals, e.g. PD-L1, but lacks sufficient expression of OX40 ligand. Persistent priming of T cells in this context can result in attenuation of T cell activation, exhaustion and evasion of immune surveillance (Sharpe et al., Nat Rev 2002) (Keir ME et al., 2008 Annu.  
20 Rev. Immunol. 26:677).

          One means to restore OX40 costimulation specifically in the tumor microenvironment, are bispecific antibodies comprised of at least one antigen binding domain for a tumor associated antigen, for example fibroblast activating protein (FAP) in the tumor stroma, and at least one antigen binding domain for OX40. For example, such bispecific antibodies have  
25 been described in WO 2017/055398 A2 and WO 2017/060144 A1. Crosslinking and surface immobilization of such bispecific molecules by cell surface FAP creates a highly agonistic matrix for OX40 positive T cells, where it supports NF $\kappa$ B mediated effector functions and can replace ligation by OX40 Ligand. High FAP expression is reported for a plethora of human tumor indications, either on tumor cells themselves or on immune suppressive cancer  
30 associated fibroblasts (CAFs).

          In certain patients with a strong immunosuppressed 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  
35 microenvironment towards a more immune-activating and less immune-suppressive 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.

5 In the present patent application *in vitro* and *in vivo* data for the combination of TCBs (anti-CEA/anti-CD3 bispecific antibodies and anti-FolR/anti-CD3 bispecific antibodies) with bispecific anti-FAP/anti-OX40 antibodies are provided which support the rationale of combining T cell recruiters with a tumor targeted OX40 agonist to improve the quantity and quality of an anti-tumor response.

## 10 SUMMARY OF THE INVENTION

The present invention relates to bispecific OX40 antibodies comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular anti-Fibroblast activation protein (FAP)/anti-OX40 bispecific antibodies and their use in combination with T-cell activating anti-CD3 bispecific antibodies specific for a tumor-associated antigen, in particular to their use in a method for treating or delaying progression of cancer, more particularly for treating or delaying progression of solid tumors. It has been found that the combination therapy described herein is more effective in inhibiting tumor growth and eliminating tumor cells than treatment with the anti-CD3 bispecific antibodies alone.

20 In one aspect, the invention provides a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method for treating or delaying progression of cancer, wherein the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is used in combination with a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen. In one aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method for treating or delaying progression of cancer, wherein the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is used in combination with a T-cell activating anti-CD3 bispecific antibody specific for another tumor-associated antigen. In one aspect, the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is an anti-CEA/anti-CD3 bispecific antibody or an anti-FolR1/anti-CD3 bispecific antibody. Particularly, the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is an anti-CEA/anti-CD3 bispecific antibody.

In a further aspect, the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is for use in a method as described herein before, wherein the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen and the  
5 T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen are administered together in a single composition or administered separately in two or more different compositions.

In another aspect, the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is for use in a method as  
10 described herein before, wherein the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen acts synergistically with the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen.

In another aspect, provided is a bispecific OX40 antibody comprising at least one  
15 antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method for treating or delaying progression of cancer, wherein the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is administered concurrently with, prior to, or subsequently to the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen.

20 In particular, the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is an anti-Fibroblast activation protein (FAP)/anti-OX40 bispecific antibody. In one aspect, the anti-FAP/anti-OX40 antibody is an OX40 agonist. In one aspect, the anti-FAP/anti-OX40 antibody is an antigen binding molecule comprising a Fc domain. In a particular aspect, the anti-FAP/anti-  
25 OX40 antibody is an antigen binding molecule comprising a Fc domain with modifications reducing Fc $\gamma$  receptor binding and/or effector function. The crosslinking by a tumor associated antigen makes it possible to avoid unspecific Fc $\gamma$ R-mediated crosslinking and thus higher and more efficacious doses of the anti-FAP/anti-OX40 antibody may be administered in comparison to common OX40 antibodies.

30 In one aspect, the invention provides a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the bispecific OX40 antibody is used in combination with a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen  
35 and wherein the bispecific OX40 antibody comprises at least one antigen binding domain

capable of specific binding to FAP comprising

(a) a heavy chain variable region (V<sub>H</sub>FAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:1, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:3, and a light chain variable region (V<sub>L</sub>FAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:4, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:5, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:6, or

(b) a heavy chain variable region (V<sub>H</sub>FAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:9, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:10, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:11, and a light chain variable region (V<sub>L</sub>FAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:12, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:13, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:14.

In a further aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as defined herein before, wherein the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to FAP comprising a heavy chain variable region (V<sub>H</sub>FAP) comprising an amino acid sequence of SEQ ID NO:7 and a light chain variable region (V<sub>L</sub>FAP) comprising an amino acid sequence of SEQ ID NO:8 or an antigen binding domain capable of specific binding to FAP comprising a heavy chain variable region (V<sub>H</sub>FAP) comprising an amino acid sequence of SEQ ID NO:15 and a light chain variable region (V<sub>L</sub>FAP) comprising an amino acid sequence of SEQ ID NO:16. In a particular aspect, the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to FAP comprising a heavy chain variable region (V<sub>H</sub>FAP) comprising an amino acid sequence of SEQ ID NO:7 and a light chain variable region (V<sub>L</sub>FAP) comprising an amino acid sequence of SEQ ID NO:8. In another aspect, the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to FAP comprising a heavy chain variable region (V<sub>H</sub>FAP) comprising an amino acid sequence of SEQ ID NO:15 and a light chain variable region (V<sub>L</sub>FAP) comprising an amino acid sequence of SEQ ID NO:16.

In a further aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as defined herein before, wherein the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to OX40 comprising

[illegible]

ID NO:20, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:27, and a light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:30, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:33, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:39.

- 5           More particularly, the the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to OX40 comprising
- (a) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:22, and a
- 10 light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:35.

- In a further aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in
- 15 particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to OX40 comprising
- (a) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:40 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of
- 20 SEQ ID NO:41, or
- (b) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:42 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:43, or
- (c) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID
- 25 NO:44 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:45, or
- (d) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:46 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:47, or
- 30 (a) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:48 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:49, or
- (a) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID
- 35 NO:50 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:51, or
- (a) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID

NO:52 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:53.

In a particular aspect, the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to OX40 comprising

- 5 (a) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:40 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:41.

In one aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an  
10 anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is an antigen binding molecule further comprising a Fc domain composed of a first and a second subunit capable of stable association. In particular, the bispecific OX40 antibody is an antigen binding  
15 molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain. More particularly, the bispecific OX40 antibody is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function. In a particular aspect, the bispecific OX40 antibody comprises an IgG1 Fc domain comprising the amino acid substitutions L234A,  
20 L235A and P329G.

In another aspect of the invention, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the bispecific OX40  
25 antibody comprises monovalent binding to a tumor associated target and and at least bivalent binding to OX40. In one aspect, the anti-FAP/anti-OX40 bispecific antibody comprises monovalent binding to a tumor associated target and and bivalent binding to OX40. In a particular aspect, the anti-FAP/anti-OX40 bispecific antibody comprises monovalent binding to a tumor associated target and and tetravalent binding to OX40.

30 In another aspect, the invention provides a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the bispecific OX40 antibody comprises a first Fab fragment capable of specific binding to OX40 fused at the C-  
35 terminus of the CH1 domain to the VH domain of a second Fab fragment capable of specific

binding to OX40 and a third Fab fragment capable of specific binding to OX40 fused at the C-terminus of the CH1 domain to the VH domain of a fourth Fab fragment capable of specific binding to OX40.

In one aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the bispecific OX40 antibody comprises

- (i) a first heavy chain comprising an amino acid sequence of SEQ ID NO:54, a second heavy chain comprising an amino acid sequence of SEQ ID NO:55, and four light chains comprising an amino acid sequence of SEQ ID NO:56, or
- (ii) a first heavy chain comprising an amino acid sequence of SEQ ID NO:57, a second heavy chain comprising an amino acid sequence of SEQ ID NO:58, and four light chains comprising an amino acid sequence of SEQ ID NO:56, or
- (i) a first heavy chain comprising an amino acid sequence of SEQ ID NO:59, a second heavy chain comprising an amino acid sequence of SEQ ID NO:60, and four light chains comprising an amino acid sequence of SEQ ID NO:56, or
- (ii) a first heavy chain comprising an amino acid sequence of SEQ ID NO:61, a second heavy chain comprising an amino acid sequence of SEQ ID NO:62, and four light chains comprising an amino acid sequence of SEQ ID NO:56.

In another aspect, the invention provides a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the bispecific OX40 antibody is used in combination with a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen and wherein the T-cell activating anti-CD3 bispecific antibody is an anti-CEA/anti-CD3 bispecific antibody.

In one aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region ( $V_{HCD3}$ ) and a light chain variable region ( $V_{LCD3}$ ), and a second antigen binding domain comprising a heavy chain variable region ( $V_{HCEA}$ ) and a light chain variable region ( $V_{LCEA}$ ).

In another aspect, the invention provides a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CD3) comprising CDR-H1 sequence of SEQ ID NO:63, CDR-H2 sequence of SEQ ID NO:64, and CDR-H3 sequence of SEQ ID NO:65; and/or a light chain variable region (V<sub>L</sub>CD3) comprising CDR-L1 sequence of SEQ ID NO:66, CDR-L2 sequence of SEQ ID NO:67, and CDR-L3 sequence of SEQ ID NO:68.

In a further aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CD3) comprising the amino acid sequence of SEQ ID NO:69 and/or a light chain variable region (V<sub>L</sub>CD3) comprising the amino acid sequence of SEQ ID NO:70. In one aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method for treating or delaying progression of cancer, wherein the T-cell activating anti-CD3 bispecific antibody comprises a second antigen binding domain comprising

(a) a heavy chain variable region (V<sub>H</sub>CEA) comprising CDR-H1 sequence of SEQ ID NO:71, CDR-H2 sequence of SEQ ID NO:72, and CDR-H3 sequence of SEQ ID NO:73, and/or a light chain variable region (V<sub>L</sub>CEA) comprising CDR-L1 sequence of SEQ ID NO:74, CDR-L2 sequence of SEQ ID NO:75, and CDR-L3 sequence of SEQ ID NO:76, or

(b) a heavy chain variable region (V<sub>H</sub>CEA) comprising CDR-H1 sequence of SEQ ID NO:79, CDR-H2 sequence of SEQ ID NO:80, and CDR-H3 sequence of SEQ ID NO:81, and/or a light chain variable region (V<sub>L</sub>CEA) comprising CDR-L1 sequence of SEQ ID NO:82, CDR-L2 sequence of SEQ ID NO:83, and CDR-L3 sequence of SEQ ID NO:84.

In a particular aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the T-cell activating anti-CD3 bispecific antibody comprises a second antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CEA) comprising the amino acid sequence of SEQ ID NO:77 and/or a light chain variable region (V<sub>L</sub>CEA) comprising the amino acid sequence of SEQ ID NO:78 or a second antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CEA)



comprising the amino acid sequence of SEQ ID NO:85 and/or a light chain variable region (V<sub>L</sub>CEA) comprising the amino acid sequence of SEQ ID NO:86.

In another aspect, the invention further provides a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the anti-CEA/anti-CD3 bispecific antibody further comprises a third antigen binding domain that binds to CEA. In particular, the third antigen binding domain comprises (a) a heavy chain variable region (V<sub>H</sub>CEA) comprising CDR-H1 sequence of SEQ ID NO:71, CDR-H2 sequence of SEQ ID NO:72, and CDR-H3 sequence of SEQ ID NO:73, and/or a light chain variable region (V<sub>L</sub>CEA) comprising CDR-L1 sequence of SEQ ID NO:74, CDR-L2 sequence of SEQ ID NO:75, and CDR-L3 sequence of SEQ ID NO:76, or (b) a heavy chain variable region (V<sub>H</sub>CEA) comprising CDR-H1 sequence of SEQ ID NO:79, CDR-H2 sequence of SEQ ID NO:80, and CDR-H3 sequence of SEQ ID NO:81, and/or a light chain variable region (V<sub>L</sub>CEA) comprising CDR-L1 sequence of SEQ ID NO:82, CDR-L2 sequence of SEQ ID NO:83, and CDR-L3 sequence of SEQ ID NO:84. More particularly, the third antigen binding domain comprises a heavy chain variable region (V<sub>H</sub>CEA) comprising the amino acid sequence of SEQ ID NO:77 and/or a light chain variable region (V<sub>L</sub>CEA) comprising the amino acid sequence of SEQ ID NO:78 or wherein the second antigen binding domain comprises a heavy chain variable region (V<sub>H</sub>CEA) comprising the amino acid sequence of SEQ ID NO:85 and/or a light chain variable region (V<sub>L</sub>CEA) comprising the amino acid sequence of SEQ ID NO:86.

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

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

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

In one aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the anti-CEA/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to CEA. In a further aspect, the anti-CEA/anti-CD3 bispecific antibody comprises a Fc domain composed of a first and a second subunit capable of stable association. In particular, the anti-CEA/anti-CD3 bispecific antibody comprises an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain. More particularly, the anti-CEA/anti-CD3 bispecific antibody comprises a Fc domain that comprises one or more amino acid substitutions that reduce binding to an Fc receptor and/or effector function. In a particular aspect, the anti-CEA/anti-CD3 bispecific antibody comprises an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

In another aspect, the invention provides a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the bispecific OX40 antibody is used in combination with a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen and wherein the T-cell activating anti-CD3 bispecific antibody is an anti-FolR1/anti-CD3 bispecific antibody.

In one aspect, the invention provides a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region ( $V_{HCD3}$ ), a second antigen binding domain comprising a heavy chain variable region ( $V_{HFolR1}$ ) and a common light chain variable region.

In another aspect of the invention, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region ( $V_{HCD3}$ ) comprising CDR-H1 sequence of SEQ ID NO:95, CDR-H2

sequence of SEQ ID NO:96, and CDR-H3 sequence of SEQ ID NO:97; a second antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>FoIR1) comprising CDR-H1 sequence of SEQ ID NO:98, CDR-H2 sequence of SEQ ID NO:99, and CDR-H3 sequence of SEQ ID NO:100; and a common light chain comprising a CDR-L1 sequence of SEQ ID NO:101, CDR-L2 sequence of SEQ ID NO:102, and CDR-L3 sequence of SEQ ID NO:103.

In a further aspect, the invention provides a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CD3) comprising the sequence of SEQ ID NO:104 and a second antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>FoIR1) comprising the sequence of SEQ ID NO:105; and wherein the common light chain comprises the sequence of SEQ ID NO:106.

In one aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the anti-FoIR1/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to FoIR1.

In a further aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the anti-FoIR1/anti-CD3 bispecific antibody comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO:107, a second heavy chain comprising the amino acid sequence of SEQ ID NO:108 and a common light chain of SEQ ID NO: 109.

In another aspect, the invention provides a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the bispecific OX40 antibody is used in combination with a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen and wherein the combination is administered at intervals from about one week to three weeks.

In yet another aspect, the invention provides a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in

particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the bispecific OX40 antibody is used in combination with a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen and in combination with an agent blocking PD-L1/PD-1 interaction. In particular, the agent  
5 blocking PD-L1/PD-1 interaction is an anti-PD-L1 antibody or an anti-PD1 antibody. More particularly, the agent blocking PD-L1/PD-1 interaction is selected from the group consisting of atezolizumab, durvalumab, pembrolizumab and nivolumab. In a specific aspect, the agent blocking PD-L1/PD-1 interaction is atezolizumab.

In a further aspect, the invention provides a pharmaceutical product comprising (A) a  
10 first composition comprising as active ingredient a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, and a pharmaceutically acceptable excipient; and (B) a second composition comprising as active ingredient a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-  
15 CEA/anti-CD3 bispecific antibody or anti-FolR1/anti-CD3 bispecific antibody, and a pharmaceutically acceptable excipient, for use in the combined, sequential or simultaneous treatment of a disease, in particular for the treatment of cancer.

In another aspect, provided is a pharmaceutical composition comprising a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to  
20 a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, and a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody or anti-FolR1/anti-CD3 bispecific antibody. In one aspect, the pharmaceutical composition further comprises blocking PD-L1/PD-1 interaction. In particular, the agent blocking PD-L1/PD-1 interaction is an anti-PD-  
25 L1 antibody or an anti-PD1 antibody. More particularly, the agent blocking PD-L1/PD-1 interaction is selected from the group consisting of atezolizumab, durvalumab, pembrolizumab and nivolumab. In a specific aspect, the agent blocking PD-L1/PD-1 interaction is atezolizumab. In one particular aspect, the pharmaceutical composition is for use in the treatment of solid tumors.

In an additional aspect, the invention provides a kit for treating or delaying progression of cancer in a subject, comprising a package comprising (A) a first composition comprising as  
30 active ingredient a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, and a pharmaceutically acceptable excipient; (B) a second  
35 composition comprising as active ingredient a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific

antibody or anti-FolR1/anti-CD3 bispecific antibody, and a pharmaceutically acceptable excipient, and (C) instructions for using the compositions in a combination therapy. In one aspect, provided is a kit for treating or delaying progression of cancer in a subject, comprising a package comprising (A) a first composition comprising as active ingredient a bispecific  
5 OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, and a pharmaceutically acceptable excipient; (B) a second composition comprising as active ingredient a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody or anti-FolR1/anti-CD3  
10 bispecific antibody, and a pharmaceutically acceptable excipient, (c) a third composition comprising as active ingredient an agent blocking PD-L1/PD-1 interaction, in particular atezolizumab, and a pharmaceutically acceptable excipient, and (C) instructions for using the compositions in a combination therapy.

In a further aspect, the invention relates to the use of a combination of a bispecific  
15 OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, and a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody or anti-FolR1/anti-CD3 bispecific antibody, in the manufacture of a medicament for treating or delaying progression of a  
20 proliferative disease, in particular cancer.

In particular, provided is the use of a combination of a T bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, and a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular  
25 an anti-CEA/anti-CD3 bispecific antibody or anti-FolR1/anti-CD3 bispecific antibody in the manufacture of a medicament for treating a disease selected from the group consisting of colon cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, breast cancer, kidney cancer, esophageal cancer, or prostate cancer.

In another aspect, the invention provides a method for treating or delaying progression  
30 of cancer in a subject comprising administering to the subject an effective amount of a T bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, and a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody or anti-FolR1/anti-CD3  
35 bispecific antibody. In another aspect, provided is a method for treating or delaying progression of cancer in a subject comprising administering to the subject an effective amount

of a T bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody or anti-FolR1/anti-CD3  
 5 bispecific antibody, and an agent blocking PD-L1/PD-1 interaction, in particular an anti-PD-L1 antibody or an anti-PD1 antibody.

In a further aspect, provided is an anti-FAP/anti-OX40 bispecific antibody for use in a method for treating or delaying progression of cancer, wherein the anti-FAP/anti-OX40 bispecific antibody is used in combination with an agent blocking PD-L1/PD-1 interaction. In  
 10 particular, the agent blocking PD-L1/PD-1 interaction is an anti-PD-L1 antibody or an anti-PD1 antibody. More particularly, the agent blocking PD-L1/PD-1 interaction is selected from the group consisting of atezolizumab, durvalumab, pembrolizumab and nivolumab. In a specific aspect, the agent blocking PD-L1/PD-1 interaction is atezolizumab.

### BRIEF DESCRIPTION OF THE DRAWINGS

15 **Figures 1A and 1B** show a particular anti-FAP/anti-OX40 bispecific antibody and a particular anti-CEA/anti-CD3 bispecific antibody, respectively, as used in the Examples. These molecules are described in more detail in Examples 1 and 2, respectively. The thick black point stands for the knob-into-hole modification. \* symbolizes amino acid  
 20 modifications in the CH1 and CL domain (so-called charged residues). **Figure 1A** shows a particular anti-FAP/anti-OX40 bispecific antibody with tetravalent binding to OX40 and monovalent binding to FAP (4+1 format, FAP VH and VL fused to the C-termini of the Fc domain). The molecule is called herein FAP OX40 iMab. In **Figure 1B** an exemplary bispecific anti-CEA/anti-CD3 antibody in 2+1 format is shown (named CEACAM5 CD3 TCB). Another anti-CEA/anti-CD3 antibody in 2+1 format (called CEA CD3 TCB) is shown  
 25 in **Figure 1C**.

In **Figure 2** TCB mediated lysis of MKN45 NucLight red tumor cells by various human immune cell preparations is shown (Example 3). Different human immune effector cell preparations (resting PBMC, CD4 or CD8 T cells) were cocultured with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of  
 30 CEACAM5 CD3 TCB (CEA CD3 TCB (2)) for 48 hours. The amount of living tumor cells was quantified by fluorescence microscopy high content life imaging using the Incucyte Zoom System (Essenbioscience, HD phase-contrast, green fluorescence and red fluorescence, 10x objective) in a 3 hours interval for 48 hours at 37 °C and 5% CO<sub>2</sub>. The integrated red fluorescence of healthy tumor cells (RCU x  $\mu\text{m}^2/\text{image}$ ) of triplicates (median) was used to

calculate the specific lysis which was plotted against the used TCB concentration to show the cytolytic potential of T cells.

**Figures 3A-3D** show the expression of OX40 on T cells upon TCB stimulation. Different human immune effector cell preparations (resting PBMC, CD4 or CD8 T cells) were cocultured with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEACAM5 CD3 TCB (CEA CD3 TCB (2)) for 48 hours. The expression of OX40 was determined on CD4<sup>+</sup> and CD8<sup>+</sup> T cells by flow cytometry. The percentage of positive cells (**Figures 3A and 3C**) and MFI (**Figures 3B and 3D**) of triplicates (median) was plotted against the used TCB concentration for CD4 positive T cells (**Figures 3A and 3B**) and CD8 positive (**Figures 3C and 3D**) T cells. Error bars indicate the SEM. TCB mediated a dose dependent cell surface expression of OX40 on CD4<sup>+</sup> T cells and on CD8<sup>+</sup> T cell, albeit to a higher extent on CD4<sup>+</sup> T cells.

In **Figures 4A-4C** it is shown that OX40 costimulation did not influence the cytolytic potential of FolR1 CD3 TCB. Resting CD4 T cells were cocultured for 48 hrs with HeLa NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of FolR1 CD3 TCB with (Figure 4B) or without a fixed concentration of FAP OX40 iMAB (Figure 4A). The amount of living tumor cells was quantified by fluorescence microscopy high content life imaging using the Incucyte Zoom System (Essenbioscience, HD phase-contrast, green fluorescence and red fluorescence, 10x objective) in a 3 hours interval for 42 hrs at 37 °C and 5% CO<sub>2</sub>. The integrated red fluorescence of healthy tumor cells (RCU x μm<sup>2</sup>/image) of triplicates (median) was plotted against the used TCB concentration for various time points to show the cytolytic potential of T cells. Error bars indicate the SEM. The Area under the curve for each timepoint was calculated as measure for cytotoxicity and plotted against the time point. For comparison of the AUC values for both FolR1 CD3 TCB alone and in combination with FAP OX40 iMAB were plotted against the time in Figure 4C showing that the addition of FAP OX40 iMab had no influence on the the cytolytic potential of FolR1 CD3 TCB.

**Figures 5A-5C** show that OX40 costimulation did not influence the cytolytic potential of CEACAM5 CD3 TCB (CEA CD3 TCB (2)). Different human immune effector cell preparations (resting PBMC in **Figure 5C**, CD4 T cells in **Figure 5A** and CD8 T cells in **Figure 5B**) were cocultured for 48 hours with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEACAM5 CD3 TCB with or without a fixed concentration of FAP OX40 iMab. The amount of living tumor cells was quantified by fluorescence microscopy high content life imaging using the Incucyte Zoom System (Essenbioscience, HD phase-contrast, green fluorescence and red fluorescence, 10x objective) in a 3 hours interval for 48 hours at 37 °C and 5% CO<sub>2</sub>. The integrated red

fluorescence of healthy tumor cells ( $\text{RCU} \times \mu\text{m}^2/\text{image}$ ) of triplicates (median) was used to calculate the specific lysis which was plotted against the used TCB concentration to show the cytolytic potential of T cells. Here, the 42 hours timepoint is shown exemplary. Error bars indicate the SEM.

5 In **Figures 6A-6D** it is shown that FAP OX40 iMAB co-stimulation did increase FolR1 CD3 TCB mediated  $\text{TNF-}\alpha$  secretion and was depending on agonistic TCR stimulation. Resting CD4 T cells were cocultured for 48 hrs with irradiated  $\text{TNF-}\alpha$  sensor cells, NIH/3T3 huFAP and HeLa NucLight Red cells in the presence of a serial dilution row of FolR1 CD3 TCB with or without a fixed concentration of FAP OX40 iMAB. The amount of  $\text{TNF-}\alpha$  was  
10 quantified as GFP induction in  $\text{TNF-}\alpha$  sensor cells by fluorescence microscopy high content life imaging using the Incucyte Zoom System (Essenbioscience, HD phase-contrast, green fluorescence and red fluorescence, 10x objective) in a 3 hours interval for 42 hrs at  $37^\circ\text{C}$  and 5%  $\text{CO}_2$ . The integrated green fluorescence of  $\text{TNF-}\alpha$  sensor cells ( $\text{GCU} \times \mu\text{m}^2/\text{image}$ ) of triplicates (median) was plotted against the used TCB concentration to quantify  
15  $\text{TNF}\alpha$  secretion of T cells. Error bars indicate the SEM. The results for FolR1 CD3 TCB are shown in Figure 6A (without costimulation) and Figure 6C (with FAP OX40 iMAB co-stimulation) whereas Figures 6B and 6D show the results with a negative control CD3 TCB.

In **Figures 7A-7D** it is shown that FAP OX40 iMAB costimulation did increase CEA CD3 TCB or CEACAM5 CD3 TCB mediated  $\text{TNF-}\alpha$  secretion. Resting CD4 T cells were  
20 cocultured for 48 hrs with irradiated  $\text{TNF-}\alpha$  sensor cells, NIH/3T3 huFAP and MKN-45 NLR cells in the presence of a serial dilution row of CEA CD3 TCB and CEACAM5 CD3 TCB, respectively, with or without a fixed concentration of FAP OX40 iMAB. The amount of  $\text{TNF-}\alpha$  was quantified as GFP induction in  $\text{TNF-}\alpha$  sensor cells by fluorescence microscopy high content life imaging as described above. The integrated green fluorescence of  $\text{TNF-}\alpha$  sensor  
25 cells ( $\text{GCU} \times \mu\text{m}^2/\text{image}$ ) of triplicates (median) was plotted against the used TCB concentration to quantify  $\text{TNF-}\alpha$  secretion of T cells. Error bars indicate the SEM. The results for CEACAM5 CD3 TCB (CEA CD3 TCB (2)) are shown in Figure 7A (without costimulation) and in Figure 7C (with FAP OX40 iMAB costimulation). The results for CEA CD3 TCB are shown in Figure 7B (without costimulation) and in Figure 7D (with FAP OX40  
30 iMAB costimulation).

**Figures 8A-8D** summarize the effects seen with the different TCBs or different cell lines, respectively. Resting CD4 T cells were cocultured for 48 hrs with  $\text{TNF-}\alpha$  sensor cell, irradiated NIH/3T3 huFAP and different target cell lines HeLa NucLight Red cells (Figure 8B), MKN-45 NucLight Red cells (Figures 8A and 8C) or Skov-3 cells (Figure 8D) with or  
35 without a fixed concentration of FAP OX40 iMAB in the presence of a serial dilution row of FolR CD3 TCB (Figures 8B and 8D), CEA CD3 TCB (Figure 8C) or CEACAM5 CD3 TCB



(Figure 8A). The amount of TNF- $\alpha$  was quantified as GFP induction in TNF- $\alpha$  sensor cells by fluorescence microscopy high content life imaging 2. The AUC of GFP was calculated for each condition and time point and was plotted against each timepoint to quantify TNF- $\alpha$  secretion of T cells. OX40 costimulation did increase CEA CD3 TCB, CEACAM5 CD3 TCB and FolR CD3 TCB mediated TNF- $\alpha$  release.

In **Figures 9A-9D** it is shown that OX40 costimulation did modulate CEACAM5 CD3 TCB mediated cytokine secretion. Resting CD4 T cells were cocultured for 48 hrs with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEACAM5 CD3 TCB with or without a fixed concentration of FAP OX40 iMAB. The secreted amount of TNF- $\alpha$ , IFN- $\gamma$ , IL-2, IL-10, IL-9 and IL-17A was quantified at the 48h end point using cytometric bead array technology. The respective cytokine concentrations were plotted against the TCB concentration. Off note – secretion of proinflammatory cytokine TNF- $\alpha$  (Figure 9A), IFN- $\gamma$  (Figure 9C), and IL-2 (Figure 9B) was enhanced by OX40 costimulation, whereas that of immunosuppressive IL-10 (Figure 9D) was decreased.

In **Figures 10A-10D** it is shown that OX40 costimulation did modulate CEA CD3 TCB mediated cytokine secretion. Resting CD4 T cells were cocultured for 48 hrs with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEA CD3 TCB with or without a fixed concentration of FAP OX40 iMAB. The secreted amount of TNF- $\alpha$ , IFN- $\gamma$ , IL-2, IL-10 (Figure 10D), IL-9 and IL-17A was quantified at the 48h end point using cytometric bead array technology. The respective cytokine concentrations were plotted against the TCB concentration. Off note – secretion of proinflammatory cytokine TNF- $\alpha$  (Figure 10A), IFN- $\gamma$  (Figure 10C), and IL-2 (Figure 10B) was enhanced by OX40 costimulation.

In **Figures 11A-11D** it is shown that OX40 costimulation did modulate FolR1 CD3 TCB mediated cytokine secretion. Resting CD4 T cells were cocultured for 48 hrs with HeLa NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of FolR1 CD3 TCB with or without a fixed concentration of FAP OX40 iMAB. The secreted amount of TNF- $\alpha$ , IFN- $\gamma$ , IL-2, and IL-10 was quantified at the 48h end point using cytometric bead array technology. The respective cytokine concentrations were plotted against the TCB concentration. Off note – secretion of proinflammatory cytokine TNF- $\alpha$  (Figure 11A), IFN- $\gamma$  (Figure 11C), and IL-2 (Figure 11B) was enhanced by OX40 costimulation whereas that of immunosuppressive IL-10 (Figure 11D) was strongly decreased.

**Figures 12A-12D** show the results of the same experiment as shown in Figures 11A-11D, however here the HeLa NucLight Red cells were replaced with Skov-3 cells. The secretion of proinflammatory cytokine TNF- $\alpha$  (Figure 12A), IFN- $\gamma$  (Figure 12C), and IL-2

(Figure 12B) and IL-10 (Figure 12D) was not much changed by OX40 costimulation in this experiment.

A summary of the results shown in Figures 9A-9D, Figures 10A-10D, Figures 11A-11D and Figures 12A-12D is presented in **Figure 13**. The changes of cytokine concentration were calculated in percent, whereby the respective sample w/o FAP OX40 iMab costimulation was considered 100%. The extent of changes depended on the tumor cell line and the respective TCB used.

The ability of FAP OX40 iMab costimulation to modulate the CEACAM5 CD3 TCB mediated cytokine secretion in resting CD4 T cells (**Figures 14A-14H**), in resting CD8 T cells (**Figures 15A-15H**) and in resting human PMBCs (**Figures 16A-16H**) was compared. The graphs show the secreted amount of the cytokines IL-2 (Figures 14A, 15A and 16A), IFN- $\gamma$  (Figures 14B, 15B and 16B), TNF- $\alpha$  (Figures 14C, 15C and 16C), IL-4 (Figures 14D, 15D and 16D), IL-9 (Figures 14E, 15E and 16E), MIP-1 $\alpha$  (Figures 14F, 15F and 16F), IL-17a (Figures 14G, 15G and 16G) and IL-10 (Figures 14H, 15H and 16H). Resting CD4 or CD8 T cells or PBMC were cocultured for 72 hrs with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEACAM5 CD3 TCB (CEA CD3 TCB (2)) with or without a fixed concentration of FAP Ox40 iMAB. The secreted amount of TNF- $\alpha$ , IFN- $\gamma$ , IL-2, IL-10, IL-9, IL-4, Mip-1 $\alpha$  and IL-17A was quantified at the 48h end point using cytometric bead array technology. The respective cytokine concentrations were plotted against the TCB concentration.

A comparison of the increases in cytokine concentration caused by FAP Ox40 iMAB costimulation is shown in **Figure 17** for the TCB top concentration.

**Figures 18A and 18B** show the pharmacokinetic profile of injected compounds during the first week of treatment in the *in vivo* experiment 1 as described in Example 4.4. 2 mice per group were bled 10 min, 6h, 24h, 96h and 7d after the first therapy and the exposure of injected compounds was analysed. Blood was processed to serum and sandwich ELISAs were performed to determine the exposure of FAP OX40 iMab (Figure 18A) and CEACAM5 CD3 TCB (Figure 18B) during the first week. The systemic exposure was comparable for mice receiving monotherapy or for mice receiving combination therapy.

**Figure 19A** shows that only the combination of CEACAM5 CD3 TCB with FAP(4B9) OX40 iMab mediated regression of subcutaneous tumors compared to all other groups. This can be clearly seen from the waterfall plot as shown in **Figure 19B**. Stem cell humanized NOG mice were s.c. injected with a mixture of MKN45 gastric tumor cells and 3T3huFAP fibroblasts in matrigel. Mice were randomized on day10 for tumor size and human T-cell count with an average T-cell count/ $\mu$ l blood of 140 and an average tumor size of 170 mm<sup>3</sup>. On

the day of randomization mice were injected i.v. with Vehicle, CEACAM5 CD3 TCB (CEA CD3 TCB (2)), FAP OX40 iMAB or the combination thereof once per week for 5 consecutive weeks. The tumor volume was measured three times a week and plotted against the study time. Error bars show standard error for 6 to 8 animals per group (Figure 19A). Percent  
5 change of tumor volume at day 41 of experiment compared to tumor volume at treatment start was calculated for each animal and plotted as waterfall plot (Figure 19B).

**Figures 20A and 20B** show the pharmacokinetic profile of injected compounds during the first week of treatment in the *in vivo* experiment 2 as described in Example 4.5. 2 mice per group were bled 10 min, 6h, 24h, 96h and 7d after the first therapy and the exposure of  
10 injected compounds was analysed. Blood was processed to serum and sandwich ELISAs were performed to determine the exposure of the different doses of FAP OX40 iMab and its combinations with CEACAM5 CD3 TCB (Figure 20A) and of CEACAM5 CD3 TCB and its combination with different doses of FAP OX40 iMab (Figure 20B) during the first week. In Figure 20A a clear dose dependency of the different dosages of FAP OX40 iMab can be seen.  
15 The exposure of CEACAM CD3 TCB was comparable for mice receiving monotherapy or for mice receiving combination therapy.

**Figures 21A-21C** show that only the combination of CEACAM5 CD3 TCB with the highest dose of FAP(4B9) OX40 iMab (12.5 mg/kg, Figure 21C) showed improved efficacy in terms of tumor growth inhibition compared to all other groups. Stem cell humanized NOG  
20 mice were s.c. injected with a mixture of MKN45 gastric tumor cells and 3T3huFAP fibroblasts in matrigel. Mice were randomized day26 for tumor size and human T-cell count with an average T-cell count/ $\mu$ l blood of 115 and an average tumor size of 490mm<sup>3</sup>. One day after randomization mice were injected i.v. with Vehicle, CEACAM5 CD3 TCB (CEA CD3 TCB (2)), and different doses of FAP OX40 iMab (12.5 mg/kg, 4.2 mg/kg and 1.4 mg/kg,  
25 respectively) or the combinations of the OX40 targeted molecule with CEACAM5 CD3 TCB for 4 weeks. The tumor volume was measured three times a week and plotted against the study time. Error bars show standard error for 8 to 10 animals per group. Figure 21A shows the tumor regression obtained with FAP OX40 iMab 1.4 mg/kg, the tumor regression observed with FAP OX40 iMab 4.2 mg/kg or with FAP OX40 iMab 12.5 mg/kg are shown in  
30 Figures 21B and 21C, respectively.

**Figure 22** summarizes the dose dependency of the anti-tumor efficacy of the combination of CEACAM5 CD3 TCB with different amounts of FAP(4B9) OX40 iMab. Percent change of tumor volume at treatment day 35 of experiment 2 compared to tumor volume at treatment start was calculated for each animal and plotted as waterfall plot.

In **Figures 23A-23D** it is shown that the combination of CEACAM5 CD3 TCB and FAP(4B9) OX40 iMab significantly increases the number of intratumoral leukocytes compared to all monotherapies. On day 50 of experiment 2 described in Example 4.5, tumor infiltrating lymphocytes were isolated and evaluated for the presence of human leukocytes and T cells by flow cytometry. Living human leukocytes (DAPI-, CD45+), NON-CD3 leukocytes (DAPI-, CD45+, CD3-), CD4 and CD8 T cells (DAPI-, CD45+, CD3+, CD4 or CD8+) were gated, normalized counts (per or  $\mu\text{g}$  tumor) calculated and values plotted for the respective treatment groups: Figure 23A for living human leukocytes, Figure 23B for NON-CD3 leukocytes, Figure 23C for CD4 T cells and Figure 23D for CD8 T cells. Error bars show standard error for 5 to 8 animals per group.

In **Figures 24A and 24B** it is shown that FAP OX40 iMAB costimulation and CEA CD3 TCB (2) act tumor specific and do not change systemic leukocyte counts in spleen (Figure 24A) and in blood (Figure 24B).

The combination of CEACAM5 CD3 TCB and FAP(4B9) OX40 iMab significantly increased the number of intratumoral T cells and CD8 T cells compared to all monotherapies. The number of CD3 positive T cells as detected by huCD3 immunohistochemistry is shown in **Figure 25A** and the number of CD8 positive T cells as detected by huCD8 immunohistochemistry is shown in **Figure 25B**. HuCD8 and HuCD3 immunohistochemistry was performed on 4  $\mu\text{m}$  paraffin sections.

In **Figures 26A-26C** it is shown that the combination of CEACAM5 CD3 TCB and FAP(4B9) OX40 iMab significantly increased the concentration of intratumoral cytokines compared to all monotherapies. No significant changes were detected in the periphery. On day 50 of experiment 2, tumor, spleen and blood were sampled and snap frozen. Cytokine concentrations were determined in the homogenates using the Bio-Plex Pro™ Human Cytokine 17-plex Assay. The whole protein content was analysis by the BCA protein assay kit and concentrations were normalized to the protein content of the samples. The median cytokine concentration of 4 animals per treatment group is depicted in Figure 26A for the tumor, in Figure 26B for spleen and in Figure 26C for blood.

**Figures 27A-27F** show that intratumoral cytokine concentrations, but not the intratumoral leukocyte count, correlate inversely with the progression of tumor growth in the animals treated with the combination of FAP OX40 iMab and CEACAM5 CD3 TCB. This was not observed in animals treated with CEACAM5 CD3 TCB monotherapy. Each open symbol stands for an individual animal treated with CEACAM5 CD3 TCB monotherapy and each filled symbol stands for an individual animal treated with the combination. In Figure 27A the count of T cells is plotted against the change in tumor volume [%], the concentration

of TNF- $\alpha$  (Figure 27B), IFN- $\gamma$  (Figure 27C), MCP-1 (Figure 27D), IL-8 (Figure 27E) and IL-6 (Figure 27F) is also plotted against the change in tumor volume [%].

**Figures 28A and 28B** show that the combination of CEA CD3 TCB with anti-PD-L1 and with FAP OX40 iMab mediated improved efficacy in terms of tumor growth inhibition compared to all other therapies (Example 5). Figures 28A and 28B show the tumor growth over time either as average of tumor volume or as average fold change of tumor volume, respectively.

**Figures 29A, 29B and 29C** show the pharmacokinetic profile of injected compounds during the first week of treatment in the *in vivo* experiment as described in Example 5. 2 mice per group were bled 1h and 72h after 1<sup>st</sup> and 3<sup>rd</sup> therapy and the exposure of injected compounds was analysed. Blood was processed to serum and sandwich ELISAs were performed to determine the exposure of FAP OX40 iMab in combination with CEACAM5 CD3 TCB or the triple combination (Figure 29A), of CEA CD3 TCB and its different combinations (Figure 29B) and of CEA CD3 TCB in combination with anti-PD-L1 or the triple combination (Figure 29C). The exposure of all three compounds was comparable for mice receiving monotherapy or for mice receiving combination therapy.

The combination of CEACAM5 CD3 TCB with anti-PD-L1 and FAP(4B9) OX40 iMab significantly increased the number of intratumoral T cells and CD8 T cells compared to all mono- or doublet therapies. The number of CD3 positive T cells as detected by huCD3 immunohistochemistry is shown in **Figure 30A** and the number of CD8 positive T cells as detected by huCD8 immunohistochemistry is shown in **Figure 30B**. HuCD8 and HuCD3 immunohistochemistry was performed on 4  $\mu$ m paraffin sections.

**Figures 31 to 35** relate to the results of an *in vitro* assay testing the efficacy of the combination of CEA CD3 TCB and FAP OX40iMab as well as the triple combination of CEA CD3 TCB and FAP-4-1BBL with anti-PD-L1 antibody (atezolizumab). PBMCs were incubated for four days in the presence of MKN45-PD-L1 and NIH/3T3-huFAP cells and different combinations of T cell activator CEA CD3 TCB, checkpoint inhibitor a-PD-L1 (atezolizumab) and immunomodulator FAP OX40 iMab. At day 4, the endpoint of the experiment, cells were stained for surface or intracellular markers and supernatant was stored for cytokine analysis. Each symbol indicates an individual donor (each group was tested in triplicate), each color/pattern indicates a specific treatment combination, the bar indicates the mean with SEM. The effect of the combinations compared to the single components and combinations thereof on surface expression of CD25 on CD4 (**Figure 31A**) and CD8 T cells (**Figure 31B**), proliferation on CD4 (**Figure 32A**) and CD8 T cells (**Figure 32B**) and intracellular expression of T-bet on CD4 (**Figure 33A**) and CD8 T cells (**Figure 33B**), and

Granzyme B on CD4 (**Figure 33C**) and CD8 T cells (**Figure 33D**), respectively, is shown for 6 different donors. Statistical significance between different treatment groups was calculated using 2-way ANOVA (Tukey's multiple comparisons test), wherein the average of 6 donors with experimental triplicates per group was calculated. Stars (\*) shown in the graphs indicate p-value, \* indicates p value <0.05, \*\* indicates p value <0.01, \*\*\* indicates p values <0.001.

**Figures 31A and 31B** show that combination treatment with 100 nM CEA CD3 TCB and 2 nM FAP OX40 iMAB or triple combination treatment with anti-PD-L1 antibody increases the percentage of CD25 expressing CD4 (Figure 31A) and CD8 (Figure 31B) T cells.

**Figures 32A and 32B** show that combination treatment with 100 nM CEA CD3 TCB and 2 nM FAP OX40 iMAB or triple combination treatment with 80 nM anti-PD-L1 antibody increases the percentage of proliferating CD4 T cells (Figure 32A) and CD8 T cells (Figure 32B). PBMCs were labelled with proliferation dye CFSE prior to the start of the experiment and proliferation was measured by dilution of the CFSE dye using FACS.

**Figures 33A and 33B** show that combination treatment with 100 nM CEA CD3 TCB and 2 nM FAP OX40 iMAB or triple combination treatment with 80 nM anti-PD-L1 antibody increases the percentage of T-bet expressing CD4 T cells (Figure 33A) and MFI (mean fluorescent intensity) of T-bet on CD8 T cells (Figure 33B). **Figures 33C and 33D** show that combination treatment with 100 nM CEA CD3 TCB and 2nM FAP OX40 iMAB increases the percentage of Granzyme B expressing CD4 T cells (Figure 33C) and of Granzyme B expressing CD8 T cells (Figure 33D). Triple combination with anti-PD-L1 antibody further increases the percentages of Granzyme B expressing CD4 and CD8 T cells as compared to CEA CD3 TCB and FAP OX40 iMAb combination treatment with statistical significance. Secreted cytokines IFN $\gamma$ , GM-CSF, TNF $\alpha$ , IL-2, IL-8, Granzyme B and IL-10 were analyzed in the supernatant after 4 days of incubation using cytometric bead array according to manufacturer's instructions. Each symbol indicates one donor (pooled experimental triplicates per group), each color/pattern indicates a specific treatment combination, the bar indicates the mean with SEM.

**Figures 34A, 34B and 34C** show that combination treatment with 100 nM CEA CD3 TCB and 2 nM FAP OX40 iMAB increases the secretion of IFN $\gamma$  (Figure 34A), Granzyme B (Figure 34B) and IL-8 (Figure 34C). Triple combination with aPD-L1 significantly increases the secretion of all three cytokines stated above.

**Figures 35A, 35B and 35C** show the fold increase of cytokines in 6 donors after treatment with the triple combination of CEA CD3 TCB, FAP OX40 iMAb and a-PD-L1 as compared to cytokines after treatment with CEA CD3 TCB and aPD-L1 combination

treatment, taken as baseline. The solid black line indicates 2 fold changes. Shown is fold increase of IFN $\gamma$  (Figure 35A), Granzyme B (Figure 35B) and IL-8 (Figure 35C).

## DETAILED DESCRIPTION OF THE INVENTION

### Definitions

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

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

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

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

The term "**monospecific**" antibody as used herein denotes an antibody that has one or  
25 more binding sites each of which bind to the same epitope of the same antigen. The term "**bispecific**" means that the antigen binding molecule is able to specifically bind to at least two distinct antigenic determinants. Typically, a bispecific antigen binding molecule comprises two antigen binding sites, each of which is specific for a different antigenic determinant. In certain embodiments the bispecific antigen binding molecule is capable of  
30 simultaneously binding two antigenic determinants, particularly two antigenic determinants expressed on two distinct cells.

The term “**valent**” as used within the current application denotes the presence of a specified number of binding sites in an antigen binding molecule. As such, the terms “bivalent”, “tetravalent”, and “hexavalent” denote the presence of two binding sites, four binding sites, and six binding sites, respectively, in an antigen binding molecule.

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

      An “**antibody fragment**” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')<sub>2</sub>; diabodies, triabodies, tetrabodies, cross-Fab fragments; linear antibodies; single-chain  
25       antibody molecules (e.g. scFv); and single domain antibodies. For a review of certain antibody fragments, see Hudson et al., Nat Med 9, 129-134 (2003). For a review of scFv fragments, see e.g. Plückthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')<sub>2</sub>  
30       fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific, see, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat Med 9, 129-134 (2003); and Hollinger et al., Proc Natl Acad Sci USA 90, 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al.,  
35       Nat Med 9, 129-134 (2003). Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-



domain antibody (Domantis, Inc., Waltham, MA; see e.g. U.S. Patent No. 6,248,516 B1). Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

5       Papain digestion of intact antibodies produces two identical antigen-binding fragments, called “Fab” fragments containing each the heavy- and light-chain variable domains and also the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. As used herein, Thus, the term “**Fab fragment**” refers to an antibody fragment comprising a light chain fragment comprising a VL domain and a constant domain of a light chain (CL),  
10       and a VH domain and a first constant domain (CH1) of a heavy chain. Fab’ fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteins from the antibody hinge region. Fab’-SH are Fab’ fragments in which the cysteine residue(s) of the constant domains bear a free thiol group. Pepsin treatment yields an F(ab')<sub>2</sub> fragment that has two antigen-combining sites (two  
15       Fab fragments) and a part of the Fc region.

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

      A “single chain Fab fragment” or “**scFab**” is a polypeptide consisting of an antibody  
30       heavy chain variable domain (VH), an antibody constant domain 1 (CH1), an antibody light chain variable domain (VL), an antibody light chain constant domain (CL) and a linker, wherein said antibody domains and said linker have one of the following orders in N-terminal to C-terminal direction: a) VH-CH1-linker-VL-CL, b) VL-CL-linker-VH-CH1, c) VH-CL-linker-VL-CH1 or d) VL-CH1-linker-VH-CL; and wherein said linker is a polypeptide of at  
35       least 30 amino acids, preferably between 32 and 50 amino acids. Said single chain Fab fragments are stabilized via the natural disulfide bond between the CL domain and the CH1

domain. In addition, these single chain Fab molecules might be further stabilized by generation of interchain disulfide bonds via insertion of cysteine residues (e.g. position 44 in the variable heavy chain and position 100 in the variable light chain according to Kabat numbering).

5           A “crossover single chain Fab fragment” or “**x-scFab**” is a polypeptide consisting of an antibody heavy chain variable domain (V<sub>H</sub>), an antibody constant domain 1 (CH1), an antibody light chain variable domain (V<sub>L</sub>), an antibody light chain constant domain (CL) and a linker, wherein said antibody domains and said linker have one of the following orders in N-terminal to C-terminal direction: a) V<sub>H</sub>-CL-linker-V<sub>L</sub>-CH1 and b) V<sub>L</sub>-CH1-linker-V<sub>H</sub>-CL; 10 wherein V<sub>H</sub> and V<sub>L</sub> form together an antigen-binding site which binds specifically to an antigen and wherein said linker is a polypeptide of at least 30 amino acids. In addition, these x-scFab molecules might be further stabilized by generation of interchain disulfide bonds via insertion of cysteine residues (e.g. position 44 in the variable heavy chain and position 100 in the variable light chain according to Kabat numbering).

15           A “**single-chain variable fragment (scFv)**” is a fusion protein of the variable regions of the heavy (V<sub>H</sub>) and light chains (V<sub>L</sub>) 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<sub>H</sub> with the C-terminus of the V<sub>L</sub>, or *vice versa*. This protein retains the specificity of the original antibody, 20 despite removal of the constant regions and the introduction of the linker. scFv antibodies are, e.g. described in Houston, J.S., Methods in Enzymol. 203 (1991) 46-96). In addition, antibody fragments comprise single chain polypeptides having the characteristics of a V<sub>H</sub> domain, namely being able to assemble together with a V<sub>L</sub> domain, or of a V<sub>L</sub> domain, namely being able to assemble together with a V<sub>H</sub> domain to a functional antigen binding site and thereby 25 providing the antigen binding property of full length antibodies.

          “**Scaffold antigen binding proteins**” are known in the art, for example, fibronectin and designed ankyrin repeat proteins (DARPin) have been used as alternative scaffolds for antigen-binding domains, see, e.g., Gebauer and Skerra, Engineered protein scaffolds as next-generation antibody therapeutics. Curr Opin Chem Biol 13:245-255 (2009) and Stumpp et al., 30 Darpins: A new generation of protein therapeutics. Drug Discovery Today 13: 695-701 (2008). In one aspect of the invention, a scaffold antigen binding protein is selected from the group consisting of CTLA-4 (Evibody), Lipocalins (Anticalin), a Protein A-derived molecule such as Z-domain of Protein A (Affibody), an A-domain (Avimer/Maxibody), a serum transferrin (*trans*-body); a designed ankyrin repeat protein (DARPin), a variable domain of 35 antibody light chain or heavy chain (single-domain antibody, sdAb), a variable domain of antibody heavy chain (nanobody, aVH), V<sub>NAR</sub> fragments, a fibronectin (AdNectin), a C-type

lectin domain (Tetranectin); a variable domain of a new antigen receptor beta-lactamase ( $V_{\text{NAR}}$  fragments), a human gamma-crystallin or ubiquitin (Affilin molecules); a kunitz type domain of human protease inhibitors, microbodies such as the proteins from the knottin family, peptide aptamers and fibronectin (adnectin).

5 Lipocalins are a family of extracellular proteins which transport small hydrophobic molecules such as steroids, bilins, retinoids and lipids. They have a rigid beta-sheet secondary structure with a number of loops at the open end of the conical structure which can be engineered to bind to different target antigens. Anticalins are between 160-180 amino acids in size, and are derived from lipocalins. For further details see Biochim Biophys Acta 1482:  
10 337-350 (2000), US7250297B1 and US20070224633.

Designed Ankyrin Repeat Proteins (DARPs) are derived from Ankyrin which is a family of proteins that mediate attachment of integral membrane proteins to the cytoskeleton. A single ankyrin repeat is a 33 residue motif consisting of two alpha-helices and a beta-turn. They can be engineered to bind different target antigens by randomizing residues in the first  
15 alpha-helix and a beta-turn of each repeat. Their binding interface can be increased by increasing the number of modules (a method of affinity maturation). For further details see J. Mol. Biol. 332, 489-503 (2003), PNAS 100(4), 1700-1705 (2003) and J. Mol. Biol. 369, 1015-1028 (2007) and US20040132028A1.

A single-domain antibody is an antibody fragment consisting of a single monomeric  
20 variable antibody domain. The first single domains were derived from the variable domain of the antibody heavy chain from camelids (nanobodies or  $V_{\text{HH}}$  fragments). Furthermore, the term single-domain antibody includes an autonomous human heavy chain variable domain (aVH) or  $V_{\text{NAR}}$  fragments derived from sharks.

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

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

As used herein, the term "**antigenic determinant**" is synonymous with "antigen" and "epitope," and refers to a site (e.g. a contiguous stretch of amino acids or a conformational configuration made up of different regions of non-contiguous amino acids) on a polypeptide macromolecule to which an antigen binding moiety binds, forming an antigen binding moiety-antigen complex. Useful antigenic determinants can be found, for example, on the surfaces of tumor cells, on the surfaces of virus-infected cells, on the surfaces of other diseased cells, on the surface of immune cells, free in blood serum, and/or in the extracellular matrix (ECM). The proteins useful as antigens herein can be any native form the proteins from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g. mice and rats), unless otherwise indicated. In a particular embodiment the antigen is a human protein. Where reference is made to a specific protein herein, the term encompasses the "full-length", unprocessed protein as well as any form of the protein that results from processing in the cell. The term also encompasses naturally occurring variants of the protein, e.g. splice variants or allelic variants.

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

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

The term “**tumor-associated antigen (TAA)**” means any antigen that is highly expressed by tumor cells or in the tumor stroma. The term tumor-associated indicates that TAA are not completely specific for the tumor, but are rather over-expressed on the tumor or its stroma. Particular tumor-associated antigens are CEA or FAP, but also other targets such as Folate Receptor (FolR1), MCSP, the EGFR family (HER2, HER3 and EGFR/HER1), VEGFR, CD20, CD19, CD22, CD33, PD1, PD-L1, TenC, EpCAM, PSA, PSMA, STEAP1, MUC1 (CA15-3) MUC16 (CA125) and 5T4 (trophoblast glycoprotein). Particular TAA include FAP, CEA and FolR1.

The term “**Fibroblast activation protein (FAP)**”, also known as Prolyl endopeptidase FAP or Seprase (EC 3.4.21), refers to any native FAP from any vertebrate source, including mammals such as primates (e.g. humans) non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The term encompasses “full-length,” unprocessed FAP as well as any form of FAP which results from processing in the cell. The term also encompasses naturally occurring variants of FAP, e.g., splice variants or allelic variants. In one embodiment, the antigen binding molecule of the invention is capable of specific binding to human, mouse and/or cynomolgus FAP. The amino acid sequence of human FAP is shown in UniProt ([www.uniprot.org](http://www.uniprot.org)) accession no. Q12884 (version 149, SEQ ID NO:120), or NCBI ([www.ncbi.nlm.nih.gov/](http://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: 121. The amino acid sequence of mouse FAP is shown in UniProt accession no. P97321 (version 126, SEQ ID NO:122), or NCBI RefSeq NP\_032012.1. The extracellular domain (ECD) of mouse FAP extends from amino acid position 26 to 761. SEQ ID NO: 123 shows the amino acid sequence of a His-tagged mouse FAP ECD. SEQ ID NO: 124 shows the amino acid sequence of a His-tagged cynomolgus FAP ECD. Preferably, an anti-FAP binding molecule of the invention binds to the extracellular domain of FAP. Exemplary anti-FAP binding molecules are described in International Patent Application No. WO 2012/020006 A2.

The term “**Carcinoembryonic antigen (CEA)**”, also known as Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5), refers to any native CEA from any vertebrate source, including mammals such as primates (e.g. humans) non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The amino acid sequence of human CEA is shown in UniProt accession no. P06731 (version 151, SEQ ID NO:125). CEA has long been identified as a tumor-associated antigen (Gold and Freedman, J Exp Med., 121:439-462, 1965; Berinstein N. L., J Clin Oncol., 20:2197-2207, 2002). Originally classified as a protein expressed only in fetal tissue, CEA has now been identified in several normal adult tissues. These tissues are primarily epithelial in origin, including cells of the gastrointestinal, respiratory, and urogenital tracts, and cells of colon,

cervix, sweat glands, and prostate (Nap et al., *Tumour Biol.*, 9(2-3):145-53, 1988; Nap et al., *Cancer Res.*, 52(8):2329-2333, 1992). Tumors of epithelial origin, as well as their metastases, contain CEA as a tumor associated antigen. While the presence of CEA itself does not indicate transformation to a cancerous cell, the distribution of CEA is indicative. In normal

5 tissue, CEA is generally expressed on the apical surface of the cell (Hammarström S., *Semin Cancer Biol.* 9(2):67-81 (1999)), making it inaccessible to antibody in the blood stream. In contrast to normal tissue, CEA tends to be expressed over the entire surface of cancerous cells (Hammarström S., *Semin Cancer Biol.* 9(2):67-81 (1999)). This change of expression pattern makes CEA accessible to antibody binding in cancerous cells. In addition, CEA expression

10 increases in cancerous cells. Furthermore, increased CEA expression promotes increased intercellular adhesions, which may lead to metastasis (Marshall J., *Semin Oncol.*, 30(a Suppl. 8):30-6, 2003). The prevalence of CEA expression in various tumor entities is generally very high. In concordance with published data, own analyses performed in tissue samples confirmed its high prevalence, with approximately 95% in colorectal carcinoma (CRC), 90%

15 in pancreatic cancer, 80% in gastric cancer, 60% in non-small cell lung cancer (NSCLC, where it is co-expressed with HER3), and 40% in breast cancer; low expression was found in small cell lung cancer and glioblastoma.

CEA is readily cleaved from the cell surface and shed into the blood stream from tumors, either directly or via the lymphatics. Because of this property, the level of serum CEA

20 has been used as a clinical marker for diagnosis of cancers and screening for recurrence of cancers, particularly colorectal cancer (Goldenberg D M., *The International Journal of Biological Markers*, 7:183-188, 1992; Chau I., et al., *J Clin Oncol.*, 22:1420-1429, 2004; Flamini et al., *Clin Cancer Res*; 12(23):6985-6988, 2006).

The term “**FolR1**” refers to Folate receptor alpha and has been identified as a potential

25 prognostic and therapeutic target in a number of cancers. It refers to any native FolR1 from any vertebrate source, including mammals such as primates (e.g. humans) non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The amino acid sequence of human FolR1 is shown in UniProt accession no. P15328 (SEQ ID NO:126), murine FolR1 has the amino acid sequence of UniProt accession

30 no. P35846 (SEQ ID NO:127) and cynomolgus FolR1 has the amino acid sequence as shown in UniProt accession no. G7PR14 (SEQ ID NO:128). FolR1 is an N-glycosylated protein expressed on plasma membrane of cells. FolR1 has a high affinity for folic acid and for several reduced folic acid derivatives and mediates delivery of the physiological folate, 5-methyltetrahydrofolate, to the interior of cells. FOLR1 is a desirable target for FOLR1-

35 directed cancer therapy as it is overexpressed in vast majority of ovarian cancers, as well as in many uterine, endometrial, pancreatic, renal, lung, and breast cancers, while the expression of FOLR1 on normal tissues is restricted to the apical membrane of epithelial cells in the kidney

proximal tubules, alveolar pneumocytes of the lung, bladder, testes, choroid plexus, and thyroid. Recent studies have identified that FolR1 expression is particularly high in triple negative breast cancers (Necela et al. PloS One 2015, 10(3), e0127133).

The term “**MCSP**” refers to Melanoma-associated Chondroitin Sulfate Proteoglycan, also known as Chondroitin Sulfate Proteoglycan 4 (CSPG4). It refers to any native FolR1 from any vertebrate source, including mammals such as primates (e.g. humans) non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The amino acid sequence of human MCSP is shown in UniProt accession no. Q6UVK1 (SEQ ID NO:129). MCSP is a highly glycosylated integral membrane chondroitin sulfate proteoglycan consisting of an N-linked 280 kDa glycoprotein component and a 450-kDa chondroitin sulfate proteoglycan component expressed on the cell membrane (Ross et al., *Arch. Biochem. Biophys.* 1983, 225:370-38). MCSP is more broadly distributed in a number of normal and transformed cells. In particular, MCSP is found in almost all basal cells of the epidermis. MCSP is differentially expressed in melanoma cells, and was found to be expressed in more than 90% of benign nevi and melanoma lesions analyzed. MCSP has also been found to be expressed in tumors of nonmelanocytic origin, including basal cell carcinoma, various tumors of neural crest origin, and in breast carcinomas.

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

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

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

The term “**CD3**” refers to any native CD3 from any vertebrate source, including mammals such as primates (e.g. humans), non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The term encompasses “full-length,” unprocessed CD3 as well as any form of CD3 that results from processing in the cell. The term also encompasses naturally occurring variants of CD3, e.g., splice variants or allelic

variants. In one embodiment, CD3 is human CD3, particularly the epsilon subunit of human CD3 (CD3 $\epsilon$ ). The amino acid sequence of human CD3 $\epsilon$  is shown in UniProt (www.uniprot.org) accession no. P07766 (version 144), or NCBI (www.ncbi.nlm.nih.gov/) RefSeq NP\_000724.1. See also SEQ ID NO: 130. The amino acid sequence of cynomolgus  
5 [Macaca fascicularis] CD3 $\epsilon$  is shown in NCBI GenBank no. BAB71849.1. See also SEQ ID NO: 131.

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  
10 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  
15 regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops (“hypervariable loops”). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the “complementarity determining regions” (CDRs), the latter being of highest sequence  
20 variability and/or involved in antigen recognition. Exemplary hypervariable loops occur at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3). (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987).) Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3. (Kabat et al.,  
25 *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).) Hypervariable regions (HVRs) are also referred to as complementarity determining regions (CDRs), and these terms are used herein interchangeably in reference to portions of the variable region that form the antigen binding regions. This particular region has been described by Kabat et al., U.S. Dept. of Health and  
30 Human Services, “Sequences of Proteins of Immunological Interest” (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which  
35 encompass the CDRs as defined by each of the above cited references are set forth below in Table B as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely



determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

**TABLE A. CDR Definitions<sup>1</sup>**

| CDR                 | Kabat  | Chothia | AbM <sup>2</sup> |
|---------------------|--------|---------|------------------|
| V <sub>H</sub> CDR1 | 31-35  | 26-32   | 26-35            |
| V <sub>H</sub> CDR2 | 50-65  | 52-58   | 50-58            |
| V <sub>H</sub> CDR3 | 95-102 | 95-102  | 95-102           |
| V <sub>L</sub> CDR1 | 24-34  | 26-32   | 24-34            |
| V <sub>L</sub> CDR2 | 50-56  | 50-52   | 50-56            |
| V <sub>L</sub> CDR3 | 89-97  | 91-96   | 89-97            |

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

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

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

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

As used herein, the term "**affinity matured**" in the context of antigen binding molecules (e.g., antibodies) refers to an antigen binding molecule that is derived from a reference antigen binding molecule, e.g., by mutation, binds to the same antigen, preferably binds to the same epitope, as the reference antibody; and has a higher affinity for the antigen

than that of the reference antigen binding molecule. Affinity maturation generally involves modification of one or more amino acid residues in one or more CDRs of the antigen binding molecule. Typically, the affinity matured antigen binding molecule binds to the same epitope as the initial reference antigen binding molecule.

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

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

20        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.

25        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<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, IgG<sub>4</sub>, IgA<sub>1</sub>, and IgA<sub>2</sub>. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$ , and  $\mu$  respectively..

30        A **"humanized"** antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A **"humanized form"** of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization. Other forms of  
35        "humanized antibodies" encompassed by the present invention are those in which the constant

region has been additionally modified or changed from that of the original antibody to generate the properties according to the invention, especially in regard to C1q binding and/or Fc receptor (FcR) binding.

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

The term “Fc domain” or “**Fc region**” herein is used to define a C-terminal region of an  
10 antibody heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. An IgG Fc region comprises an IgG CH2 and an IgG CH3 domain. The “CH2 domain” of a human IgG Fc region usually extends from an amino acid residue at about position 231 to an amino acid residue at about position 340. In one embodiment, a carbohydrate chain is attached to the CH2 domain. The CH2 domain  
15 herein may be a native sequence CH2 domain or variant CH2 domain. The “CH3 domain” comprises the stretch of residues C-terminal to a CH2 domain in an Fc region (i.e. from an amino acid residue at about position 341 to an amino acid residue at about position 447 of an IgG). The CH3 region herein may be a native sequence CH3 domain or a variant CH3 domain (e.g. a CH3 domain with an introduced “protuberance” (“knob”) in one chain thereof and a  
20 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  
25 (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.

30 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  
35 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

5 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

10 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

15 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

20 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 functions**" refers to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector

25 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.

30 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 “**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<sub>4</sub>S)<sub>n</sub>, (SG<sub>4</sub>)<sub>n</sub> or G<sub>4</sub>(SG<sub>4</sub>)<sub>n</sub> peptide linkers, wherein “n” is generally a number between 1 and 10, typically  
 5 between 2 and 4, in particular 2, i.e. the peptides selected from the group consisting of GGGGS (SEQ ID NO:132), GGGGSGGGGS (SEQ ID NO:133), SGGGSGGGG (SEQ ID NO:134) and GGGGSGGGGSGGGG (SEQ ID NO:135), but also include the sequences GSPGSSSSGS (SEQ ID NO:136), (G<sub>4</sub>S)<sub>3</sub> (SEQ ID NO:137), (G<sub>4</sub>S)<sub>4</sub> (SEQ ID NO:138), GSGSGSGS (SEQ ID NO:139), GSGSGNGS (SEQ ID NO:140), GGSGSGSG (SEQ ID  
 10 NO:141), GGSGSG (SEQ ID NO:142), GGSG (SEQ ID NO:143), GGSGNGSG (SEQ ID NO:144), GGNGSGSG (SEQ ID NO:145) and GGNGSG (SEQ ID NO:146). Peptide linkers of particular interest are (G<sub>4</sub>S) (SEQ ID NO:132), (G<sub>4</sub>S)<sub>2</sub> (SEQ ID NO:133), (G<sub>4</sub>S)<sub>3</sub> (SEQ ID NO:137) and (G<sub>4</sub>S)<sub>4</sub> (SEQ ID NO:138).

The term “**amino acid**” as used within this application denotes the group of naturally  
 15 occurring carboxy  $\alpha$ -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 polypeptide and an  
 20 ectodomain of 4-1BBL) are linked by peptide bonds, either directly or via one or more peptide linkers.

“**Percent (%) amino acid sequence identity**” with respect to a reference polypeptide (protein) sequence is defined as the percentage of amino acid residues in a candidate sequence  
 25 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  
 30 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  
 35 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.

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

**TABLE B**

| <b>Original Residue</b> | <b>Exemplary Substitutions</b>      | <b>Preferred Substitutions</b> |
|-------------------------|-------------------------------------|--------------------------------|
| Ala (A)                 | Val; Leu; Ile                       | Val                            |
| Arg (R)                 | Lys; Gln; Asn                       | Lys                            |
| Asn (N)                 | Gln; His; Asp, Lys; Arg             | Gln                            |
| Asp (D)                 | Glu; Asn                            | Glu                            |
| Cys (C)                 | Ser; Ala                            | Ser                            |
| Gln (Q)                 | Asn; Glu                            | Asn                            |
| Glu (E)                 | Asp; Gln                            | Asp                            |
| Gly (G)                 | Ala                                 | Ala                            |
| His (H)                 | Asn; Gln; Lys; Arg                  | Arg                            |
| Ile (I)                 | Leu; Val; Met; Ala; Phe; Norleucine | Leu                            |
| Leu (L)                 | Norleucine; Ile; Val; Met; Ala; Phe | Ile                            |
| Lys (K)                 | Arg; Gln; Asn                       | Arg                            |
| Met (M)                 | Leu; Phe; Ile                       | Leu                            |
| Phe (F)                 | Trp; Leu; Val; Ile; Ala; Tyr        | Tyr                            |
| Pro (P)                 | Ala                                 | Ala                            |
| Ser (S)                 | Thr                                 | Thr                            |
| Thr (T)                 | Val; Ser                            | Ser                            |
| Trp (W)                 | Tyr; Phe                            | Tyr                            |
| Tyr (Y)                 | Trp; Phe; Thr; Ser                  | Phe                            |
| Val (V)                 | Ile; Leu; Met; Phe; Ala; Norleucine | Leu                            |

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

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

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

The term “**amino acid sequence variants**” includes substantial variants wherein there are amino acid substitutions in one or more hypervariable region residues of a parent antigen binding molecule (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antigen binding molecule and/or will have substantially retained certain biological properties of the parent antigen binding molecule. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more CDR residues are mutated and the variant antigen binding molecules displayed on phage and screened for a particular biological activity (e.g. binding affinity). In certain embodiments, substitutions, insertions, or deletions may occur within one or more CDRs so long as such alterations do not substantially reduce the ability of the antigen binding molecule to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in CDRs. A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antigen binding molecule complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

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

In certain embodiments, the antigen binding molecules provided herein are altered to increase or decrease the extent to which the antibody is glycosylated. Glycosylation variants of the molecules may be conveniently obtained by altering the amino acid sequence such that one or more glycosylation sites is created or removed. Where the antigen binding molecule comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies



produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the “stem” of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in the antigen binding molecules may be made in order to create variants with certain improved properties. In one aspect, variants of antigen binding molecules are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. Such fucosylation variants may have improved ADCC function, see e.g. US Patent Publication Nos. US 2003/0157108 (Presta, L.) or US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Further variants of the antigen binding molecules of the invention include those with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region is bisected by GlcNAc. Such variants may have reduced fucosylation and/or improved ADCC function., see for example WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function and are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

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

In certain aspects, the antigen binding molecules provided herein may be further modified to contain additional non-proteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either

homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide copolymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the bispecific antibody derivative will be used in a therapy under defined conditions, etc. In another aspect, conjugates of an antibody and non-proteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the non-proteinaceous moiety is a carbon nanotube (Kam, N.W. et al., Proc. Natl. Acad. Sci. USA 102 (2005) 11600-11605). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the non-proteinaceous moiety to a temperature at which cells proximal to the antibody-non-proteinaceous moiety are killed. In another aspect, immunoconjugates of the 4-1BBL-containing antigen binding molecules provided herein may be obtained. An **"immunoconjugate"** is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

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

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

nucleic acids according to the present invention further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid may be or may include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

By a nucleic acid or polynucleotide having a nucleotide sequence at least, for example,  
5 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  
10 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  
15 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).

20 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  
25 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"  
30 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  
35 stable mRNA. Once the expression vector is inside the target cell, the ribonucleic acid molecule or protein that is encoded by the gene is produced by the cellular transcription

and/or translation machinery. In one embodiment, the expression vector of the invention comprises an expression cassette that comprises polynucleotide sequences that encode bispecific antigen binding molecules of the invention or fragments thereof.

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

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

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

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

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

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

10       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.

15       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  
20       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 solid tumors, or melanoma.

### **Exemplary targeted OX40 agonists for use in the invention**

25       In particular, the targeted OX40 agonists as used in combination with the T-cell activating anti-CD3 bispecific antibodies specific for a tumor-associated antigen are bispecific OX40 antibodies comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen.

30       In particular, the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is an anti-Fibroblast activation protein (FAP)/anti-OX40 bispecific antibody. In one aspect, the anti-FAP/anti-OX40 antibody is an OX40 agonist. In one aspect, the anti-FAP/anti-OX40 antibody is an antigen binding molecule comprising a Fc domain. In a particular aspect, the anti-FAP/anti-OX40 antibody is an antigen binding molecule comprising a Fc domain with modifications

reducing Fc $\gamma$  receptor binding and/or effector function. The crosslinking by a tumor associated antigen makes it possible to avoid unspecific Fc $\gamma$ R-mediated crosslinking and thus higher and more efficacious doses of the anti-FAP/anti-OX40 antibody may be administered in comparison to common OX40 antibodies.

- 5 In one aspect, the invention provides a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer, wherein the bispecific OX40 antibody is used in combination with a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen
- 10 and wherein the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to FAP comprising
- (a) a heavy chain variable region (V<sub>H</sub>FAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:1, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:3, and a light
- 15 chain variable region (V<sub>L</sub>FAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:4, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:5, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:6, or
- (b) a heavy chain variable region (V<sub>H</sub>FAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:9, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID
- 20 NO:10, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:11, and a light chain variable region (V<sub>L</sub>FAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:12, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:13, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:14.

- In a further aspect, provided is a bispecific OX40 antibody comprising at least one
- 25 antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as defined herein before, wherein the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to FAP comprising a heavy chain variable region (V<sub>H</sub>FAP) that is at least 90%, 95%, 96%, 97%,
- 30 98%, or 99% identical to an amino acid sequence of SEQ ID NO:7 and a light chain variable region (V<sub>L</sub>FAP) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence of SEQ ID NO:8 or an antigen binding domain capable of specific binding to FAP comprising a heavy chain variable region (V<sub>H</sub>FAP) that is at least 90%, 95%, 96%, 97%,
- 35 98%, or 99% identical to an amino acid sequence of SEQ ID NO:15 and a light chain variable region (V<sub>L</sub>FAP) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence of SEQ ID NO:16.

In a particular aspect, the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to FAP comprising a heavy chain variable region (V<sub>H</sub>FAP) comprising an amino acid sequence of SEQ ID NO:7 and a light chain variable region (V<sub>L</sub>FAP) comprising an amino acid sequence of SEQ ID NO:8. In another aspect, the bispecific OX40 antibody comprises at least one an antigen binding domain capable of specific binding to FAP comprising a heavy chain variable region (V<sub>H</sub>FAP) comprising an amino acid sequence of SEQ ID NO:15 and a light chain variable region (V<sub>L</sub>FAP) comprising an amino acid sequence of SEQ ID NO:16.

In a further aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as defined herein before, wherein the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to OX40 comprising

(a) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:22, and a light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:35, or

(b) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:21, and a light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:34, or

(c) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:23, and a light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:36, or

(d) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:24, and a light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (v) CDR-L2 comprising the amino acid sequence of SEQ ID

NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:37, or  
 (e) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino  
 acid sequence of SEQ ID NO:18, (ii) CDR-H2 comprising the amino acid sequence of SEQ  
 ID NO:20, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:25, and a  
 5 light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid  
 sequence of SEQ ID NO:29, (v) CDR-L2 comprising the amino acid sequence of SEQ ID  
 NO:32, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:38, or  
 (f) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino  
 acid sequence of SEQ ID NO:18, (ii) CDR-H2 comprising the amino acid sequence of SEQ  
 10 ID NO:20, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:26, and a  
 light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid  
 sequence of SEQ ID NO:29, (v) CDR-L2 comprising the amino acid sequence of SEQ ID  
 NO:32, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:38, or  
 (g) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino  
 15 acid sequence of SEQ ID NO:18, (ii) CDR-H2 comprising the amino acid sequence of SEQ  
 ID NO:20, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:27, and a  
 light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid  
 sequence of SEQ ID NO:30, (v) CDR-L2 comprising the amino acid sequence of SEQ ID  
 NO:33, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:39.

20 More particularly, the the bispecific OX40 antibody comprises at least one antigen  
 binding domain capable of specific binding to OX40 comprising  
 (a) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino  
 acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ  
 ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:22, and a  
 25 light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid  
 sequence of SEQ ID NO:28, (v) CDR-L2 comprising the amino acid sequence of SEQ ID  
 NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:35.

In a further aspect, provided is a bispecific OX40 antibody comprising at least one  
 antigen binding domain capable of specific binding to a tumor-associated antigen, in  
 30 particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or  
 delaying progression of cancer, wherein the bispecific OX40 antibody comprises at least one  
 antigen binding domain capable of specific binding to OX40 comprising  
 (a) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID  
 NO:40 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of  
 35 SEQ ID NO:41, or  
 (b) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID  
 NO:42 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of



SEQ ID NO:43, or

(c) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:44 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:45, or

5 (d) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:46 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:47, or

(a) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:48 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of

10 SEQ ID NO:49, or

(a) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:50 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:51, or

(a) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID  
15 NO:52 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:53.

In a particular aspect, the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to OX40 comprising

(a) a heavy chain variable region (V<sub>H</sub>OX40) that is at least 90%, 95%, 96%, 97%, 98%, or  
20 99% identical to an amino acid sequence of SEQ ID NO:40 and a light chain variable region (V<sub>L</sub>OX40) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence of SEQ ID NO:41.

More particularly, bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to OX40 comprising a heavy chain variable region

25 (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:40 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:41.

In one aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying

30 progression of cancer, wherein the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is an antigen binding molecule further comprising a Fc domain composed of a first and a second subunit capable of stable association. In particular, the bispecific OX40 antibody is an antigen binding molecule comprising an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc  
35 domain. More particularly, the bispecific OX40 antibody is an antigen binding molecule comprising a Fc domain that comprises one or more amino acid substitution that reduces

binding to an Fc receptor and/or effector function. In a particular aspect, the bispecific OX40 antibody comprises an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G.

In another aspect of the invention, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the bispecific OX40 antibody comprises monovalent binding to a tumor associated target and and at least bivalent binding to OX40. In one aspect, the anti-FAP/anti-OX40 bispecific antibody comprises monovalent binding to a tumor associated target and and bivalent binding to OX40. In a particular aspect, the anti-FAP/anti-OX40 bispecific antibody comprises monovalent binding to a tumor associated target and and tetravalent binding to OX40.

In another aspect, the invention provides a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the bispecific OX40 antibody comprises a first Fab fragment capable of specific binding to OX40 fused at the C-terminus of the CH1 domain to the VH domain of a second Fab fragment capable of specific binding to OX40 and a third Fab fragment capable of specific binding to OX40 fused at the C-terminus of the CH1 domain to the VH domain of a fourth Fab fragment capable of specific binding to OX40.

In one aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein before, wherein the bispecific OX40 antibody comprises

- (i) a first heavy chain comprising an amino acid sequence of SEQ ID NO:54, a second heavy chain comprising an amino acid sequence of SEQ ID NO:55, and four light chains comprising an amino acid sequence of SEQ ID NO:56, or
- (ii) a first heavy chain comprising an amino acid sequence of SEQ ID NO:57, a second heavy chain comprising an amino acid sequence of SEQ ID NO:58, and four light chains comprising an amino acid sequence of SEQ ID NO:56, or
- (i) a first heavy chain comprising an amino acid sequence of SEQ ID NO:59, a second heavy chain comprising an amino acid sequence of SEQ ID NO:60, and four light chains comprising an amino acid sequence of SEQ ID NO:56, or
- (ii) a first heavy chain comprising an amino acid sequence of SEQ ID NO:61, a second heavy

chain comprising an amino acid sequence of SEQ ID NO:62, and four light chains comprising an amino acid sequence of SEQ ID NO:56.

In one particular aspect, provided is a bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, in particular an anti-FAP/anti-OX40 bispecific antibody, for use in a method for treating or delaying progression of cancer as described herein, wherein the bispecific OX40 antibody comprises a first heavy chain comprising an amino acid sequence of SEQ ID NO:54, a second heavy chain comprising an amino acid sequence of SEQ ID NO:55, and four light chains comprising an amino acid sequence of SEQ ID NO:56.

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

The present invention relates to targeted OX40 agonists and their use in combination with T-cell activating anti-CD3 bispecific antibodies specific for a tumor-associated antigen, in particular to their use in a method for treating or delaying progression of cancer, more particularly for treating or delaying progression of solid tumors. In particular, tumor-associated antigen is CEA. The anti-CEA/anti-CD3 bispecific antibodies as used herein are bispecific antibodies comprising a first antigen binding domain that binds to CD3, and a second antigen binding domain that binds to CEA.

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

In a particular aspect, the anti-CEA/anti-CD3 bispecific antibody for use in the combination comprises a first antigen binding domain comprising a heavy chain variable region ( $V_H$ CD3) comprising CDR-H1 sequence of SEQ ID NO:63, CDR-H2 sequence of SEQ ID NO:64, and CDR-H3 sequence of SEQ ID NO:65; and/or a light chain variable region ( $V_L$ CD3) comprising CDR-L1 sequence of SEQ ID NO:66, CDR-L2 sequence of SEQ ID NO:67, and CDR-L3 sequence of SEQ ID NO:68. More particularly, the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region ( $V_H$ CD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:69 and/or a light chain variable region ( $V_L$ CD3) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:70. In a further aspect, the anti-CEA/anti-CD3 bispecific antibody comprises a heavy chain variable region ( $V_H$ CD3) comprising the amino acid sequence of SEQ ID NO:69 and/or a light chain variable region ( $V_L$ CD3) comprising the amino acid sequence of SEQ ID NO:70.

In one aspect, the antibody that specifically binds to CD3 is a full-length antibody. In one aspect, the antibody that specifically binds to CD3 is an antibody of the human IgG class, particularly an antibody of the human IgG<sub>1</sub> class. In one aspect, the antibody that specifically binds to CD3 is an antibody fragment, particularly a Fab molecule or a scFv molecule, more particularly a Fab molecule. In a particular aspect, the antibody that specifically binds to CD3 is a crossover Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged (i.e. replaced by each other). In one aspect, the antibody that specifically binds to CD3 is a humanized antibody.

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

- (a) a heavy chain variable region (V<sub>H</sub>CEA) comprising CDR-H1 sequence of SEQ ID NO:71, CDR-H2 sequence of SEQ ID NO:72, and CDR-H3 sequence of SEQ ID NO:73, and/or a light chain variable region (V<sub>L</sub>CEA) comprising CDR-L1 sequence of SEQ ID NO:74, CDR-L2 sequence of SEQ ID NO:75, and CDR-L3 sequence of SEQ ID NO:76, or
- (b) a heavy chain variable region (V<sub>H</sub>CEA) comprising CDR-H1 sequence of SEQ ID NO:79, CDR-H2 sequence of SEQ ID NO:80, and CDR-H3 sequence of SEQ ID NO:81, and/or a light chain variable region (V<sub>L</sub>CEA) comprising CDR-L1 sequence of SEQ ID NO:82, CDR-L2 sequence of SEQ ID NO:83, and CDR-L3 sequence of SEQ ID NO:84.

More particularly, the anti-CEA/anti-CD3 bispecific comprises a second antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CEA) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:77 and/or a light chain variable region (V<sub>L</sub>CEA) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:78. In a further aspect, the anti-CEA/anti-CD3 bispecific comprises a second antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CEA) comprising the amino acid sequence of SEQ ID NO:77 and/or a light chain variable region (V<sub>L</sub>CEA) comprising the amino acid sequence of SEQ ID NO:78. In another aspect, the anti-CEA/anti-CD3 bispecific comprises a second antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CEA) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:85 and/or a light chain variable region (V<sub>L</sub>CEA) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:86. In a further aspect, the anti-CEA/anti-CD3 bispecific comprises a second antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CEA) comprising the amino acid sequence of SEQ ID NO:85 and/or a light chain variable region (V<sub>L</sub>CEA) comprising the amino acid sequence of SEQ ID NO:86.

In another particular aspect, the anti-CEA/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to CEA. In particular, the anti-CEA/anti-CD3 bispecific antibody comprises a third antigen binding domain comprising

- (a) a heavy chain variable region ( $V_H$ CEA) comprising CDR-H1 sequence of SEQ ID NO:71, CDR-H2 sequence of SEQ ID NO:72, and CDR-H3 sequence of SEQ ID NO:73, and/or a light chain variable region ( $V_L$ CEA) comprising CDR-L1 sequence of SEQ ID NO:74, CDR-L2 sequence of SEQ ID NO:75, and CDR-L3 sequence of SEQ ID NO:76, or
- 5 (b) a heavy chain variable region ( $V_H$ CEA) comprising CDR-H1 sequence of SEQ ID NO:79, CDR-H2 sequence of SEQ ID NO:80, and CDR-H3 sequence of SEQ ID NO:81, and/or a light chain variable region ( $V_L$ CEA) comprising CDR-L1 sequence of SEQ ID NO:82, CDR-L2 sequence of SEQ ID NO:83, and CDR-L3 sequence of SEQ ID NO:84.

More particularly, the anti-CEA/anti-CD3 bispecific comprises a third antigen binding domain comprising a heavy chain variable region ( $V_H$ CEA) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:77 and/or a light chain variable region ( $V_L$ CEA) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:78. In a further aspect, the anti-CEA/anti-CD3 bispecific comprises a third antigen binding domain comprising a heavy chain variable region ( $V_H$ CEA) comprising the amino acid sequence of SEQ ID NO:77 and/or a light chain variable region ( $V_L$ CEA) comprising the amino acid sequence of SEQ ID NO:78. In another particular aspect, the anti-CEA/anti-CD3 bispecific comprises a third antigen binding domain comprising a heavy chain variable region ( $V_H$ CEA) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:85 and/or a light chain variable region ( $V_L$ CEA) that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:86. In a further aspect, the anti-CEA/anti-CD3 bispecific comprises a third antigen binding domain comprising a heavy chain variable region ( $V_H$ CEA) comprising the amino acid sequence of SEQ ID NO:85 and/or a light chain variable region ( $V_L$ CEA) comprising the amino acid sequence of SEQ ID NO:86.

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

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

30 (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the

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

The Fab molecules may be fused to the Fc domain or to each other directly or through a peptide linker, comprising one or more amino acids, typically about 2-20 amino acids. Peptide linkers are known in the art and are described herein. Suitable, non-immunogenic peptide linkers include, for example,  $(G_4S)_n$ ,  $(SG_4)_n$ ,  $(G_4S)_n$  or  $G_4(SG_4)_n$  peptide linkers. "n" is generally an integer from 1 to 10, typically from 2 to 4. In one embodiment said peptide linker has a length of at least 5 amino acids, in one embodiment a length of 5 to 100, in a further embodiment of 10 to 50 amino acids. In one embodiment said peptide linker is  $(G_xS)_n$  or  $(G_xS)_nG_m$  with G=glycine, S=serine, and  $(x=3, n=3, 4, 5 \text{ or } 6, \text{ and } m=0, 1, 2 \text{ or } 3)$  or  $(x=4, n=2, 3, 4 \text{ or } 5 \text{ and } m=0, 1, 2 \text{ or } 3)$ , in one embodiment  $x=4$  and  $n=2 \text{ or } 3$ , in a further embodiment  $x=4$  and  $n=2$ . In one embodiment said peptide linker is  $(G_4S)_2$ . A particularly suitable peptide linker for fusing the Fab light chains of the first and the second Fab molecule to each other is  $(G_4S)_2$ . An exemplary peptide linker suitable for connecting the Fab heavy chains of the first and the second Fab fragments comprises the sequence (D)-(G<sub>4</sub>S)<sub>2</sub>. Another suitable such linker comprises the sequence  $(G_4S)_4$ . Additionally, linkers may comprise (a portion of) an immunoglobulin hinge region. Particularly where a Fab molecule is fused to the N-terminus of an Fc domain subunit, it may be fused via an immunoglobulin hinge region or a portion thereof, with or without an additional peptide linker.

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

In a particular aspect, the anti-CEA/anti-CD3 bispecific antibody comprises two polypeptides that are at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 87, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 88, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 89, and a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 90. In a further particular embodiment, the bispecific antibody comprises two polypeptides of SEQ ID NO: 87, a polypeptide of SEQ ID NO: 88, a polypeptide of SEQ ID NO: 89 and a polypeptide of SEQ ID NO: 90 (CEA CD3 TCB).

In a further particular aspect, the anti-CEA/anti-CD3 bispecific antibody comprises two polypeptides that are at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO:91, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO:92, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO:93, and a polypeptide that is at least 95%, 96%, 97%,

98%, or 99% identical to the sequence of SEQ ID NO:94. In a further particular embodiment, the bispecific antibody comprises two polypeptides of SEQ ID NO:91, a polypeptide of SEQ ID NO:92, a polypeptide of SEQ ID NO:93 and a polypeptide of SEQ ID NO:94 (CEACAM5 CD3 TCB).

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

In a further aspect, the anti-CEA/anti-CD3 bispecific antibody may also comprise a bispecific T cell engager (BiTE®). In a further aspect, the anti-CEA/anti-CD3 bispecific antibody is a bispecific antibody as described in WO 2007/071426 or WO 2014/131712. In  
10 another aspect, the bispecific antibody is MEDI565 (AMG211).

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

The present invention also relates to anti-FolR1/anti-CD3 bispecific antibodies and their use in combination with targeted OX40 agonists, in particular to their use in a method for treating or delaying progression of cancer, more particularly for treating or delaying  
15 progression of solid tumors. The anti-FolR1/anti-CD3 bispecific antibodies as used herein are bispecific antibodies comprising a first antigen binding domain that binds to CD3, and a second antigen binding domain that binds to FolR1. In a particular, the anti-FolR1/anti-CD3 bispecific antibodies as used herein comprise a third antigen binding domain that binds to FolR1.

20 In one aspect, the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CD3), a second antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>FolR1), a third antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>FolR1) and three times a common light chain variable region.

25 In another aspect, the first antigen binding domain comprises a heavy chain variable region (V<sub>H</sub>CD3) comprising CDR-H1 sequence of SEQ ID NO:95, CDR-H2 sequence of SEQ ID NO:96, and CDR-H3 sequence of SEQ ID NO:97; the second antigen binding domain comprises a heavy chain variable region (V<sub>H</sub>FolR1) comprising CDR-H1 sequence of SEQ ID NO:98, CDR-H2 sequence of SEQ ID NO:99, and CDR-H3 sequence of SEQ ID  
30 NO:100; the third antigen binding domain comprises a heavy chain variable region (V<sub>H</sub>FolR1) comprising CDR-H1 sequence of SEQ ID NO:98, CDR-H2 sequence of SEQ ID NO:99, and CDR-H3 sequence of SEQ ID NO:100; and the common light chains comprise a CDR-L1 sequence of SEQ ID NO:101, CDR-L2 sequence of SEQ ID NO:102, and CDR-L3 sequence of SEQ ID NO:103. In another aspect, the first antigen binding domain comprises a heavy

chain variable region (V<sub>H</sub>CD3) comprising the sequence of SEQ ID NO:104; the second antigen binding domain comprises a heavy chain variable region (V<sub>H</sub>FolR1) comprising the sequence of SEQ ID NO:105; the third antigen binding domain comprises a heavy chain variable region (V<sub>H</sub>FolR1) comprising the sequence of SEQ ID NO:105; and the common light chains comprise the sequence of SEQ ID NO:106.

In a particular aspect, the anti-FolR1/anti-CD3 bispecific antibody comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO:107, a second heavy chain comprising the amino acid sequence of SEQ ID NO:108 and three times a common light chain of SEQ ID NO: 109.

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

In one aspect of the invention, the targeted OX40 agonists, in particular bispecific OX40 antibodies comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen are for use in a method for treating or delaying progression of cancer, wherein the targeted OX40 agonists are used in combination with T-cell activating anti-CD3 bispecific antibodies specific for a tumor-associated antigen, in particular anti-CEA/anti-CD3 bispecific antibodies or anti-FolR1/anti-CD3 bispecific antibodies, and additionally they are combined with an agent blocking PD-L1/PD-1 interaction. In one aspect, an agent blocking PD-L1/PD-1 interaction is a PD-L1 binding antagonist or a PD-1 binding antagonist. In particular, the agent blocking PD-L1/PD-1 interaction is an anti-PD-L1 antibody or an anti-PD-1 antibody.

The term “**PD-L1**”, also known as CD274 or B7-H1, refers to any native PD-L1 from any vertebrate source, including mammals such as primates (e.g. humans) non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), in particular to “human PD-L1”. The amino acid sequence of complete human PD-L1 is shown in UniProt (www.uniprot.org) accession no. Q9NZQ7 (SEQ ID NO:110). The term “**PD-L1 binding antagonist**” refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L1 with either one or more of its binding partners, such as PD-1, B7-1. In some embodiments, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, the PD-L1 binding antagonist inhibits binding of PD-L1 to PD-1 and/or B7-1. In some embodiments, the PD-L1 binding antagonists include anti-PD-L1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L1 with one or more of its binding partners, such as PD-1, B7-1. In one embodiment, a PD-L1 binding antagonist reduces the negative co-stimulatory signal mediated



by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L1 so as to render a dysfunctional T-cell less dysfunctional (e.g., enhancing effector responses to antigen recognition). In particular, a PD-L1 binding antagonist is an anti-PD-L1 antibody. The term “**anti-PD-L1 antibody**” or “antibody binding to human PD-L1” or  
5 “antibody that specifically binds to human PD-L1” or “antagonistic anti-PD-L1” refers to an antibody specifically binding to the human PD-L1 antigen with a binding affinity of KD-value of  $1.0 \times 10^{-8}$  mol/l or lower, in one aspect of a KD-value of  $1.0 \times 10^{-9}$  mol/l or lower. The binding affinity is determined with a standard binding assay, such as surface plasmon resonance technique (BIAcore®, GE-Healthcare Uppsala, Sweden).

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

The term “**PD-1**”, also known as CD279, PD1 or programmed cell death protein 1,  
25 refers to any native PD-L1 from any vertebrate source, including mammals such as primates (e.g. humans) non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), in particular to the human protein PD-1 with the amino acid sequence as shown in UniProt ([www.uniprot.org](http://www.uniprot.org)) accession no. Q15116 (SEQ ID NO:111). The term “**PD-1 binding antagonist**” refers to a molecule that inhibits the binding of PD-1 to its ligand  
30 binding partners. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2. In particular, a PD-L1 binding antagonist is an anti-PD-L1 antibody. The term “**anti-PD-1 antibody**” or “antibody binding to human PD-1” or “antibody  
35 that specifically binds to human PD-1” or “antagonistic anti-PD-1” refers to an antibody specifically binding to the human PD1 antigen with a binding affinity of KD-value of  $1.0 \times 10^{-8}$  mol/l or lower, in one aspect of a KD-value of  $1.0 \times 10^{-9}$  mol/l or lower. The binding

affinity is determined with a standard binding assay, such as surface plasmon resonance technique (BIAcore®, GE-Healthcare Uppsala, Sweden).

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

### **Preparation of bispecific antibodies for use in the invention**

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

Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, Nature 305: 537 (1983)), WO 93/08829, and Traunecker et al., EMBO J. 10: 3655 (1991)), and “knob-in-hole” engineering (see, e.g., U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking of two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., Science, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., J. Immunol., 148(5):1547-1553 (1992)); using “diabody” technology for making bispecific antibody fragments (see, e.g., Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see, e.g., Gruber et al., J. Immunol., 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. J. Immunol. 147: 60 (1991).

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

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

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

As it is a single polypeptide, the bispecific T cell engager may be expressed using any  
20 prokaryotic or eukaryotic cell expression system known in the art, e.g., a CHO cell line. However, specific purification techniques (see, e.g., EP1691833) may be necessary to separate monomeric bispecific T cell engagers from other multimeric species, which may have biological activities other than the intended activity of the monomer. In one exemplary purification scheme, a solution containing secreted polypeptides is first subjected to a metal  
25 affinity chromatography, and polypeptides are eluted with a gradient of imidazole concentrations. This eluate is further purified using anion exchange chromatography, and polypeptides are eluted using with a gradient of sodium chloride concentrations. Finally, this eluate is subjected to size exclusion chromatography to separate monomers from multimeric species. In one aspect, the bispecific bispecific antibodies used in the invention are composed  
30 of a single polypeptide chain comprising two single chain FV fragments (scFV) fused to each other by a peptide linker.

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

The Fc domain of the antigen binding molecules of the invention consists of a pair of polypeptide chains comprising heavy chain domains of an immunoglobulin molecule. For  
35 example, the Fc domain of an immunoglobulin G (IgG) molecule is a dimer, each subunit of

which comprises the CH2 and CH3 IgG heavy chain constant domains. The two subunits of the Fc domain are capable of stable association with each other.

The Fc domain confers favorable pharmacokinetic properties to the antigen binding molecules of the invention, including a long serum half-life which contributes to good accumulation in the target tissue and a favorable tissue-blood distribution ratio. At the same time it may, however, lead to undesirable targeting of the bispecific antibodies of the invention to cells expressing Fc receptors rather than to the preferred antigen-bearing cells. Accordingly, in particular aspects, the Fc domain of the antigen binding molecules of the invention exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG1 Fc domain. In one aspect, the Fc does not substantially bind to an Fc receptor and/or does not induce effector function. In a particular aspect the Fc receptor is an Fc $\gamma$  receptor. In one aspect, the Fc receptor is a human Fc receptor. In a specific aspect, the Fc receptor is an activating human Fc $\gamma$  receptor, more specifically human Fc $\gamma$ RIIIa, Fc $\gamma$ RI or Fc $\gamma$ RIIa, most specifically human Fc $\gamma$ RIIIa. In one aspect, the Fc domain does not induce effector function. The reduced effector function can include, but is not limited to, one or more of the following: reduced complement dependent cytotoxicity (CDC), reduced antibody-dependent cell-mediated cytotoxicity (ADCC), reduced antibody-dependent cellular phagocytosis (ADCP), reduced cytokine secretion, reduced immune complex-mediated antigen uptake by antigen-presenting cells, reduced binding to NK cells, reduced binding to macrophages, reduced binding to monocytes, reduced binding to polymorphonuclear cells, reduced direct signaling inducing apoptosis, reduced dendritic cell maturation, or reduced T cell priming.

In certain aspects, one or more amino acid modifications may be introduced into the Fc 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.

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

In one aspect, the Fc domain of the antibody of the invention comprises one or more amino acid mutation that reduces the binding affinity of the Fc domain to an Fc receptor and/or effector function. Typically, the same one or more amino acid mutation is present in each of the two subunits of the Fc domain. In particular, the Fc domain comprises an amino acid substitution at a position of E233, L234, L235, N297, P331 and P329 (EU numbering).

In particular, the Fc domain comprises amino acid substitutions at positions 234 and 235 (EU numbering) and/or 329 (EU numbering) of the IgG heavy chains. More particularly, provided is an antibody according to the invention which comprises an Fc domain with the amino acid substitutions L234A, L235A and P329G ("P329G LALA", EU numbering) in the IgG heavy chains. The amino acid substitutions L234A and L235A refer to the so-called LALA mutation. The "P329G LALA" combination of amino acid substitutions almost completely abolishes Fc $\gamma$  receptor binding of a human IgG1 Fc domain and is described in International Patent Appl. Publ. No. WO 2012/130831 A1 which also describes methods of preparing such mutant Fc domains and methods for determining its properties such as Fc receptor binding or effector functions.

Fc domains with reduced Fc receptor binding and/or effector function also include those with substitution of one or more of Fc domain residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

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

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

Binding to Fc receptors can be easily determined e.g. by ELISA, or by Surface Plasmon Resonance (SPR) using standard instrumentation such as a BIAcore instrument (GE Healthcare), and Fc receptors such as may be obtained by recombinant expression. Alternatively, binding affinity of Fc domains or cell activating antibodies comprising an Fc domain for Fc receptors may be evaluated using cell lines known to express particular Fc receptors, such as human NK cells expressing Fc $\gamma$ IIIa receptor.

Effector function of an Fc domain, or antibodies of the invention comprising an Fc domain, can be measured by methods known in the art. A suitable assay for measuring ADCC

is described herein. Other examples of in vitro assays to assess ADCC activity of a molecule of interest are described in U.S. Patent No. 5,500,362; Hellstrom et al. Proc Natl Acad Sci USA 83, 7059-7063 (1986) and Hellstrom et al., Proc Natl Acad Sci USA 82, 1499-1502 (1985); U.S. Patent No. 5,821,337; Bruggemann et al., J Exp Med 166, 1351-1361 (1987).

5 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  
10 may be assessed in vivo, e.g. in a animal model such as that disclosed in Clynes et al., Proc Natl Acad Sci USA 95, 652-656 (1998).

In some aspects, binding of the Fc domain to a complement component, specifically to C1q, is reduced. Accordingly, in some embodiments wherein the Fc domain is engineered to have reduced effector function, said reduced effector function includes reduced CDC. C1q  
15 binding assays may be carried out to determine whether the bispecific antibodies of the invention are able to bind C1q and hence has CDC activity. See e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J Immunol Methods 202, 163 (1996); Cragg et al., Blood 101, 1045-1052 (2003); and Cragg and Glennie, Blood 103,  
20 2738-2743 (2004)).

### **Fc domain modifications promoting heterodimerization**

The bispecific antigen binding molecules of the invention comprise different antigen-binding sites, fused to one or the other of the two subunits of the Fc domain, thus the two subunits of the Fc domain may be comprised in two non-identical polypeptide chains.

25 Recombinant co-expression of these polypeptides and subsequent dimerization leads to several possible combinations of the two polypeptides. To improve the yield and purity of the bispecific antibodies of the invention in recombinant production, it will thus be advantageous to introduce in the Fc domain of the bispecific antigen binding molecules of the invention a modification promoting the association of the desired polypeptides.

30 Accordingly, in particular aspects the invention relates to the bispecific antigen binding molecule comprising (a) at least one antigen binding domain capable of specific binding to a tumor-associated antigen, (b) at least one antigen binding domain capable of specific binding to OX40, 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  
35 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”  
5 modification in the other one of the two subunits of the Fc domain. Thus, the invention relates to an antigen binding molecule comprising (a) at least one antigen binding domain capable of specific binding to a tumor-associated antigen, (b) at least one antigen binding domain capable of specific binding to OX40, 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  
10 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).

15 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  
20 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).

25 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  
30 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  
35 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).

5 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  
10 (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  
15 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.

20 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  
25 antibody comprising a heavy chain including a C-terminal CH3 domain as specified herein, comprises the C-terminal glycine-lysine dipeptide (G446 and K447, numbering according to Kabat EU index). In one embodiment of all aspects as reported herein, a bispecific antibody comprising a heavy chain including a C-terminal CH3 domain, as specified herein, comprises a C-terminal glycine residue (G446, numbering according to Kabat EU index).

### 30 **Modifications in the Fab domains**

In one aspect, the invention relates to bispecific antibodies comprising at least one Fab fragment, wherein either the variable domains VH and VL or the constant domains CH1 and CL are exchanged. The bispecific antibodies are prepared according to the Crossmab technology.



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

In one aspect, the invention relates to a bispecific antigen binding molecule comprising a Fab fragment, wherein 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 another aspect, the invention relates to a bispecific antigen binding molecule comprising a Fab fragment, wherein the variable domains VL and VH are replaced by each other so that the VH domain is part of the light chain and the VL domain is part of the heavy chain.

In another aspect, and to further improve correct pairing, the bispecific antigen binding 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 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 at position 213 (EU numbering) have been substituted by glutamic acid (E).

### **Polynucleotides**

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

The isolated polynucleotides encoding the antibodies of the invention may be expressed as a single polynucleotide that encodes the entire antigen binding molecule or as multiple (e.g., two or more) polynucleotides that are co-expressed. Polypeptides encoded by polynucleotides that are co-expressed may associate through, e.g., disulfide bonds or other means to form a functional antigen binding molecule. For example, the light chain portion of an immunoglobulin may be encoded by a separate polynucleotide from the heavy chain portion of the immunoglobulin. When co-expressed, the heavy chain polypeptides will associate with the light chain polypeptides to form the immunoglobulin.

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

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

### **Recombinant Methods**

Bispecific antibodies as used in the invention may be obtained, for example, by solid-  
10   state peptide synthesis (e.g. Merrifield solid phase synthesis) or recombinant production. For recombinant production one or more polynucleotide encoding the antibody or polypeptide fragments thereof, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such polynucleotide may be readily isolated and sequenced using conventional procedures. In one aspect of the invention, a vector,  
15   preferably an expression vector, comprising one or more of the polynucleotides of the invention is provided. Methods which are well known to those skilled in the art can be used to construct expression vectors containing the coding sequence of the antibody (fragment) along with appropriate transcriptional/translational control signals. These methods include in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination/genetic  
20   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  
25   expression cassette into which the polynucleotide encoding the antibody or polypeptide fragments thereof (i.e. the coding region) is cloned in operable association with a promoter and/or other transcription or translation control elements. As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it may be considered  
30   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,  
35   or may comprise two or more coding regions, e.g. a vector of the present invention may encode one or more polypeptides, which are post- or co-translationally separated into the final

proteins via proteolytic cleavage. In addition, a vector, polynucleotide, or nucleic acid of the invention may encode heterologous coding regions, either fused or unfused to a polynucleotide encoding the antibody of the invention or polypeptide fragments thereof, or variants or derivatives thereof. Heterologous coding regions include without limitation

5 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

10 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

15 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

20 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

25 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  $\alpha$ -globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-

30 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).

35 The expression cassette may also include other features such as an origin of replication, and/or chromosome integration elements such as retroviral long terminal repeats (LTRs), or adeno-associated viral (AAV) inverted terminal repeats (ITRs).

Polynucleotide and nucleic acid coding regions of the present invention may be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide of the present invention. For example, if secretion of the antibody or polypeptide fragments thereof is desired, DNA  
5 encoding a signal sequence may be placed upstream of the nucleic acid an antibody of the invention or polypeptide fragments thereof. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that  
10 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  
15 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  $\beta$ -glucuronidase.

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

In a further aspect of the invention, a host cell comprising one or more polynucleotides of the invention is provided. In certain embodiments a host cell comprising one or more  
25 vectors of the invention is provided. The polynucleotides and vectors may incorporate any of the features, singly or in combination, described herein in relation to polynucleotides and vectors, respectively. In one aspect, a host cell comprises (e.g. has been transformed or transfected with) a vector comprising a polynucleotide that encodes (part of) an antibody of the invention of the invention. As used herein, the term "host cell" refers to any kind of  
30 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  
35 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., YO, NS0, Sp20 cell). Standard technologies are known in the art to express foreign genes in these systems. Cells expressing a polypeptide comprising either the heavy or the light chain of an immunoglobulin, may be engineered so as to also express the other of the immunoglobulin

chains such that the expressed product is an immunoglobulin that has both a heavy and a light chain.

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

In certain embodiments the moieties capable of specific binding to a target cell antigen  
10 (e.g. Fab fragments) forming part of the antigen binding molecule comprise at least an immunoglobulin variable region capable of binding to an antigen. Variable regions can form part of and be derived from naturally or non-naturally occurring antibodies and fragments thereof. Methods to produce polyclonal antibodies and monoclonal antibodies are well known in the art (see e.g. Harlow and Lane, "Antibodies, a laboratory manual", Cold Spring Harbor  
15 Laboratory, 1988). Non-naturally occurring antibodies can be constructed using solid phase-peptide synthesis, can be produced recombinantly (e.g. as described in U.S. patent No. 4,186,567) or can be obtained, for example, by screening combinatorial libraries comprising variable heavy chains and variable light chains (see e.g. U.S. Patent. No. 5,969,108 to McCafferty).

20 Any animal species of immunoglobulin can be used in the invention. Non-limiting immunoglobulins useful in the present invention can be of murine, primate, or human origin. If the fusion protein is intended for human use, a chimeric form of immunoglobulin may be used wherein the constant regions of the immunoglobulin are from a human. A humanized or fully human form of the immunoglobulin can also be prepared in accordance with methods  
25 well known in the art (see e. g. U.S. Patent No. 5,565,332 to Winter). Humanization may be achieved by various methods including, but not limited to (a) grafting the non-human (e.g., donor antibody) CDRs onto human (e.g. recipient antibody) framework and constant regions with or without retention of critical framework residues (e.g. those that are important for retaining good antigen binding affinity or antibody functions), (b) grafting only the non-  
30 human specificity-determining regions (SDRs or a-CDRs; the residues critical for the antibody-antigen interaction) onto human framework and constant regions, or (c) transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues. Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, Front Biosci 13, 1619-1633 (2008), and  
35 are further described, e.g., in Riechmann et al., Nature 332, 323-329 (1988); Queen et al., Proc Natl Acad Sci USA 86, 10029-10033 (1989); US Patent Nos. 5,821,337, 7,527,791,

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

In certain aspects, the antibodies are engineered to have enhanced binding affinity 25 according to, for example, the methods disclosed in PCT publication WO 2012/020006 (see Examples relating to affinity maturation) or U.S. Pat. Appl. Publ. No. 2004/0132066. The ability of the antigen binding molecules of the invention to bind to a specific antigenic determinant can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. surface plasmon resonance technique 30 (Liljeblad, et al., *Glyco J* 17, 323-329 (2000)), and traditional binding assays (Heeley, *Endocr Res* 28, 217-229 (2002)). Competition assays may be used to identify an antigen binding molecule that competes with a reference antibody for binding to a particular antigen. In certain embodiments, such a competing antigen binding molecule binds to the same epitope (e.g. a linear or a conformational epitope) that is bound by the reference antigen binding 35 molecule. Detailed exemplary methods for mapping an epitope to which an antigen binding molecule binds are provided in Morris (1996) “Epitope Mapping Protocols”, in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ). In an exemplary competition assay,

immobilized antigen is incubated in a solution comprising a first labeled antigen binding molecule that binds to the antigen and a second unlabeled antigen binding molecule that is being tested for its ability to compete with the first antigen binding molecule for binding to the antigen. The second antigen binding molecule may be present in a hybridoma supernatant.

5 As a control, immobilized antigen is incubated in a solution comprising the first labeled antigen binding molecule but not the second unlabeled antigen binding molecule. After incubation under conditions permissive for binding of the first antibody to the antigen, excess unbound antibody is removed, and the amount of label associated with immobilized antigen is measured. If the amount of label associated with immobilized antigen is substantially reduced  
10 in the test sample relative to the control sample, then that indicates that the second antigen binding molecule is competing with the first antigen binding molecule for binding to the antigen. See Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

Antibodies of the invention prepared as described herein may be purified by art-known  
15 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  
20 be used to which the antigen binding molecule binds. For example, for affinity chromatography purification of bispecific antibodies of the invention, a matrix with protein A or protein G may be used. Sequential Protein A or G affinity chromatography and size exclusion chromatography can be used to isolate an antigen binding molecule essentially as described in the Examples. The purity of the antigen binding molecule or fragments thereof  
25 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 antibodies as described in the Examples were shown to be intact and properly assembled as demonstrated by reducing and non-reducing SDS-PAGE.

## Assays

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

### 1. Affinity assays

The affinity of the bispecific antigen binding molecules provided herein for the  
35 corresponding receptor can be determined in accordance with the methods set forth in the



Examples by surface plasmon resonance (SPR), using standard instrumentation such as a BIAcore instrument (GE Healthcare), and receptors or target proteins such as may be obtained by recombinant expression. The affinity of the bispecific antigen binding molecule for the target cell antigen can also be determined by surface plasmon resonance (SPR), using  
5 standard instrumentation such as a BIAcore instrument (GE Healthcare), and receptors or target proteins such as may be obtained by recombinant expression. For the FAP-OX40 bispecific antibodies the methods have been described in more detail in International Patent Appl. Publ. No. WO 2017/055398 A2 or WO 2017/060144 A1. According to one aspect,  $K_D$  is measured by surface plasmon resonance using a BIACORE® T100 machine (GE  
10 Healthcare) at 25 °C.

## **2. Binding assays and other assays**

In one aspect, the FAP-OX40 bispecific antibody as reported herein is tested for its antigen binding activity as described in more detail in International Patent Appl. Publ. No. WO 2017/055398 A2 or WO 2017/060144 A1.

## **3. Activity assays**

In one aspect, assays are provided for identifying the biological activity of targeted OX40 bispecific antigen binding molecules.

In certain embodiments, an antibody as reported herein is tested for such biological activity.

## **Pharmaceutical Compositions, Formulations and Routes of Administration**

In a further aspect, the invention provides pharmaceutical compositions comprising the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen and the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific  
25 antibody or anti-FolR1/anti-CD3 bispecific antibody, provided herein, e.g., for use in any of the below therapeutic methods. In one embodiment, a pharmaceutical composition comprises an antibody provided herein and at least one pharmaceutically acceptable excipient. In another embodiment, a pharmaceutical composition comprises the antibody provided herein and at least one additional therapeutic agent, e.g., as described below.

30 In one aspect, the invention provides pharmaceutical compositions comprising an anti-FAP/anti-OX40 bispecific antibody and the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody or anti-FolR1/anti-CD3 bispecific antibody.

In another aspect, the invention provides pharmaceutical compositions comprising the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen and an agent blocking PD-L1/PD-1 interaction. In particular, the agent blocking PD-L1/PD-1 interaction is an antagonistic anti-PD-L1 antibody or an antagonistic anti-PD1 antibody. More particularly, the agent blocking PD-L1/PD-1 interaction is selected from the group consisting of atezolizumab, durvalumab, pembrolizumab and nivolumab. In a specific aspect, the agent blocking PD-L1/PD-1 interaction is atezolizumab.

Pharmaceutical compositions of the present invention comprise a therapeutically effective amount of one or more antibodies dissolved or dispersed in a pharmaceutically acceptable excipient. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that are generally non-toxic to recipients at the dosages and concentrations employed, i.e. do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation of a pharmaceutical composition comprising the active ingredients (e.g. an bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen, a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen and/or an agent blocking PD-L1/PD-1 interaction) 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, intralesional, intravenous, intraarterial intramuscular, intrathecal or intraperitoneal injection. For injection, the antigen binding molecules of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. The solution may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the fusion proteins may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. Sterile injectable solutions are prepared by incorporating the fusion proteins of the invention in the required amount in the appropriate solvent with various of the other ingredients enumerated below, as required. Sterility may be readily accomplished,

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

(18th Ed. Mack Printing Company, 1990). Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the polypeptide, which matrices are in the form of shaped articles, e.g. films, or microcapsules. In particular embodiments, prolonged absorption of an injectable composition can be brought about by the use in the compositions of agents delaying absorption, such as, for example, aluminum monostearate, gelatin or combinations thereof.

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

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

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

Pharmaceutical compositions comprising the active ingredients 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 antibody of the invention may be formulated into a composition in a free acid or base, neutral or salt form. Pharmaceutically acceptable salts are salts that substantially retain the biological activity of the free acid or base. These include the acid addition salts, e.g. those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also

be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine. Pharmaceutical salts tend to be more soluble in aqueous and other protic solvents than are the corresponding free base forms.

5       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.

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

**Administration of the anti-FAP/anti-OX40 bispecific antibody and the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody**

15       Both the anti-FAP/anti-OX40 bispecific antibody and the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody (both called substance herein) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. The methods of the present invention are particularly useful, however, in relation to therapeutic agents administered by parenteral, particularly intravenous, 20       infusion.

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

30       Both the anti-FAP/anti-OX40 bispecific antibody and the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method

of administration, the scheduling of administration, and other factors known to medical practitioners. Both the anti-FAP/anti-OX40 bispecific antibody and the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, need not be, but are optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of therapeutic agent present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of the anti-FAP/anti-OX40 bispecific antibody and the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody (when used in their combination or with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of the anti-FAP/anti-OX40 bispecific antibody, the severity and course of the disease, whether both agents are administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the therapeutic agent, and the discretion of the attending physician. Each substance is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1  $\mu\text{g/kg}$  to 15 mg/kg (e.g. 0.1 mg/kg – 10 mg/kg) of the substance can be an initial candidate dosage for administration to the subject, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1  $\mu\text{g/kg}$  to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of each substance would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the subject. Such doses may be administered intermittently, e.g. every week, every two weeks, or every three weeks (e.g. such that the subject receives from about two to about twenty, or e.g. about six doses of the therapeutic agent). An initial higher loading dose, followed by one or more lower doses, or an initial lower dose, followed by one or more higher doses may be administered. An exemplary dosing regimen comprises administering an initial dose of about 10 mg, followed by a bi-weekly dose of about 20 mg of the therapeutic agent. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

In one aspect, the administration of both the anti-FAP/anti-OX40 bispecific antibody and the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen, in particular an anti-CEA/anti-CD3 bispecific antibody, is a single administration. In certain aspects, the administration of the therapeutic agent is two or more administrations. In one  
5 such aspect, the substances are administered every week, every two weeks, or every three weeks, particularly every two weeks. In one aspect, the substance is administered in a therapeutically effective amount. In one aspect, the substance is administered at a dose of about 50 µg/kg, about 100 µg/kg, about 200 µg/kg, about 300 µg/kg, about 400 µg/kg, about 500 µg/kg, about 600 µg/kg, about 700 µg/kg, about 800 µg/kg, about 900 µg/kg or about  
10 1000 µg/kg. In one embodiment, the anti-CEA/anti-CD3 bispecific antibody is administered at a dose which is higher than the dose of the anti-CEA/anti-CD3 bispecific antibody in a corresponding treatment regimen without the administration of the anti-FAP/anti-OX40 bispecific antibody. In one aspect the administration of the anti-CEA/anti-CD3 bispecific antibody comprises an initial administration of a first dose of the the anti-CEA/anti-CD3  
15 bispecific antibody, and one or more subsequent administrations of a second dose of the anti-CEA/anti-CD3 bispecific antibody, wherein the second dose is higher than the first dose. In one aspect, the administration of the anti-CEA/anti-CD3 bispecific antibody comprises an initial administration of a first dose of the anti-CEA/anti-CD3 bispecific antibody, and one or more subsequent administrations of a second dose of the anti-CEA/anti-CD3 bispecific  
20 antibody, wherein the first dose is not lower than the second dose.

In one aspect, the administration of the anti-CEA/anti-CD3 bispecific antibody in the treatment regimen according to the invention is the first administration of that the anti-CEA/anti-CD3 bispecific antibody to the subject (at least within the same course of treatment). In one aspect, no administration of the anti-FAP/anti-OX40 bispecific antibody is made to the  
25 subject prior to the administration of the anti-CEA/anti-CD3 bispecific antibody.

In the present invention, the combination of the anti-CEA/anti-CD3 bispecific antibody and the anti-FAP/anti-OX40 bispecific antibody can be used in combination with further agents in a therapy. For instance, at least one additional therapeutic agent may be co-administered. In certain aspects, an additional therapeutic agent is an immunotherapeutic  
30 agent.

In one aspect, the combination of the anti-FAP/anti-OX40 bispecific antibody and the anti-CEA/anti-CD3 bispecific antibody can be used in combination with a PD-1 axis binding antagonist. In one aspect, the PD-1 axis binding antagonist is selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding  
35 antagonist. In a particular aspect, PD-1 axis binding antagonist is a PD-1 binding antagonist, in particular an antagonistic PD-1 antibody. In one aspect, the PD-1 axis binding antagonist is

selected MDX 1106 (nivolumab, CAS Reg. No. 946414-94-4), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, and BGB-108. In another particular aspect, the PD-1 axis binding antagonist is a PD-L1 binding antagonist, in particular an antagonistic PD-L1 antibody. In one aspect, the PD-1 axis binding antagonist is selected from MPDL3280A (atezolizumab), YW243.55.S70, MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab). In one aspect, the PD-L1 antagonistic antibody is selected from the group consisting of atezolizumab, durvalumab and avelumab. More particularly, the combination of the anti-FAP/anti-OX40 bispecific antibody and the anti-CEA/anti-CD3 bispecific antibody can be used in combination with MPDL3280A (atezolizumab). In some aspects, atezolizumab may be administered at a dose of about 800 mg to about 1500 mg every three weeks (e.g., about 1000 mg to about 1300 mg every three weeks, e.g., about 1100 mg to about 1200 mg every three weeks). In one particular aspect, atezolizumab is administered at a dose of about 1200 mg every three weeks.

The period of time between the administration of the PD-1 axis binding antagonist and the administration of the combination therapy comprising the anti-CEA/anti-CD3 bispecific antibody and the anti-FAP/anti-OX40 bispecific antibody and the doses are chosen such as to effectively shrink the tumor in the subject prior to administration of the combination therapy.

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

## **Therapeutic methods and compositions**

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

In one aspect, provided is a method for treating or delaying progression of cancer in a subject comprising administering to the subject an effective amount of an anti-FAP/anti-OX40 bispecific antibody and an anti-CEA/anti-CD3 antibody.

In one such aspect, the method further comprises administering to the subject an effective amount of at least one additional therapeutic agent. In further embodiments, herein is provided a method for tumor shrinkage comprising administering to the subject an effective



amount of an anti-FAP/anti-OX40 bispecific antibody and an anti-CEA/anti-CD3 antibody. An “individual” or a “subject” according to any of the above aspects is preferably a human.

In further aspects, a composition for use in cancer immunotherapy is provided comprising an anti-FAP/anti-OX40 bispecific antibody and an anti-CEA/anti-CD3 antibody.

- 5 In certain embodiments, a composition comprising an anti-FAP/anti-OX40 bispecific antibody and an anti-CEA/anti-CD3 antibody for use in a method of cancer immunotherapy is provided.

In a further aspect, herein is provided the use of a composition comprising an anti-FAP/anti-OX40 bispecific antibody and an anti-CEA/anti-CD3 antibody in the manufacture  
10 or preparation of a medicament. In one embodiment, the medicament is for treatment of solid tumors. In a further embodiment, the medicament is for use in a method of tumor shrinkage comprising administering to an individual having a solid tumor an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent. In a further  
15 embodiment, the medicament is for treating solid tumors. In some aspects, the individual has CEA positive cancer. In some aspects, CEA positive cancer is colon cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, breast cancer, kidney cancer, esophageal cancer, or prostate cancer. In some aspects, the breast cancer is a breast carcinoma or a breast adenocarcinoma. In some aspects, the breast  
20 carcinoma is an invasive ductal carcinoma. In some aspects, the lung cancer is a lung adenocarcinoma. In some embodiments, the colon cancer is a colorectal adenocarcinoma. An “individual” according to any of the above embodiments may be a human.

The combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate  
25 administration, in which case, administration of the antibody as reported herein can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent or agents. In one aspect, administration of an anti-FAP/anti-OX40 bispecific antibody and an anti-CEA/anti-CD3 antibody and optionally the administration of an additional therapeutic agent occur within about one month, or within about one, two or three weeks, or within about  
30 one, two, three, four, five, or six days, of each other.

Both the anti-FAP/anti-OX40 bispecific antibody and the anti-CEA/anti-CD3 bispecific antibody as reported herein (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular,  
35 intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by

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

5 Both the anti-FAP/anti-OX40 bispecific antibody and the anti-CEA/anti-CD3 bispecific antibody as reported herein would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method  
10 of administration, the scheduling of administration, and other factors known to medical practitioners. The antibodies need not be, but are optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibodies present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same  
15 dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

### **Articles of Manufacture (Kits)**

In another aspect of the invention, a kit containing materials useful for the treatment,  
20 prevention and/or diagnosis of the disorders described above is provided. The kit comprises at least one container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating,  
25 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). In one aspect, at least two active agents in the kit are an anti-CEA/anti-CD3 bispecific antibody and an anti-FAP/anti-OX40 bispecific antibody of the invention.

30 In a particular aspect, provided is a kit for treating or delaying progression of cancer in a subject, comprising a package comprising (A) a first composition comprising as active ingredient an anti-FAP/anti-OX40 bispecific antibody and a pharmaceutically acceptable excipient, (B) a second composition comprising as active ingredient the anti-CEA/anti-CD3 bispecific antibody and a pharmaceutically acceptable excipient, and (C) instructions for  
35 using the compositions in a combination therapy.

In one further aspect, provided is a kit for treating or delaying progression of cancer in a subject, comprising a package comprising (A) a first composition comprising as active ingredient an anti-FAP/anti-OX40 bispecific antibody and a pharmaceutically acceptable excipient, (B) a second composition comprising as active ingredient the anti-CEA/anti-CD3 bispecific antibody and a pharmaceutically acceptable excipient, (C) a third composition comprising as active ingredient the agent blocking PD-L1/PD-1 interaction and a pharmaceutically acceptable excipient, and (C) instructions for using the compositions in a combination therapy.

The label or package insert indicates how the composition is used for treating the condition of choice and provides the instructions for using the compositions in a combination therapy. Moreover, the kit may comprise (a) a first container with a composition contained therein, wherein the composition comprises an anti-FAP/anti-OX40 bispecific antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises an anti-CEA/anti-CD3 bispecific antibody of the invention. In addition, the kit may comprise one or more further containers comprising further active ingredients that can be used in combination. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition.

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

**Table C (Sequences):**

| SEQ ID NO: | Name            | Sequence                                                                                                                           |
|------------|-----------------|------------------------------------------------------------------------------------------------------------------------------------|
| 1          | FAP(4B9) CDR-H1 | SYAMS                                                                                                                              |
| 2          | FAP(4B9) CDR-H2 | AIIGSGASTYYADSVKG                                                                                                                  |
| 3          | FAP(4B9) CDR-H3 | GWFGGFNY                                                                                                                           |
| 4          | FAP(4B9) CDR-L1 | RASQSVTSSYLA                                                                                                                       |
| 5          | FAP(4B9) CDR-L2 | VGSRRAT                                                                                                                            |
| 6          | FAP(4B9) CDR-L3 | QQGIMLPPT                                                                                                                          |
| 7          | FAP(4B9) VH     | EVQLLES GGGLVQPGGSLRLSCAASGFTFSSYA<br>MSWVRQAPGKGLEWVSAIIGSGASTYYADSVK<br>GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK<br>GWFGGFNYWGQGTLLTVSS |
| 8          | FAP(4B9) VL     | EIVLTQSPGTLSPGERATLSCRASQSVTSSYLA<br>WYQQKPGQAPRLLINVGSRRTATGIPDRFSGSGSG                                                           |

| SEQ ID NO: | Name                                | Sequence                                                                                                                |
|------------|-------------------------------------|-------------------------------------------------------------------------------------------------------------------------|
|            |                                     | TDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGT KVEIK                                                                                |
| 9          | FAP (28H1) CDR-H1                   | SHAMS                                                                                                                   |
| 10         | FAP (28H1) CDR-H2                   | AIWASGEQYYADSVKG                                                                                                        |
| 11         | FAP (28H1) CDR-H3                   | GWLGNFYD                                                                                                                |
| 12         | FAP (28H1) CDR-L1                   | RASQSVSRSYLA                                                                                                            |
| 13         | FAP (28H1) CDR-L2                   | GASTRAT                                                                                                                 |
| 14         | FAP (28H1) CDR-L3                   | QQGQVIPPT                                                                                                               |
| 15         | FAP(28H1) VH                        | EVQLLESGLLVQPGGSLRLSCAASGFTFSSHA MSWVRQAPGKGLEWVSAIWASGEQYYADSVK GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAK GWLGNFYDYGQGT LVTSS |
| 16         | FAP(28H1) VL                        | EIVLTQSPGTL SLSPGERATL SCRASQSVSRSYLA WYQQKPGQAPRL LIIGASTRATGIPDRFSGSGG TDFTLTISRLEPEDFAVYYCQQGQVIPPTFGQGT KVEIK       |
| 17         | OX40(8H9,49B4,1G4, 20B7) CDR-H1     | SYAIS                                                                                                                   |
| 18         | OX40(CLC-563, CLC-564, 17A9) CDR-H1 | SYAMS                                                                                                                   |
| 19         | OX40(8H9,49B4,1G4, 20B7) CDR-H2     | GIPIFGTANYAQKFQG                                                                                                        |
| 20         | OX40(CLC-563, CLC-564, 17A9) CDR-H2 | AISGSGGSTYYADSVKG                                                                                                       |
| 21         | OX40(8H9) CDR-H3                    | EYGWMDY                                                                                                                 |
| 22         | OX40(49B4) CDR-H3                   | EYYRGPYDY                                                                                                               |
| 23         | OX40(1G4) CDR-H3                    | EYGSM DY                                                                                                                |
| 24         | OX40(20B7) CDR-H3                   | VNYPYSYWGDFDY                                                                                                           |
| 25         | OX40(CLC-563) CDR-H3                | DVGAFDY                                                                                                                 |
| 26         | OX40(CLC-564) CDR-H3                | DVGPF DY                                                                                                                |
| 27         | OX40(17A9)-CDR-H3                   | VFYRGGVSM DY                                                                                                            |
| 28         | OX40(8H9,49B4,1G4, 20B7) CDR-L1     | RASQSISSWLA                                                                                                             |
| 29         | OX40(CLC-563, CLC564) CDR-L1        | RASQSVSSSYLA                                                                                                            |
| 30         | OX40(17A9) CDR-L1                   | QGDSLRSYYAS                                                                                                             |
| 31         | OX40(8H9,49B4,1G4, 20B7) CDR-L2     | DASSLES                                                                                                                 |
| 32         | OX40(CLC-563, CLC564) CDR-L2        | GASSRAT                                                                                                                 |
| 33         | OX40(17A9) CDR-L2                   | GKNNRPS                                                                                                                 |
| 34         | OX40(8H9) CDR-L3                    | QQYLTYSRFT                                                                                                              |
| 35         | OX40(49B4) CDR-L3                   | QQYSSQPYT                                                                                                               |
| 36         | OX40(1G4) CDR-L3                    | QQYISYSMLT                                                                                                              |
| 37         | OX40(20B7) CDR-L3                   | QQYQAFSLT                                                                                                               |
| 38         | OX40(CLC-563, CLC-                  | QQYGSSPLT                                                                                                               |

| SEQ ID NO: | Name              | Sequence                                                                                                                                                                                 |
|------------|-------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|            | 564) CDR-L3       |                                                                                                                                                                                          |
| 39         | OX40(17A9) CDR-L3 | NSRVMPHNRV                                                                                                                                                                               |
| 40         | OX40(49B4) VH     | <u>QVQLVQSGAEVKKPGSSVKV</u> <u>SCKASGGTFSSYA</u><br><u>ISWVRQAPGQGLEWMGGIPIFGTANYAQKFQ</u><br><u>RVTITADKSTSTAYMELSSLRSED</u> <u>TAVYYCAREY</u><br><u>YRGPYDYWGQGT</u> <u>TVTVSS</u>     |
| 41         | OX40(49B4) VL     | <u>DIQMTQSPSTLSASVGDRVTITCRASQSISSWLA</u><br><u>WYQQKPGKAPKLLIYDASSLESGVPSRFS</u> <u>SGSGSG</u><br><u>TEFTLTISSLQPDDFATYYCQQYSSQPYTFGQGT</u><br><u>KVEIK</u>                             |
| 42         | OX40(8H9) VH      | <u>QVQLVQSGAEVKKPGSSVKV</u> <u>SCKASGGTFSSYA</u><br><u>ISWVRQAPGQGLEWMGGIPIFGTANYAQKFQ</u><br><u>RVTITADKSTSTAYMELSSLRSED</u> <u>TAVYYCAREY</u><br><u>GWMDYWGQGT</u> <u>TVTVSS</u>       |
| 43         | OX40(8H9) VL      | <u>DIQMTQSPSTLSASVGDRVTITCRASQSISSWLA</u><br><u>WYQQKPGKAPKLLIYDASSLESGVPSRFS</u> <u>SGSGSG</u><br><u>TEFTLTISSLQPDDFATYYCQQYLTYSRFTFGQG</u><br><u>TKVEIK</u>                            |
| 44         | OX40(1G4) VH      | <u>QVQLVQSGAEVKKPGSSVKV</u> <u>SCKASGGTFSSYA</u><br><u>ISWVRQAPGQGLEWMGGIPIFGTANYAQKFQ</u><br><u>RVTITADKSTSTAYMELSSLRSED</u> <u>TAVYYCAREY</u><br><u>GSMDYWGQGT</u> <u>TVTVSS</u>       |
| 45         | OX40(1G4) VL      | <u>DIQMTQSPSTLSASVGDRVTITCRASQSISSWLA</u><br><u>WYQQKPGKAPKLLIYDASSLESGVPSRFS</u> <u>SGSGSG</u><br><u>TEFTLTISSLQPDDFATYYCQQYISYSMLTFGQGT</u><br><u>KVEIK</u>                            |
| 46         | OX40(20B7) VH     | <u>QVQLVQSGAEVKKPGSSVKV</u> <u>SCKASGGTFSSYA</u><br><u>ISWVRQAPGQGLEWMGGIPIFGTANYAQKFQ</u><br><u>RVTITADKSTSTAYMELSSLRSED</u> <u>TAVYYCARV</u><br><u>NYPYSYWGDFDYWGQGT</u> <u>TVTVSS</u> |
| 47         | OX40(20B7) VL     | <u>DIQMTQSPSTLSASVGDRVTITCRASQSISSWLA</u><br><u>WYQQKPGKAPKLLIYDASSLESGVPSRFS</u> <u>SGSGSG</u><br><u>TEFTLTISSLQPDDFATYYCQQYQAFSLTFGQGT</u><br><u>KVEIK</u>                             |
| 48         | OX40(CLC-563) VH  | <u>EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYA</u><br><u>MSWVRQAPGKGLEWVSAISGSGGSTYYADSVK</u><br><u>GRFTISRDN SKNTLYLQMNSLRAED</u> <u>TAVYYCAL</u><br><u>DVGAFDYWGQ</u> <u>GALVTVSS</u>            |
| 49         | OX40(CLC-563) VL  | <u>EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLA</u><br><u>WYQQKPGQAPRLLIYGASSRATGIPDRFS</u> <u>SGSGSG</u><br><u>TDFTLTISRLEPEDFAVYYCQQYGSSPLTFGQGT</u><br><u>KVEIK</u>                            |
| 50         | OX40(CLC-564) VH  | <u>EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYA</u><br><u>MSWVRQAPGKGLEWVSAISGSGGSTYYADSVK</u><br><u>GRFTISRDN SKNTLYLQMNSLRAED</u> <u>TAVYYCAF</u><br><u>DVGPFEDYWGQGT</u> <u>LVTVSS</u>           |
| 51         | OX40(CLC-564) VL  | <u>EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLA</u><br><u>WYQQKPGQAPRLLIYGASSRATGIPDRFS</u> <u>SGSGSG</u><br><u>TDFTLTISRLEPEDFAVYYCQQYGSSPLTFGQGT</u><br><u>KVEIK</u>                            |

| SEQ ID NO: | Name                                           | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|------------|------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 52         | OX40(17A9) VH                                  | EVQLLES <del>GGGLVQPGGSLRLS</del> CAASGFTFSSYA<br>MSWVRQAPGKGLEWVSAISGSGGSTYYADSVK<br>GRFTISRDN <del>SKNTLYLQMNSLRAEDTAVYYCAR</del><br>VFYRGGVSM <del>DYWGQGT</del> LVTVSS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| 53         | OX40(17A9) VL                                  | SSELTQDPAVSVALGQTVRITCQGD <del>SLRSYYAS</del><br>WYQQKPGQAPVLVIY <del>GKNNRPSGIPDRFSGSSSG</del><br>NTASLTITGAQAED <del>EADYYCNSRVMPHNRV</del> FGG<br>GTKLTV                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 54         | HC 1<br>(49B4) VHCH1_VHCH1<br>Fc knob VH (4B9) | QVQLVQSGAEVKKPGSSVKVSC <del>KASGGTFSSYA</del><br>ISWVRQAPGQGLEWMGGIPIFGTANYAQKFQ<br>RVTITADKSTSTAYMELSSLRSEDTAVYYCAREY<br>YRGPYDYWGQGT <del>TVTVSSASTKGPSVFPLAPSS</del><br>KSTSGGTAALGCLVKDYFPEPVT <del>SVWNSGALTS</del><br>GVHTFPAVLQSSGLYSLSSV <del>TVPS</del> SSLGTQTYIC<br>NVN <del>HKPSNTKVDKKVEPKSCDGGGGSGGGGSQ</del><br>VQLVQSGAEVKKPGSSVKVSC <del>KASGGTFSSYAIS</del><br>WVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV<br>TITADKSTSTAYMELSSLRSEDTAVYYCAREYYR<br>GPYDYWGQGT <del>TVTVSSASTKGPSVFPLAPSSKST</del><br>SGGTAALGCLVKDYFPEPVT <del>SVWNSGALTSGVH</del><br>TFPAVLQSSGLYSLSSV <del>TVPS</del> SSLGTQTYICNVN<br>HKPSNTKVDKKVEPKSCDK <del>THTCPPCPAPEAAG</del><br>GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED<br>PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV<br>VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK<br>TISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL<br>VKGFYPSDIAVEWESNGQPENNYK <del>TTTPVLDSD</del><br>GSFFLYSKLTVDKSRWQQGNV <del>FSCSVMHEALH</del><br>NHYTQKSLSLSPGGGGGGSGGGGSGGGGSGGGGS<br>EVQLLES <del>GGGLVQPGGSLRLS</del> CAASGFTFSSYA<br>MSWVRQAPGKGLEWVSAIIGSGASTYYADSVK<br>GRFTISRDN <del>SKNTLYLQMNSLRAEDTAVYYCAK</del><br>GWFGGFNYWGQGT <del>LVTVSS</del> |
| 55         | HC 2<br>(49B4) VHCH1_VHCH1<br>Fc hole VL (4B9) | QVQLVQSGAEVKKPGSSVKVSC <del>KASGGTFSSYA</del><br>ISWVRQAPGQGLEWMGGIPIFGTANYAQKFQ<br>RVTITADKSTSTAYMELSSLRSEDTAVYYCAREY<br>YRGPYDYWGQGT <del>TVTVSSASTKGPSVFPLAPSS</del><br>KSTSGGTAALGCLVKDYFPEPVT <del>SVWNSGALTS</del><br>GVHTFPAVLQSSGLYSLSSV <del>TVPS</del> SSLGTQTYIC<br>NVN <del>HKPSNTKVDKKVEPKSCDGGGGSGGGGSQ</del><br>VQLVQSGAEVKKPGSSVKVSC <del>KASGGTFSSYAIS</del><br>WVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV<br>TITADKSTSTAYMELSSLRSEDTAVYYCAREYYR<br>GPYDYWGQGT <del>TVTVSSASTKGPSVFPLAPSSKST</del><br>SGGTAALGCLVKDYFPEPVT <del>SVWNSGALTSGVH</del><br>TFPAVLQSSGLYSLSSV <del>TVPS</del> SSLGTQTYICNVN<br>HKPSNTKVDKKVEPKSCDK <del>THTCPPCPAPEAAG</del><br>GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED<br>PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV<br>VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK<br>TISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA                                                                                                                                                                                                                                                                                                                  |

| SEQ ID NO: | Name                                            | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|------------|-------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|            |                                                 | VKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSVTSSYLAWYQQKPGQAPRLLINVGSRRTATGIPDRFSGSGGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGT KVEIK                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| 56         | LC (49B4)                                       | DIQMTQSPSTLSASVGDRVTITCRASQSISSWLA WYQQKPGKAPKLLIYDASSLESQVPSRFSGSGGSTEFTLTISSLQPDDFATYYCQQYSSQPYTFGQGT KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSITLTLTKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| 57         | HC 1<br>(49B4) VHCH1_VHCH1<br>Fc knob VH (28H1) | QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV TITADKSTSTAYMELSSLRSED TAVYYCAREY YRGPYDYWGQGT TTVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVT VSWNSGALTS GVHTFPAVLQSSGLYSLSSV VTPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSGQ VQLVQSGAEVKKPGSSVKVSCASGGTFSSYAIS WVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV TITADKSTSTAYMELSSLRSED TAVYYCAREYYR GPYDYWGQGT TTVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVT VSWNSGALTSGVH TFP AVLQSSGLYSLSSV VTPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLT VLVHQLDNLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL VKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSSHAMSWVRQAPGKGLEWVSAIWASGEQYYADSVKGRFTISRDN SKNTLYLQMNSLR AEDTAVYYCAK GWLGNFDYWGQGT LTVVSS |
| 58         | HC 2<br>(49B4) VHCH1_VHCH1<br>Fc hole VL (28H1) | QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV TITADKSTSTAYMELSSLRSED TAVYYCAREY YRGPYDYWGQGT TTVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVT VSWNSGALTS GVHTFPAVLQSSGLYSLSSV VTPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSGQ VQLVQSGAEVKKPGSSVKVSCASGGTFSSYAIS WVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV TITADKSTSTAYMELSSLRSED TAVYYCAREYYR GPYDYWGQGT TTVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVT VSWNSGALTSGVH                                                                                                                                                                                                                                                                                                                                                                                                                                            |

| SEQ ID NO: | Name                                           | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|------------|------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|            |                                                | TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN<br>HKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG<br>GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED<br>PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV<br>VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK<br>TISKAKGQPREPQVCTLPISRDELTKNQVSLSCA<br>VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD<br>GSFLLVSKLTVDKSRWQQGNVFSCSVMHEALH<br>NHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGS<br>EIVLTQSPGTLSLSPGERATLSCRASQSVRSYLA<br>WYQQKPGQAPRLLIIGASTRATGIPDRFSGSGSG<br>TDFTLTISRLEPEDFAVYYCQQGQVIPPTFGQGT<br>KVEIK                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| 59         | HC 1<br>(49B4) VHCH1_VHCH1<br>Fc knob VL (4B9) | QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYA<br>ISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV<br>RVTITADKSTSTAYMELSSLRSEDTAVYYCAREY<br>YRGPYDYWGQGTITVTVSSASTKGPSVFPLAPSS<br>KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS<br>GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC<br>NVNHHKPSNTKVDKKVEPKSCDGGGGSGGGGSQ<br>VQLVQSGAEVKKPGSSVKVSCASGGTFSSYAIS<br>WVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV<br>TITADKSTSTAYMELSSLRSEDTAVYYCAREYYR<br>GPYDYWGQGTITVTVSSASTKGPSVFPLAPSSKST<br>SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH<br>TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN<br>HKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG<br>GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED<br>PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV<br>VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK<br>TISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL<br>VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD<br>GSFLLYSLKLTVDKSRWQQGNVFSCSVMHEALH<br>NHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGS<br>EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLA<br>WYQQKPGQAPRLINVGSRRTATGIPDRFSGSGSG<br>TDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGT<br>KVEIK |
| 60         | HC 2<br>(49B4) VHCH1_VHCH1<br>Fc hole VH (4B9) | QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYA<br>ISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV<br>RVTITADKSTSTAYMELSSLRSEDTAVYYCAREY<br>YRGPYDYWGQGTITVTVSSASTKGPSVFPLAPSS<br>KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS<br>GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC<br>NVNHHKPSNTKVDKKVEPKSCDGGGGSGGGGSQ<br>VQLVQSGAEVKKPGSSVKVSCASGGTFSSYAIS<br>WVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV<br>TITADKSTSTAYMELSSLRSEDTAVYYCAREYYR<br>GPYDYWGQGTITVTVSSASTKGPSVFPLAPSSKST<br>SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH<br>TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN                                                                                                                                                                                                                                                                                                                                                                                                                                     |



| SEQ ID NO: | Name                                              | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|------------|---------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|            |                                                   | HKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG<br>GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED<br>PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV<br>VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK<br>TISKAKGQPREPQVCTLPISRDELTKNQVSLSCA<br>VKGFYPSDIAVEWESNGQPENNYKTTPVLDSD<br>GSFFLVSKLTVDKSRWQQGNVFSCSVMHEALH<br>NHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGS<br>EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYA<br>MSWVRQAPGKGLEWVSAIIGSGASTYYADSVK<br>GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK<br>GWFGGFNYWGQGTLLTVSS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| 61         | HC 1<br>(49B4) VHCH1_VHCH1<br>Fc wt knob VH (4B9) | QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYA<br>ISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV<br>RVTITADKSTSTAYMELSSLRSEDVAVYYCAREY<br>YRGPYDYWGQGTITVTVSSASTKGPSVFPLAPSS<br>KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS<br>GVHTFPAVLQSSGLYSLSVVTVPSSSLGTQTYIC<br>NVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSQ<br>VQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAIS<br>WVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV<br>TITADKSTSTAYMELSSLRSEDVAVYYCAREYYR<br>GPYDYWGQGTITVTVSSASTKGPSVFPLAPSSKST<br>SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH<br>TFPAPVLQSSGLYSLSVVTVPSSSLGTQTYICNVN<br>HKPSNTKVDKKVEPKSCDKTHTCPPCPAPPELLG<br>GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED<br>PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV<br>VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT<br>ISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL<br>VKGFYPSDIAVEWESNGQPENNYKTTPVLDSD<br>GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH<br>NHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGS<br>EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYA<br>MSWVRQAPGKGLEWVSAIIGSGASTYYADSVK<br>GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK<br>GWFGGFNYWGQGTLLTVSS |
| 62         | HC 2<br>(49B4) VHCH1_VHCH1<br>Fc wt hole VL (4B9) | QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYA<br>ISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV<br>RVTITADKSTSTAYMELSSLRSEDVAVYYCAREY<br>YRGPYDYWGQGTITVTVSSASTKGPSVFPLAPSS<br>KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS<br>GVHTFPAVLQSSGLYSLSVVTVPSSSLGTQTYIC<br>NVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSQ<br>VQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAIS<br>WVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV<br>TITADKSTSTAYMELSSLRSEDVAVYYCAREYYR<br>GPYDYWGQGTITVTVSSASTKGPSVFPLAPSSKST<br>SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH<br>TFPAPVLQSSGLYSLSVVTVPSSSLGTQTYICNVN<br>HKPSNTKVDKKVEPKSCDKTHTCPPCPAPPELLG                                                                                                                                                                                                                                                                                                                                                                                                       |

| SEQ ID NO: | Name                | Sequence                                                                                                                                                                                                                                                                                                                                        |
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|            |                     | GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGGGGGGSGGGGGSGGGGGSEIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPGQAPRLLINVGSRRTATGIPDRFSGSGGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGTKVEIK |
| 63         | CD3-HCDR1           | TYAMN                                                                                                                                                                                                                                                                                                                                           |
| 64         | CD3-HCDR2           | RIRSKYNNYATYYADSVKG                                                                                                                                                                                                                                                                                                                             |
| 65         | CD3-HCDR3           | HGNFGNSYVSWFAY                                                                                                                                                                                                                                                                                                                                  |
| 66         | CD3-LCDR1           | GSSTGAVTTSNYAN                                                                                                                                                                                                                                                                                                                                  |
| 67         | CD3-LCDR2           | GTNKRAP                                                                                                                                                                                                                                                                                                                                         |
| 68         | CD3-LCDR3           | ALWYSNLWV                                                                                                                                                                                                                                                                                                                                       |
| 69         | CD3 VH              | EVQLLESGLLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLLTVSS                                                                                                                                                                                                                    |
| 70         | CD3 VL              | QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL                                                                                                                                                                                                                                    |
| 71         | CEA-HCDR1           | EFGMN                                                                                                                                                                                                                                                                                                                                           |
| 72         | CEA-HCDR2           | WINTKTGEATYVEEFKG                                                                                                                                                                                                                                                                                                                               |
| 73         | CEA-HCDR3           | WDFAYYVEAMDY                                                                                                                                                                                                                                                                                                                                    |
| 74         | CEA-LCDR1           | KASAAVGTYVA                                                                                                                                                                                                                                                                                                                                     |
| 75         | CEA-LCDR2           | SASYRKR                                                                                                                                                                                                                                                                                                                                         |
| 76         | CEA-LCDR3           | HQYYTYPLFT                                                                                                                                                                                                                                                                                                                                      |
| 77         | CEA VH              | <u>QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFGMNWVRQAPGQGLEWMGWINTKTGEATYVEEFKGRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARWDFAYYVEAMDYWGQGTITVTVSS</u>                                                                                                                                                                                                               |
| 78         | CEA VL              | <u>DIQMTQSPSSLSASVGDRTITCKASAAVGTYVAWYQQKPGKAPKLLIYSASYRKRGVPSRFSGSGSGTDFLTISLQPEDFATYYCHQYYTYPLFTFGQGTKLEIK</u>                                                                                                                                                                                                                                |
| 79         | CEA-HCDR1 (CEACAM5) | DTYMH                                                                                                                                                                                                                                                                                                                                           |
| 80         | CEA-HCDR2 (CEACAM5) | RIDPANGNSKYVPKFQG                                                                                                                                                                                                                                                                                                                               |
| 81         | CEA-HCDR3 (CEACAM5) | FGYYVSDYAMAY                                                                                                                                                                                                                                                                                                                                    |
| 82         | CEA-LCDR1 (CEACAM5) | RAGESVDIFGVGFLH                                                                                                                                                                                                                                                                                                                                 |
| 83         | CEA-LCDR2           | RASNRAT                                                                                                                                                                                                                                                                                                                                         |

| SEQ ID NO: | Name                                                                                                            | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
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|            | (CEACAM5)                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 84         | CEA-LCDR3 (CEACAM5)                                                                                             | QQTNEDPYT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| 85         | CEA VH (CEACAM5)                                                                                                | QVQLVQSGAEVKKPGSSVKVSCKASGFNIKDTY<br>MHWVRQAPGQGLEWMGRIDPANGNSKYVPKF<br>QGRVTITADTSTSTAYMELSSLRSEDTAVYYCAP<br>FGYYVSDYAMAYWGQGTLVTVSS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| 86         | CEA VL (CEACAM5)                                                                                                | EIVLTQSPATLSLSPGERATLSCRAGESVDIFGVG<br>FLHWYQQKPGQAPRLLIYRASNRATGIPARFSGS<br>GSGTDFTLTISSLEPEDFAVYYCQQTNEDPYTFG<br>QGKLEIK                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| 87         | Light chain „CEA <sub>2F1</sub> ”<br>(CEA TCB)                                                                  | DIQMTQSPSSLSASVGDRVTITCKASAAVGTYYVA<br>WYQQKPGKAPKLLIYSASYRKRGVPSRFSGSGS<br>GTDFTLTISLQPEDFATYYCHQYYTYPLFTFGQ<br>GTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL<br>LNNFYPREAKVQWKVDNALQSGNSQESVTEQD<br>SKDSTYSLSTLTLSKADYEKHKVYACEVTHQG<br>LSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| 88         | Light Chain humanized CD3 <sub>CH2527</sub> (Crossfab, VL-CH1)<br>(CEA TCB)                                     | QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNY<br>ANWVQEKPGQAFRGLIGGTNKRAPGTPARFSGS<br>LLGGKAALTLGAQPEDEAEYYCALWYSNLWV<br>FGGGTKLTVLSSASTKGPSVFPLAPSSKSTSGGT<br>AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA<br>VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP<br>SNTKVDKKVEPKSC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 89         | CEA <sub>CH1A1A 98/99</sub> — humanized CD3 <sub>CH2527</sub> (Crossfab VH-Ck)—<br>Fc(knob) P329GLALA (CEA TCB) | QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFG<br>MNWVRQAPGQGLEWMGWINTKTGEATYVEEF<br>KGRVTFITDTSTSTAYMELRSLRSDDTAVYYCA<br>RWDFAYYVEAMDYWGQGTITVTVSSASTKGPSV<br>FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN<br>SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL<br>GTQTYICNVNHKPSNTKVDKKVEPKSCDGGGGS<br>GGGGSEVQLLESGLLVQPGSLRLSCAASGFT<br>FSTYAMNWRQAPGKGLEWVSRIISKYNNYAT<br>YYADSVKGRFTISRDDSKNTLYLQMNSLRADT<br>AVYYCVRHGFNFGNSYVSWFAYWGQTLVTVSS<br>ASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP<br>REAKVQWKVDNALQSGNSQESVTEQDSKDSTY<br>SLSTLTLSKADYEKHKVYACEVTHQGLSSPVT<br>KSFNRGECDKTHTCPPCPAPEAAGGPSVFLFPPK<br>PKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV<br>DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ<br>DWLNGKEYKCKVSNKALGAPIEKTISKAKGQPR<br>EPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIA<br>VEWESNGQPENNYKTTPVLDSDGSFFLYSKLT<br>VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL<br>SPGK |
| 90         | CEA <sub>CH1A1A 98/99</sub> (VH-CH1)—<br>Fc(hole) P329GLALA (CEA TCB)                                           | QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFG<br>MNWVRQAPGQGLEWMGWINTKTGEATYVEEF<br>KGRVTFITDTSTSTAYMELRSLRSDDTAVYYCA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

| SEQ ID NO: | Name                                                                    | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
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|            |                                                                         | RWDFAYYVEAMDYWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCTLPSSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK                                                                                               |
| 91         | CD3 VH-CL (CEACAM5 TCB)                                                 | EVQLLESGLLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVSRIISKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFNGNSYVSWFAYWGQGLTVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC                                                                                                                                                                                                                         |
| 92         | humanized CEA VH-CH1(EE)-Fc (hole, P329G LALA) (CEACAM5 TCB)            | QVQLVQSGAEVKKPGSSVKVSCKASGFNIKDTYMHWVRQAPGQGLEWMGRIDPANGNSKYVPKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYCAPFGYYVSDYAMAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCTLPSSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 93         | humanized CEA VH-CH1(EE)-CD3 VL-CH1-Fc (knob, P329G LALA) (CEACAM5 TCB) | QVQLVQSGAEVKKPGSSVKVSCKASGFNIKDTYMHWVRQAPGQGLEWMGRIDPANGNSKYVPKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYCAPFGYYVSDYAMAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL                                                                              |

| SEQ ID NO: | Name                                              | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
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|            |                                                   | VKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSP                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| 94         | humanized CEA VL-CL(RK)<br>(CEACAM5 TCB)          | EIVLTQSPATLSLSPGERATLSCRAGESVDIFGVGFLHWYQQKPGQAPRLLIYRASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQTNEDPYTFGQGTKLEIKRTVAAPSVFIFPPSDRKLKSGTASVVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC                                                                                                                                                                                                                                                                                                                    |
| 95         | (CH2527) CD3-HCDR1                                | TYAMN                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 96         | (CH2527) CD3-HCDR2                                | RIRSKYNNYATYYADSVKG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| 97         | (CH2527) CD3-HCDR3                                | HGNFGNSYVSWFAY                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| 98         | (16D5) FoIR1-HCDR1                                | NAWMS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 99         | (16D5) FoIR1-HCDR2                                | RIKSKTDGGTTDYAAPVKG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| 100        | (16D5) FoIR1-HCDR3                                | PWEWSWYDY                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| 101        | (CH2527-VL7-46-13)-<br>LCDR1                      | GSSTGAVTTSNYAN                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| 102        | (CH2527-VL7-46-13)-<br>LCDR2                      | GTNKRAP                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 103        | (CH2527-VL7-46-13)-<br>LCDR3                      | ALWYSNLWV                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| 104        | (CH2527) CD3 VH                                   | EVQLLES GGGLVQPGGSLRLSCAASGFTFSTYAMNWRQAPGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGT LTVSS                                                                                                                                                                                                                                                                                                                                                                                                                |
| 105        | (16D5) FoIR1 VH                                   | EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEWVGRIKSKTDGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCTTPWEWSWYDYWGQGT LTVSS                                                                                                                                                                                                                                                                                                                                                                                                                     |
| 106        | (CH2527-VL7-46-13)VL                              | QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| 107        | (16D5)VH-CH1-<br>(CH2527)VH-CH1 Fc<br>knob PGLALA | EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEWVGRIKSKTDGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCTTPWEWSWYDYWGQGT LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSEVQLLES GGGLVQPGGSLRLSCAASGFTFTYAMNWRQAPGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGT LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN |

| SEQ ID NO: | Name                                                | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
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|            |                                                     | AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE<br>YKCKVSNKALGAPIEKTISKAKGQPREPQVYTLTP<br>PCRDELTKNQVSLWCLVKGFYPSDIAVEWESNG<br>QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQ<br>QGNVFSCSVMHEALHNHYTQKSLSLSPGK                                                                                                                                                                                                                                                                                                                                     |
| 108        | (16D5)VH-CH1-Fc hole<br>PGLALA H435R-Y436F          | EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAW<br>MSWVRQAPGKGLEWVGRIKSKTDGGTTDYAAP<br>VKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYC<br>TTPWEWSWYDYWGQGTLVTVSSASTKGPSVFP<br>LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS<br>GALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG<br>TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP<br>PCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV<br>VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE<br>QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK<br>ALGAPIEKTISKAKGQPREPQVCTLPSPRDELTKN<br>QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTT<br>PPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSV<br>MHEALHNRFTHQKSLSLSPGK |
| 109        | (CH2527-VL7-46-13)VL-<br>CL<br>(common light chain) | QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNY<br>ANWVQEKPGQAFRGLIGGTNKRAPGTPARFSGS<br>LLGGKAALTLGAQPEDEAEYYCALWYSNLWV<br>FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA<br>TLVCLISDFYPGAVTVAWKADSSPVKAGVETTT<br>PSKQSNKYAASSYLSLTPEQWKSHRSYSCQVT<br>HEGSTVEKTVAPTECS                                                                                                                                                                                                                                                                          |
| 110        | human PD-L1 (Uniprot<br>Q9NZQ7)                     | MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYG<br>SNMTIECKFPVEKQLDLAALIVWEMEDKNIIQF<br>VHGEEDLKVQHSSYRQRARLLKDQLSLGNAAL<br>QITDVKLQDAGVYRCMISYGGADYKRITVKVNA<br>PYNKINQRILVVDPTSEHELTCQAEGYPKAEVI<br>WTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRIN<br>TTTNEIFYCTFRRLDPEENHTAELVIPELP<br>LAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR<br>MMDVKKCGIQDTNSKKQSDTHLEET                                                                                                                                                                                       |
| 111        | human PD-1 (Uniprot<br>Q15116)                      | MQIPQAPWPVWVAVLQLGWRPGWFLDSPDRP<br>WNPPTFSPALLVVTEDGNATFTCSFSNTSESVL<br>NWYRMSPSNQTDKLAAPEDRSQPGQDCRFRVT<br>QLPNGRDFHMSVVRARRNDSTYLCGAISLAPK<br>AQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQ<br>FQTLVVGVGGLLGSLLVLLVWVLAVICSRARG<br>TIGARRTGQPLKEDPSAVPVFSVDYGELDFQWR<br>EKTPEPPVPCVPEQTEYATIVFPSGMGTSSPARRG<br>SADGPRSAQPLRPEDGHCSWPL                                                                                                                                                                                           |
| 112        | VH (PD-L1)                                          | EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWI<br>HWVRQAPGKGLEWVAWISPYGGSTYYADSVKG<br>RFTISADTSKNTAYLQMNSLRAEDTAVYYCARR<br>HWPGGFDYWGQGTLVTVSS                                                                                                                                                                                                                                                                                                                                                                                     |
| 113        | VL (PD-L1)                                          | DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVA<br>WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSG<br>TDFLTISLQPEDFATYYCQQYLYHPATFGQGT                                                                                                                                                                                                                                                                                                                                                                                                           |

| SEQ ID NO: | Name                                                     | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
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|            |                                                          | KVEIK                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 114        | VH (PD-L1)                                               | EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYW<br>MSWVRQAPGKGLEWVANIKQDGSEKYYVDSV<br>KGRFTISRDNKNSLYLQMNSLRAEDTAVYYCA<br>REGGWFGELAFDYWGQGLTVTVSS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| 115        | VL (PD-L1)                                               | EIVLTQSPGTLSPGERATLSCRASQRVSSSYLA<br>WYQQKPGQAPRLLIYDASSRATGIPDRFSGSGSG<br>TDFLTISRLEPEDFAVYYCQYGSPLPWTFGQGT<br>KVEIK                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 116        | VH (PD-1)                                                | QVQLVQSGVEVKKPGASVKVSCKASGYTFTNY<br>YMYWVRQAPGQGLEWMGGINPSNGGTNFNEKF<br>KNRVTLTDSSTTTAYMELKSLQFDDTAVYYCA<br>RRDYRFDMGFDYWGQGTITVTVSS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| 117        | VL (PD-1)                                                | EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYS<br>YLHWYQQKPGQAPRLLIYLA SYLESQVPA RFSG<br>SGSGTDFLTITISLEPEDFAVYYCQH SRDLPLTFG<br>GGTKVEIK                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| 118        | VH (PD-1)                                                | QVQLVESGGGVVQPGRSLRLDCKASGITFSNSG<br>MHWVRQAPGKGLEWVA VIWYDGS KRY YADSV<br>KGRFTISRDN SKNTLFLQMNSLRAEDTAVYYCA<br>TNDDYWGQGLTVTVSS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| 119        | VL (PD-1)                                                | EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAW<br>YQQKPGQAPRLLIYDASN RATGIPARFSGSGSGT<br>DFTLTITISLEPEDFAVYYCQQSSNWPRTFGQGTK<br>VEIK                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| 120        | Human (hu) FAP                                           | UniProt no. Q12884                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| 121        | hu FAP ectodomain+poly-<br>lys-tag+his <sub>6</sub> -tag | RPSRVHNSEENTMRALTKDILNGTFSYKTFPPN<br>WISGQEYLHQ SADNNIVLYNIETGQSYTILSNRT<br>MKS VNASNYGLSPDRQFVYLEDYSKLWRYSY<br>TATYYIYDLN GEFVRGNELPRPIQYLCWSPVGS<br>KLAYVYQNNIYLKQRP GDPPFQITFNGRENKIFN<br>GIPDWVYEEEMLATKYALWWSPNGKFLAYAEF<br>NDTDIPVIAYSYYGDEQYPR TINIPYPKAGAKNP<br>VVRIFIIDTTPAYVGPQEV PVPAMIASSDYYS<br>WLTWVTDERVCLQWLKRVQNVSVLSICDFRED<br>WQTWDCPKTQE HIEESRTGWAGGFFVSTPVFSY<br>DAISYYKIFSDK DGYKHHYIKDTVENAIQITSGK<br>WEAINIFRVTQDSL FYSSNEFEEYPGRRN IYRISIG<br>SYPPSKKCVTCHLRKERCQYYTASFSDYAKYYA<br>LVCYGP GIPISTLHDGR TDQEIKILEENKELENAL<br>KNIQLPKEEIKKLEVDEITLWYKMILPPQFDRSK<br>KYPLLIQVYGGPCSQS VRSVFVNWISYLASKEG<br>MVIALVDGRGTA FQGDKLLYAVYRKLGVYEVE<br>DQITAVRKFIEMGFIDEKRIAIWGSYGGYVSSL<br>ALASGTGLFKCGI AVPVSSWEYYASVYTERFM<br>GLPTKDDNLEHYKNSTVMARAEYFRNV DYLLIH<br>GTADDNVHFQNSAQIAKALVNAQVDFQAMWY<br>SDQNHGLSGLSTNHL YTHMTHFLKQCFSLSDGK<br>KKKKKGHHHHHH |
| 122        | mouse FAP                                                | UniProt no. P97321                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |

| SEQ ID NO: | Name                                                         | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|------------|--------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 123        | Murine FAP ectodomain+poly-lys-tag+his <sub>6</sub> -tag     | RPSRVYKPEGNTKRALTLKDILNGTFSYKTYFPN<br>WISEQEYLHQSEDDNIVFYNIETRESYIILSNSTM<br>KSVNATDYGLSPDRQFVYLESDYSKLWRYSYTA<br>TYYIYDLQNGEFVRGYELPRPIQYLCWSPVGSKL<br>AYVYQNNIYLKQRPDPPFQITYTGRENRIENGIP<br>DWVYEEEMLATKYALWWSPDGKFLAYVEFND<br>SDIPIIAYSYYGDGQYPRITINIPYKAGAKNPVVR<br>VFIVDTTYPHHVGPMEVPVPEMIASSDYYFSWLT<br>WVSSERVCLQWLKRVQNVSVLSICDFREDWHA<br>WECPKNQEHVEESRTGWAGGFFVSTPAFSQDAT<br>SYYKIFSDKDGKHHIHYIKDTVENAIQITSGKWE<br>AIYIFRVTQDSLFISSNEFEGYPGRRNIYRISIGNS<br>PPSKKCVTCHLRKERCQYYTASFYSYKAKYYALV<br>CYGPGLPISTLHDGRTDQEIQVLEENKELENSLR<br>NIQLPKVEIKKLDGGLTFWYKMILPPQFDRSKK<br>YPLLIQVYGGPCSQSVKSVFAVNWITYLASKEGI<br>VIALVDGRGTAQGDKFLHAVYRKLGVEVED<br>QLTAVRKFIEMGFIDEERIAIWGWSYGGYVSSLA<br>LASGTGLFKCGIAVAPVSSWEYYASIYSERFMGL<br>PTKDDNLEHYKNSTVMARAIFYRNVYLLIHGT<br>ADDNVHFQNSAQIAKALVNAQVDFQAMWYSD<br>QNHGILSGRSQNHLYTHMTHFLKQCFSLSDGKK<br>KKKKGHHHHHHH  |
| 124        | Cynomolgus FAP ectodomain+poly-lys-tag+his <sub>6</sub> -tag | RPPRVHNSEENTMRALTLKDILNGTFSYKTFFPN<br>WISGQEYLHQSAADNNIVLYNIETGQSYTILSNRT<br>MKS VNASNYGLSPDRQFVYLESDYSKLWRYSY<br>TATYYIYDLNNGEFVRGNELPRPIQYLCWSPVGS<br>KLAYVYQNNIYLKQRPDPPFQITFNGRENKIFN<br>GIPDWVYEEEMLATKYALWWSPNGKFLAYAEF<br>NDTDIPVIAYSYYGDEQYPRITINIPYKAGAKNPF<br>VRIFIIDTTPAYVGPQEVVPVAMIASSDYYFSWL<br>TWVTDERVCLQWLKRVQNVSVLSICDFREDWQ<br>TWDCPKTQEHIEESRTGWAGGFFVSTPVFSYDAI<br>SYYKIFSDKDGKHHIHYIKDTVENAIQITSGKWE<br>AINIFRVTQDSLFISSNEFEDYPGRRNIYRISIGSY<br>PPSKKCVTCHLRKERCQYYTASFSDYAKYYALV<br>CYGPGIPISTLHDGRTDQEIKILEENKELENALKN<br>IQLPKEEIKKLEVDEITLWYKMILPPQFDRSKKYP<br>LLIQVYGGPCSQSVRSVFAVNWISYLASKEGMVI<br>ALVDGRGTAQGDKLLYAVYRKLGVEVEDQI<br>TAVRKFIEMGFIDEKRIAIWGWSYGGYVSSLALA<br>SGTGLFKCGIAVAPVSSWEYYASVYTERFMGLP<br>TKDDNLEHYKNSTVMARAIFYRNVYLLIHGT<br>ADDNVHFQNSAQIAKALVNAQVDFQAMWYSD<br>QNHGLSGLSTNHLYTHMTHFLKQCFSLSDGKKK<br>KKKKGHHHHHHH |
| 125        | human CEA                                                    | UniProt no. P06731                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| 126        | Human FolR1                                                  | UniProt no. P15328                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| 127        | Murine FolR1                                                 | UniProt no. P35846                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| 128        | Cynomolgus FolR1                                             | UniProt no. G7PR14                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |



| SEQ ID NO: | Name                              | Sequence                                   |
|------------|-----------------------------------|--------------------------------------------|
| 129        | human MCSP                        | UniProt no. Q6UVK1                         |
| 130        | human CD3ε                        | UniProt No. P07766                         |
| 131        | cynomolgus CD3ε                   | NCBI GenBank no. BAB71849.1 Uniprot Q05LI5 |
| 132        | G4S peptide linker                | GGGGS                                      |
| 133        | (G4S) <sub>2</sub>                | GGGGS GGGGS                                |
| 134        | (SG4) <sub>2</sub>                | SGGGGS GGGG                                |
| 135        | peptide linker                    | GGGGS GGGGS GGGG                           |
| 136        | peptide linker                    | GSPGSSSSGS                                 |
| 137        | (G4S) <sub>3</sub> peptide linker | GGGGS GGGGS GGGGS <sub>3</sub>             |
| 138        | (G4S) <sub>4</sub> peptide linker | GGGGS GGGGS GGGGS GGGGS                    |
| 139        | peptide linker                    | GSGSGSGS                                   |
| 140        | peptide linker                    | GSGSGNGS                                   |
| 141        | peptide linker                    | GGSGSGSG                                   |
| 142        | peptide linker                    | GGSGSG                                     |
| 143        | peptide linker                    | GGSG                                       |
| 144        | peptide linker                    | GGSGNGSG                                   |
| 145        | peptide linker                    | GGNGSGSG                                   |
| 146        | peptide linker                    | GGNGSG                                     |

General information regarding the nucleotide sequences of human immunoglobulins light and heavy chains is given in: Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991). Amino acids of antibody chains are numbered and referred to according to the numbering systems according to Kabat (Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)) as defined above.

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## EXAMPLES

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

### 5        **Recombinant DNA techniques**

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

#### **DNA sequencing**

DNA sequences were determined by double strand sequencing.

#### **Gene synthesis**

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

#### **Cell culture techniques**

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

### Protein purification

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

### SDS-PAGE

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

### Analytical size exclusion chromatography

Size exclusion chromatography (SEC) for the determination of the aggregation and oligomeric state of antibodies was performed by HPLC chromatography. Briefly, Protein A  
20 purified antibodies were applied to a Tosoh TSKgel G3000SW column in 300 mM NaCl, 50 mM KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub>, pH 7.5 on an Agilent HPLC 1100 system or to a Superdex 200 column (GE Healthcare) in 2 x PBS on a Dionex HPLC-System. The eluted protein was quantified by UV absorbance and integration of peak areas. BioRad Gel Filtration Standard 151–1901 served as a standard.

### 25 Mass spectrometry

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

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

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

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

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

### **Example 1**

#### **Preparation, purification and characterization of anti-FAP/anti-OX40 bispecific antibodies**

Anti-FAP/anti-OX40 bispecific antibodies were prepared as described in International  
25 Patent Appl. Publ. No. WO 2017/055398 A2 or WO 2017/060144 A1.

In particular, the molecules according to Example 4.4 of WO 2017/060144 A1 were made, that possess tetravalent binding to OX40 and monovalent binding to FAP. The knob-into-hole technology was applied to allow the assembling of two different heavy chains. A schematic scheme of the bispecific antibodies in 4+1 format is shown in **Figure 1A**.

30 In Molecule A, the first heavy chain (HC 1) was comprised of two Fab units (VHCH1\_VHCH1) of the anti-OX40 binder 49B4 followed by Fc knob chain fused by a (G4S) linker to a VH domain of the anti-FAP binder 4B9. The second heavy chain (HC 2) of the construct was comprised of two Fab units (VHCH1\_VHCH1) of the anti-OX40 binder

49B4 followed Fc hole chain fused by a (G4S) linker to a VL domain of the anti-FAP binder 4B9. Molecule A (FAP OX40 iMAB) thus comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO:54, a second heavy chain comprising the amino acid sequence of SEQ ID NO:55 and four times a light chain of SEQ ID NO: 56.

5 Molecule B was prepared in analogy to Molecule A, however the FAP binder 4B9 was replaced by FAP binder 28H1. Molecule B comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO:57, a second heavy chain comprising the amino acid sequence of SEQ ID NO:58 and four times a light chain of SEQ ID NO: 56.

10 In Molecule C, the first heavy chain (HC 1) was comprised of two Fab units (VHCH1\_VHCH1) of the anti-OX40 binder 49B4 followed by Fc knob chain fused by a (G4S) linker to the VL domain of the anti-FAP binder 4B9. The second heavy chain (HC 2) of the construct was comprised of two Fab units (VHCH1\_VHCH1) of the anti-OX40 binder 49B4 followed Fc hole chain fused by a (G4S) linker to the VH domain of the anti-FAP binder 4B9. Molecule C comprises a first heavy chain comprising the amino acid sequence of  
15 SEQ ID NO:59, a second heavy chain comprising the amino acid sequence of SEQ ID NO:60 and four times a light chain of SEQ ID NO: 56.

In all these molecules the Pro329Gly, Leu234Ala and Leu235Ala mutations were introduced in the constant region of the knob and hole heavy chains to abrogate binding to Fc gamma receptors according to the method described in WO 2012/130831, whereas in  
20 Molecule D, the wildtype human IgG1 Fc domain with knob into hole mutations was used. Molecule D comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO:61, a second heavy chain comprising the amino acid sequence of SEQ ID NO:62 and four times a light chain of SEQ ID NO: 56.

The production and characterization of the molecules is described in detail in WO  
25 2017/060144 A1.

## Example 2

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

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

30 The preparation of the anti-CEA/anti-CD3 bispecific antibody (CEA CD3 TCB or CEA TCB) used in the experiments is described in Example 3 of WO 2014/131712 A1. CEA CD3 TCB is a "2+1 IgG CrossFab" antibody and is comprised of two different heavy chains and two different light chains (one of them is two times present in the molecule). Point mutations

in the CH3 domain (“knobs into holes”) were introduced to promote the assembly of the two different heavy chains. Exchange of the VH and VL domains in the CD3 binding Fab were made in order to promote the correct assembly of the two different light chains. 2 +1 means that the molecule has two antigen binding domains specific for CEA and one antigen binding domain specific for CD3. CEACAM5 CD3 TCB has a similar format, but comprises another CEA binder and comprises point mutations in the CH and CL domains of the CD3 binder in order to support correct pairing of the light chains.

CEA CD3 TCB comprises two times a light chain of the amino acid sequence of SEQ ID NO:87, a heavy chain comprising the amino acid sequence of SEQ ID NO:88, a heavy chain comprising the amino acid sequence of SEQ ID NO:89 and a light chain comprising the amino acid sequence of SEQ ID NO:90. A schematic scheme of the bispecific antibody in 2+1 format is shown in **Figure 1C**. CEACAM5 CD TCB comprises two times a light chain of the amino acid sequence of SEQ ID NO:91, a heavy chain comprising the amino acid sequence of SEQ ID NO:92, a heavy chain comprising the amino acid sequence of SEQ ID NO:93 and a light chain comprising the amino acid sequence of SEQ ID NO:94. A schematic scheme of the bispecific antibody in 2+1 format is shown in **Figure 1B**.

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

FolR1 CD3 TCB comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO:107, a second heavy chain comprising the amino acid sequence of SEQ ID NO:108 and three times a common light chain of SEQ ID NO: 109.

### Example 3

#### ***In vitro* co-culture assays with human immune effector cells**

The immune functions of T cells were tested in *in vitro* co-culture assays with human immune effector cells (resting PBMC, CD4 or CD8 T cells), target antigen positive tumor cells and FAP positive fibroblasts in the presence of TCBs (CEA CD3 TCB, CEACAM5 CD3

TCB and FolR1 CD3 TCB) and FAP OX40 iMab. Evaluated tumor cell lines were the gastric cancer cell line MKN-45, the ovarian adenocarcinoma cell line SK-OV-3 and the cervical cancer cell line HeLa. The mouse embryonic fibroblast cell line NIH/3T3 transduced to express human FAP was used as FAP positive fibroblast. Effector cells were resting human PBMC and isolated resting CD4 or CD8 T cells. In some assays, TNF- $\alpha$  sensor cells were added to monitor TNF- $\alpha$  induction. Tumor cell lysis (kinetic high content life imaging, endpoint flow cytometry), expression of cell surface activation and maturation markers (endpoint flow cytometry) and cytokine secretion (kinetic high content life imaging, endpoint cytometric bead array) was used to monitor the extent of T cell function induced by TCBs and modulated by FAP OX40 iMAB (Molecule A).

*a) Target cell lines and fibroblasts*

The SK-OV-3 cells (ATCC, Ca.No. HTP-77) naturally express the folate receptor. HeLa NLR cells (EssenBioscience, Ca.No. 4489) naturally express the folate receptor and MKN45 NLR cells naturally express CEA. Both cell lines harbour the Essen CellPlayer NucLight Red Lentivirus (Essenbioscience, Cat. No. 4476; EF1 $\alpha$ , puromycin) to stable express the NucLight Red fluorescent protein restricted to the nucleus. This enables easy separation from non-fluorescent effector T cells or fibroblasts. As the red fluorescence measured per well is directly proportional to the number of red nuclei, and thus healthy tumor cells, real-time assessment of tumor cell lysis or proliferation by high through put life fluorescence microscopy is possible.

HeLa NucLight Red (NLR) cells were cultured in DMEM (GIBCO, Cat.No. 42430-082) containing 10 % Fetal Bovine Serum (FBS, Gibco by Life Technology, Cat. No. 16000-044, Lot 941273, gamma-irradiated, mycoplasma-free and heat inactivated at 56 °C for 35 min), 1 % (v/v) GlutaMAX I (GIBCO by Life Technologies, Cat. No. 35050 038) and 1 mM Sodium-Pyruvate (SIGMA, Cat. No. S8636).

The MKN45 NucLight Red (NLR) cells naturally express CEA. MKN45 NucLight Red cells were cultured in DMEM (GIBCO, Cat.No.42430-082) containing 10 % Fetal Bovine Serum (FBS, Gibco by Life Technology, Cat. No. 16000-044, gamma-irradiated, mycoplasma-free and heat inactivated at 56 °C for 35 min), 1 % (v/v) GlutaMAX I (GIBCO by Life Technologies, Cat. No. 35050 038), 1 mM sodium pyruvate (SIGMA, Cat. No. S8636) and 0.5  $\mu$ g/mL Puromycin (Sigma-Aldrich, Cat. No. ant-pr-1). MKN-45 (DSMZ; ACC409) were transduced with the Essen CellPlayer NucLight Red Lentivirus Reagent (Essenbioscience, Cat. No. 4476; EF1 $\alpha$ , puromycin) at an MOI of 5 (TU/cell) in the presence of 8  $\mu$ g/mL polybrene following the manufacturer's instructions to stable express a nuclear-restricted NucLight Red fluorescent protein. This enables easy separation from non-

fluorescent effector T cells or fibroblasts and monitoring of the tumor cell growth by high through put life fluorescence microscopy. Quantification per well over time allows thus real-time assessment of tumor cell lysis or proliferation.

The crosslinking of FAP-binding antibodies by cell surface FAP was provided by  
5 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. Cells were cultured in DMEM (GIBCO, Cat.No. 42430-082) containing 10 % calf serum (Sigma-Aldrich, Cat. No. C8056-500ml, gamma-irradiated, mycoplasma-free and heat inactivated at 56 °C for  
10 35 min) and 1.5 µg/mL Puromycin (Sigma-Aldrich, Cat. No. ant-pr-1).

#### b) Preparation of Effector cells

Buffy coats were obtained from the Zürich blood donation center. To isolate fresh peripheral blood mononuclear cells (PBMCs) the buffy coat was diluted with the same volume of DPBS (Gibco by Life Technologies, Cat. No. 14190 326). 50 mL polypropylene  
15 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  
20 DPBS and resuspended in T cell medium consisting of RPMI 1640 medium (Gibco by Life Technology, Cat. No. 42401-042) supplied with 10 % Fetal Bovine Serum (FBS, Gibco by Life Technology, Cat. No. 16000-044, Lot 941273, gamma-irradiated, mycoplasma-free and heat inactivated at 56 °C for 35 min), 1 % (v/v) GlutaMAX I (GIBCO by Life Technologies, Cat. No. 35050 038), 1 mM Sodium Pyruvate (SIGMA, Cat. No. S8636), 1 % (v/v) MEM  
25 non-essential amino acids (SIGMA, Cat.-No. M7145) and 50 µM β-Mercaptoethanol (SIGMA, M3148). In some cases, RPMI1640 was replaced by FluoroBrite DMEM media (GIBCO, Invitrogen, Cat No A18967-01) for improved high content live microscopy with reduced background fluorescence.

PBMCs were used as effector cells directly after isolation (resting human PBMCs) or  
30 certain subfractions, as resting CD4 T cells or CD8 T cells, were isolated using the untouched human CD4+ T cell isolation kit (Miltenyi, Ca.No. 130-096-533) and untouched human CD8+ T cell isolation kit (Miltenyi, Ca.No. 130-096-495) according to manufacturers instructions, respectively. Briefly, human PBMC were centrifuged for 8 min at 400 x g, 4°C and were washed once with MACS buffer (PBS + BSA (0.5 % v/w, Sigma-Aldrich, Cat. No.  
35 A9418) + EDTA ([2nM], Ambion, AM9261)). The pellet was resuspended with the



respective provided streptavidin labeled negative antibody cocktail and incubated for 5 minutes at 4 °C (per 1\*10<sup>7</sup> cells 40 µL MACS buffer and 10 µL antibody mix) followed by a subsequent incubation with biotinylated magnetic capture beads (per 1\*10<sup>7</sup> cells 30 µL MACS buffer and 20 µL bead mix) for 10 min at 4 °C. Labeled non-CD4 or non-CD8 T cells were removed by magnetic separation using an LS column (Miltenyi, Ca.No. 130-042-401) according to manufacturer's instructions. The column flow through, containing unlabeled resting CD4 and CD8 T cells, respectively, was centrifuged and washed once with MACS buffer as described above. Cells were adjusted to 2 mio cells/mL in RPMI1640 or Fuorobright DMEM based T cell media.

10 c) *TNF-α sensor cells*

TNF-α sensor cells were HEK 293T cells (ATCC, Cat. No. xxx) transduced with the reporter plasmid pETR14327 encoding for green fluorescent protein (GFP) under the control of an NFκB sensitive promotor element. HEK 293T cells express naturally the TNF receptor to which TNF-α secreted by activated T cells can bind. This leads to dose dependent activation of NFκB and translocation to the nucleus, which in turn switches on dose dependent GFP production. The GFP fluorescence can be quantified by high through put life fluorescence microscopy over time and allows thus real-time assessment of TNF-α secretion.

TNF-α sensor cell line was generated by lentiviral transduction of HEK293T cells (ATCC; CRL-3216). Lentivirus-based viral vectors were produced by co-transfection of HEK293T cells with lentiviral packaging plasmids and a lentiviral expression vector (pETR14372) coding for green fluorescent protein (GFP) coupled with the minimal cytomegalovirus (mCMV) promoter in conjunction with the NFκB consensus transcriptional response elements. Plasmid transfections into HEK293T cells were performed with Lipofectamine LTX (Life Technologies) according the manufacturer's instructions. Transfections were done in 6-well plates seeded with 6 × 10<sup>5</sup> cells/well the day before transfection and 2.5 µg of plasmid DNA. The lentiviral vector-containing supernatant was collected after 48 h and filtered through a 0.45 µm pore-sized polyethersulfone membrane. To generate stable expressing cell lines, HEK293T cells were seeded at 1.0 × 10<sup>6</sup> cells/well in 6-well plates and overlaid with 1 mL of viral vector-containing supernatant. Transductions were carried out by spinoculation at 800 x g and at 32 °C for 30 min in an Eppendorf centrifuge 5810 table-top centrifuge (Eppendorf). A TNF-α inducible cell clone was obtained by FACS sorting (FACS ARIA, Becton, Dickinson and Company).

d) *Cytotoxicity and T cell activation assay*

Mouse embryonic fibroblast NIH/3T3-huFAP cells, TNF-α sensor cells and MKN45 NLR cells were harvested using cell dissociation buffer (Invitrogen, Cat.-No. 13151-014) for

10 minutes at 37°C. Cells were washed once with DPBS. TNF- $\alpha$  sensor cells or fibroblasts were irradiated in an xRay irradiator using a dose of 4500 RAD to prevent later overgrowth of effector or tumor cell lines. Target cell lines, NIH/3T3-huFAP and in some assays TNF- $\alpha$  sensor cells were cultured at a density of  $0.1 \times 10^5$  cells per well in T cell media in a sterile 96-well flat bottom adhesion tissue culture plate (TPP, Cat. No. 92097) overnight at 37 °C and in 5% CO<sub>2</sub> in an incubator (Hera Cell 150).

Resting human PBMC, human CD4 T cells, human CD8 T cells or NLV-specific T cells were prepared as described above and were added at a density of  $0.5 \times 10^5$  cells per well. A serial dilution row of TCBs (CEA CD3 TCB or CEA CD3 TCB (2)) and a fixed concentration of FAP OX40 iMab (2 nM) was added to a total volume of 200 uL per well. Cells were cocultured for up to 72 hours at 37 °C and 5% CO<sub>2</sub> in an incubator (Hera Cell 150).

In some assays, plates were monitored by fluorescence microscopy high content life imaging using the Incucyte Zoom System (Essenbioscience, HD phase-contrast, green fluorescence and red fluorescence, 10 x objective) in a 3 hours interval for up to 72 hours at 37 °C and 5% CO<sub>2</sub>. The integrated red fluorescence of healthy tumor cells (RCUxum2/image), which is proportional to the amount of NLR<sup>+</sup> cells per well, was quantified using the IncucyteZoom Software to monitor tumor cell growth vs lysis by T cells. Values were plotted for the respective time point and conditions against the used TCB concentration to analyse effects on the cytolytic potential of T cells.

In some assays where TNF- $\alpha$  sensor cells were present, the integrated green RCUxum2/image was quantified using the IncucyteZoom Software to monitor TNF- $\alpha$  induced production of GFP by the TNF- $\alpha$  sensor cells. Values were plotted for the respective time point and conditions against the used TCB concentration to analyze effects on TNF- $\alpha$  secretion by T cells.

After 72 hrs, the supernatant was collected for subsequent analysis of selected cytokine using the cytometric bead array according to manufacturer's instructions. Evaluated cytokines were IL-2 (Human IL-2 CBA Flex- set (Bead A4), BD Bioscience, Ca.No. 558270), IL-17A (Human IL-17A CBA Flex- set (Bead B5), BD Bioscience, Ca.No. 560383), TNF- $\alpha$  (Human TNF- $\alpha$  CBA Flex- set (Bead C4), BD Bioscience, Ca.No. 560112), IFN- $\gamma$  (IFN- $\gamma$  CBA Flex- set (Bead E7), BD Bioscience, Ca.No. 558269), IL-4 (Human IL-4 CBA Flex- set (Bead A5), BD Bioscience, Ca.No. 558272), IL-10 (Human IL-10 CBA Flex- set (Bead B7), BD Bioscience, Ca.No. 558274) and IL-9 (Human IL-9 CBA Flex- set (Bead B6), BD Bioscience, Ca.No. 558333).

Thereafter, all cells were detached from the wells by incubation with cell dissociation buffer for 10 minutes at 37°C followed by centrifugation at 400xg at 4°C. Pellets were

washed with ice cold FACS buffer (DPBS (Gibco by Life Technologies, Cat. No. 14190 326) w/ BSA (0.1 % v/w, Sigma-Aldrich, Cat. No. A9418). Cells were surface-stained with fluorescent dye-conjugated antibodies anti-human CD4 (clone RPA-T4, BioLegend, Cat.-No. 300532), CD8 (clone RPa-T8, BioLegend, Cat.-No. 3010441), CD62L (clone DREG-56, BioLegend, Cat.-No. 304834), CD127 (clone 019D5, BioLegend, Cat.-No. A019D5), CD134 (clone Ber-ACT35, BioLegend, Cat.-No. 350008), CD137 (clone 4B4-1, BioLegend, Cat.-No. 309814), GITR (clone 621, BioLegend, Cat.-No. 3311608) and CD25 (clone M-A251, BioLegend, Cat.-No. 356112) for 20 min at 4°C in FACS buffer. Then, they were washed once with FACS buffer before being resuspended in 85 µL/well FACS buffer containing 0.2 µg/mL DAPI (Santa Cruz Biotech, Cat. No. Sc-3598) before they were acquired the same day using 5-laser LSR-Fortessa (BD Bioscience with DIVA software). Living CD4 and CD8 T cells were gated (DAPI-, NucLight RED-, CD4 or CD8+) and counts, the mean fluorescence intensity (MFI) of activation marker (CD134, CD137, GITR, CD25) or maturation marker (CD127, CD62L) or percentage of positive cells were plotted for the respective conditions against the used TCB concentration to analyze effects on T activation.

## Results

### 3.1 T cell bispecific antibodies induce a dose dependent upregulation of OX40 on CD8 and CD4 T cells

Different human immune effector cell preparations (resting PBMC, CD4 or CD8 T cells, NLV specific CD8 T effector memory cells) were cocultured with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEACAM5 CD3 TCB for 48 hrs. The amount of living tumor cells was quantified by fluorescence microscopy high content life imaging using the Incucyte Zoom System and the integrated red fluorescence of healthy tumor cells was used to calculate the specific lysis (**Figure 2**). The expression of OX40 was evaluated by flow cytometry on CD4 and CD8 positive T cells (**Figures 3A-3D**).

CEACAM5 CD3 TCB was able to induce lysis of MKN45 NucLight red cells in all used immune effector cell preparations, as shown in **Figure 2** for the 42 hours time point. The EC<sub>50</sub> values and the magnitude of lysis differed slightly between the different effector cell preparations and were highest for isolated CD8 T cells. Concomitant to tumor cell lysis, T cells increased surface expression of activation markers including OX40 (**Figures 3A-3D**). Surface expression of OX40 was highest on CD4 positive T cells, but was also detected to a lower extent on CD8 positive T cells. The extent of OX40 expression was not depending on the presence of helper cells (no difference of expression levels in PBMC vs isolated populations for CD4 or CD8 T cells).

### 3.2 The presence of FAP-targeted OX40 agonists does not influence the cytolytic potential of T cells

Next we evaluated the influence of OX40 costimulation on TCB mediated tumor cell lysis. As described in 3.1, T cells were cocultured for 48 hours with MKN-45 NucLight Red  
 5 cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEACAM5 CD3 TCB with or without a fixed concentration of FAP OX40 iMab, respectively.

The amount of living tumor cells was quantified by fluorescence microscopy high content life imaging using the Incucyte Zoom System in 3 hour intervals and the integrated red fluorescence of healthy tumor cells was used to calculate the specific lysis.

10 No influence of FAP OX40 iMAB costimulation on the extent of tumor cell lysis was observed for FolR1 CD3 TCB at all evaluated time points (**Figures 4A-4C**). For an easier comparison over time the area under curve (AUC) was calculated for each time point with and w/o FAP OX40 iMAB costimulation and was plotted against time. The AUC was increasing over time as tumor cells were proliferating in the absence of TCB but clearly no costimulation  
 15 dependent difference in the AUC was detected.

The presence of OX40 costimulation did also neither speed up tumor cell lysis nor increase the magnitude of tumor cell lysis by CEACAM5 CD3 TCB nor decrease the TCB concentration necessary to achieve lysis of a certain percentage of tumor cells (e.g. shift in EC<sub>50</sub> values). This was true for all evaluated effector cell preparations and is shown  
 20 exemplary for the 42 hrs time point in **Figures 5A-5C**.

Similar findings were obtained using CEA CD3 TCB (data not shown).

### 3.3 The presence of FAP targeted OX40 agonists does influence the secretion of cytokines

In some assays, TNF- $\alpha$  sensor cells were cultured additionally to the above described setting. TNF- $\alpha$  sensor cells naturally express the TNF- $\alpha$  receptor and were genetically  
 25 modified with GFP under the control of an NF $\kappa$ B sensitive promotor element. Binding of TNF- $\alpha$  secreted by activated T cells leads to dose dependent activation of NF $\kappa$ B and subsequently to expression of GFP. The GFP fluorescence can be quantified by high through put life fluorescence microscopy over time and allows thus real-time assessment of TNF- $\alpha$  secretion. As described in 3.1 above, CD4 T cells were cocultured for 48 hours with MKN-45  
 30 NucLight Red cells as target cells and irradiated NIH/3T3 huFAP in the presence of fixed concentration of FAP OX40 iMAB and a serial dilution row of CEACAM5 CD3 TCB, FolR1 CD3 TCB and CEA CD3 TCB, respectively.

Activation of T cells by the present TCB led to dose dependent release of TNF- $\alpha$ , which led to a dose dependent increase of GFP fluorescence over time in the TNF- $\alpha$  sensor cells. Additional costimulation with FAP OX40 iMab further increased the GFP fluorescence and thus the TNF- $\alpha$  secretion by activated T cells (**Figures 6A-6D and 7A-7D**). This effect was  
5 mostly on the extent of TCB mediated TNF- $\alpha$  secretion but did not lower the TCB concentration at which TNF- $\alpha$  secretion was induced (shift in EC<sub>50</sub> values). Also, agonistic TCR stimulation was needed to observe this positive impact on cytokine secretion and no unspecific TNF- $\alpha$  secretion was detected in control TCB treated samples.

For an easier comparison over time the area under curve (AUC) was calculated for each  
10 time point with and w/o OX40 costimulation and was plotted against time (**Figures 8A-8D**). An increased AUC was observed for all tested TCBs (CEA CD3 TCB, FolR1 TCB and CEACAM5 CD3 TCB) and in the presence of different tumor cell lines (MKN45 NLR, HeLa NLR red, Skov-3).

The supernatants of all samples were evaluated at the end point (48 hours) using the  
15 cytometric bead array system (BD Bioscience) to quantify the effect on secretion of several cytokines beyond TNF- $\alpha$ . Evaluated cytokines were IL-2 and TNF- $\alpha$  as marker for general T cell activation, IFN- $\gamma$  (Th1 cytokine), IL-4 (Th2 cytokine), IL-9 (Th9 cytokine) and IL-17A (Th17 cytokine) to monitor a differentiation towards a certain Th subclass, and IL-10 as immunosuppressive cytokine.

20 Activation of T cells by the present TCB led, next to TNF- $\alpha$ , to a dose dependent release of all evaluated cytokines, namely IL-2, IL-4, IFN- $\gamma$ , IL-17a and IL-10 (**Figures 9A-9D, Figures 10A-10D, Figures 11A-11D and Figures 12A-12D**). The extent of this cytokine release differed for the TCBs, when the same target cell line was used. This can be seen from a comparison of Figures 9A-9D (CEACAM5 CD3 TCB) and Figures 10A-10D (CEA CD3  
25 TCB). But also when the same TCB (FolR CD3 TCB) was used a difference could be observed when different target cell lines were used. Figures 11A-11D show the cytokine release with HeLa NLR cells whereas Skov-3 cells were used in Figures 12A-12D.

Additional co-stimulation with FAP OX40 iMab modulated the extent of dose-  
dependent cytokine secretion, but did not lower the TCB threshold concentration needed for  
30 cytokine secretion. Thereby, an increase of pro-inflammatory IL-2, TNF- $\alpha$  and IFN- $\gamma$  secretion was observed, whereby the concentration of immunosuppressive IL-10 was lowered. For an easier comparison, the changes in cytokine concentration in samples with OX40 costimulation were calculated relative to those without costimulation for the TCB plateau concentration (**Figure 13**).

Thereby, an increase of pro-inflammatory IL-2, TNF- $\alpha$  and IFN- $\gamma$  secretion was evident, whereby the concentration of immunosuppressive IL-10 was lowered only in some target cell/TCB combinations. There was a trend visible that a more forceful T cell activation with strong dose-dependent cytokine secretion was also stronger modulated by OX40 costimulation. Especially, the decrease in immunosuppressive IL-10 release was coupled to a strong T cell activation.

We also tested the ability of OX40 costimulation to modulate the cytokine secretion of resting CD4 and CD8 T cells and of resting human PBMC. As described in 3.1, resting human PBMC, isolated CD4 or CD8 T cells were co-cultured for 72 hrs with MKN-45 NucLight Red cells and irradiated NIH/3T3 huFAP in the presence of a serial dilution row of CEACAM5 CD3 TCB with or without a fixed concentration of FAP OX40 iMAB. The supernatant was evaluated at 72 hrs using the cytometric bead array (CBA) as described above.

OX40 costimulation supported the secretion of pro-inflammatory cytokines in resting human PBMC and to a lower extent also on CD8 T cells (dose dependency, see **Figures 14A-14H** for resting CD4 T cells, **Figures 15A-15H** for resting CD8 T cells and **Figures 16A-16H** for resting PBMCs). A comparison for top TCB concentration is shown in **Figure 17**. Remarkable was especially the impact on IL-2 and TNF- $\alpha$  production by resting CD8 T cells.

Thus, costimulation via OX40 does not increase directly the cytolytic potential of T cells in a 48-72 hour *in vitro* cytotoxicity assay, but it increased the ability to secrete cytokines and modulated the cytokine microenvironment. A more proinflammatory cytokine milieu in the tumor can shift the tumor microenvironment towards a more immune-activating and less immune-suppressive state, e.g. lower level of IL-10 and increased concentrations of IFN- $\gamma$  can allow myeloid cells in the tumor to mature to Th1 and cytotoxic T cell supporting antigen presenting cells. A shift to a supportive cytokine network will restore a successfully and sustained tumor cell elimination where before the tumor achieved to escape immune control. In line with the preferential expression of OX40 on CD4 T cells, a stronger modulation was observed for cytokine secretion on CD4 T cells vs that of CD8 T cells. However, both cell types were influenced.

## Example 4

### Combination therapy of FAP OX40 iMab and CEACAM5 TCB *in vivo*

#### 4.1 Methods

In the following examples we tested if the combination of TCBs and FAP Ox40 iMab  
5 leads to a superior anti-tumor efficacy *in vivo* compared to the respective monotherapies.

Human monovalent FAP targeted, tetravalent OX40 bispecific antibodies (FAP OX40  
iMab) were tested as single agent and in combination with the human CEACAM5 CD3 TCB  
(CEA CD3 TCB (2)) against vehicle and CEACAM5 CD3 TCBonly treated animals treated  
with CEACAM5 CD3 TCB only. Human gastric MKN45 cancer cells were cografed sub  
10 cutaneously with a mouse fibroblast cell line (3T3) in NOG humaniced mice.

#### 4.2 Cell lines and tumor model

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<sub>2</sub>. In vitro passage 7  
15 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 1.5ug/ml Puromycin.  
Clone 39 was used at an *in vitro* passage number 9 (Experint 1, Table 1) and 7 (Experiment 2,  
Table 2), respectively, at a viability of 98.8% and 98.4%, respectively.

20 50 microliters cell suspension ( $1 \times 10^6$  MKN45 cells +  $1 \times 10^6$  3T3-huFAP) mixed with 50  
microliters Matrigel were injected subcutaneously in the flank of anaesthetized mice with a  
22G to 30G needle.

#### 4.3 Mouse model

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

#### 4.4 Treatment and Experimental Handling of Experiment 1

The human monovalent FAP-targeted OX40 bispecific antibody with tetravalent binding to OX40 (FAP OX40 iMab, Molecule A as described in Example 1) was tested as single agent and in combination with the human CEACAM5 CD3 TCB. The FAP binder used  
5 in the FAP OX40 iMab construct was 4B9. Human gastric MKN45 cancer cells were cografted subcutaneously with a mouse fibroblast cell line (3T3) in NOG humanized mice.

7 days before cell injection mice were bled and screened for the amount of human T-cells in the blood. Mice were injected subcutaneously on study day 0 with  $1 \times 10^6$  MKN45 cells mixed with  $1 \times 10^6$  3T3 fibroblasts. Tumors were measured 2 to 3 times per week during  
10 the whole experiment by Caliper. On day 10 mice were randomized for tumor size and human T-cell count with an average T-cell count/ $\mu$ l blood of 140 and an average tumor size of 170  $\text{mm}^3$ . On the day of randomization mice were injected i.v. with Vehicle, CEACAM5 CD3 TCB, FAP(4B9) OX40 iMab or the combination of the FAP(4B9) OX40 iMab with CEACAM5 CD3 TCB for 5 weeks.

15 All mice were injected i.v. with 200  $\mu$ l of the appropriate solution. The mice in the vehicle group were injected with Histidine Buffer and the treatment groups with OX40 agonizing construct, the CEACAM5 CD3 TCB or the combination. To obtain the proper amount of compound per 200  $\mu$ l, the stock solutions were diluted with Histidine Buffer when necessary. The dose and schedule used for CEACAM5 TCB was 0.5 mg/kg, once/week  
20 whereas the FAP OX40iMab was given at a dose of 12.5 mg/kg, once/week.

2 mice/group were bled 10 min, 4 h, 72 h and 168 h after the first therapy to determine the exposure of compounds during the first week. FAP OX40 iMab was measured by sandwich ELISA, binding of the construct to human OX40 and detection of huCH1-domain. CEACAM5 CD3 TCB was detected by sandwich ELISA, binding of the TCB to an anti CD3-  
25 CDR specific antibody and detection of human Fc (see **Figures 18A and 18B**).

The experiment was terminated at study day 44. Tumors, blood and spleen were harvested in PBS, single cell suspensions were generated and stained for different immune cell markers and analysed by FACS. Erythrolysis of whole blood samples were performed for 3 minutes at room temperature using the BD Pharm Lyse buffer (BD, Ca.No. 555899)  
30 according to manufacturers instructions. Splenocytes were isolated by homogenization of the spleen through a cell strainers (nylon filter 70 $\mu$ m, BD Falcon) followed by erythrolysis as described above. Tumor single cell suspensions were prepared by using the gentleMACS Dissociator (Miltenyi) and digest the homogenate for 30 minutes at 37°C with DNase I ([0.025mG/mL], RocheDiagnostics, Ca.No. 11284932001) and Collagenase D ([1 mG/mL],  
35 RocheDiagnostics, Ca.No. 11088882001). Afterwards cell suspensions were filtered through



cell strainers (nylon filter 70um, BD Falcon) to remove debris. All preparations were washed with excess ice cold FACS buffer. Cells were surface-stained with fluorescent dye-conjugated antibodies anti-mouse CD4 (clone GK 1.5, BioLegend, Cat.-No. 100422), CD8 (clone 53-6.7, BioLegend, Cat.-No. 100730), CD45 (clone 30-F11, BioLegend, Cat.-No. 103116), and CD3 (clone 145-2C11, BioLegend, Cat.- 100351) in the presence of purified Rat anti-mouse CD16/CD32 (clone 2.4G2, BD, Ca.No. 553142) for 30 min at 4°C, dark, in FACS buffer. Samples were resuspendend in FACS buffer containing 0.2 µg/mL DAPI (Santa Cruz Biotec, Cat. No. Sc-3598) before they were acquired the same day using 5-laser LSR-Fortessa (BD Bioscience with DIVA software). Living CD4 and CD8 T cells were gated (DAPI-, CD45+, CD3+, CD4 or CD8+), normalized counts (per uL blood, mg spleen or mg tumor) calculated and values plotted for the respective treatmentgroups.

**Table 1: Compositions used in the *in vivo* experiment**

| Compound                                        | Dose (mg/kg) | Formulation buffer                                   | Concentration (mg/mL)          |
|-------------------------------------------------|--------------|------------------------------------------------------|--------------------------------|
| FAP(4B9) OX40 iMab<br>(Molecule A of Example 1) | 12.5         | 20 mM Histidine, 140 mM NaCl, pH 6.0, 0.01% Tween-20 | 4.41<br>(=stock concentration) |
| CEACAM5 CD3 TCB<br>(Example 2)                  | 0.5          | 20 mM Histidine, 140 mM NaCl, pH 6.0, 0.01% Tween20  | 1.72<br>(=stock concentration) |

#### 4.5 Treatment and Experimental Handling of Experiment 2

The human monovalent anti-FAP(4B9)/anti-OX40 bispecific antibody (FAP OX40 iMab) was tested in 3 different doses as single agent and in combination with the human CEACAM5 CD3 TCB. Human gastric MKN45 cancer cells were cografed subcutaneously with a mouse fibroblast cell line (3T3) in NOG humanized mice with human stem cells as described above.

7 days before cell injection mice were bled and screened for the amount of human T-cells in the blood. Mice were injected subcutaneously on study day 0 with  $1 \times 10^6$  MKN45 cells mixed with  $1 \times 10^6$  3T3 fibroblasts. Tumors were measured 2 to 3 times per week during the whole experiment by Caliper. On day 26, mice were randomized for tumor size and human T-cell count with an average T-cell count/ $\mu$ l blood of 115 and an average tumor size of 490 mm<sup>3</sup>. One day after randomization mice were injected i.v. with Vehicle, CEACAM5 CD3 TCB, FAP OX40 iMab or the combinations of FAP OX40 iMab with CEACAM5 CD3 TCB for 4 weeks.

All mice were injected i.v. with 200  $\mu$ l of the appropriate solution. The mice in the vehicle group were injected with Histidine Buffer and the treatment groups with the OX40 agonizing constructs, the CEACAM5 CD3 TCB or the combination. To obtain the proper amount of compound per 200  $\mu$ l, the stock solutions were diluted with Histidine Buffer when  
 5 necessary. The dose and schedule used for CEACAM5 CD3 TCB was 0.5 mg/kg, once/week whereas FAP OX40 iMab was given at a dose of 12.5 mg/kg, 4.2 mg/kg or 1.4mg/kg, once/week.

The experiment was terminated at study day 50. Tumors, blood and spleen were harvested in PBS, single cell suspensions were generated and stained for different immune  
 10 cell markers and analysed by FACS.

Spleen and tumor from all remaining mice per group were analysed by flow cytometry at termination. Single cell suspensions were stained for CD45, CD3, CD4 and CD8 and the amount of cells was analysed. Parts of tumors at termination and from animals during the experiment were formalin fixed and afterwards embedded in Paraffin. Samples were cut and  
 15 stained for CD3 and CD8. Plasma as well as part of spleen and tumor was frozen for Cytokine analysis via Multiplex. Parts of tumors at termination were formalin fixed and afterwards embedded in Paraffin. Samples were cut and stained for CD3 and CD8.

**Table 2: Compositions used in the *in vivo* experiment**

| Compound                                        | Dose (mg/kg)             | Formulation buffer                                   | Concentration (mg/mL)         |
|-------------------------------------------------|--------------------------|------------------------------------------------------|-------------------------------|
| FAP(4B9) OX40 iMab<br>(Molecule A of Example 1) | 12.5 or<br>4.2 or<br>1.4 | 20 mM Histidine, 140 mM NaCl, pH 6.0, 0.01% Tween-20 | 3.2<br>(=stock concentration) |
| CEACAM5 CD3 TCB<br>(Example 2)                  | 0.5                      | 20 mM Histidine, 140 mM NaCl, pH 6.0, 0.01% Tween20  | 3.1<br>(=stock concentration) |

20 In order to determine the pharmacokinetic profiles of the injected compounds during the first week, 2 mice per Group were bled 10 min, 4 h, 72 h and 7d after the first therapy and injected compounds were analysed by ELISA. OX40 iMAbs were detected via OX40 binding (A) whereas CEACAM5 CD3 TCB was detected via binding to an anti-CD3 CDR antibody (B).

25 (A) Biotinylated human OX40, test sample, Digoxigenin labelled anti-huCH1 antibody and anti-Digoxigenin detection antibody (POD) were added stepwise to a 96-well streptavidin-coated microtiter plate and incubated after every step for 1h at room temperature.

The plate was washed three times after each step to remove unbound substances. Finally, the peroxidase-bound complex was visualized by adding ABTS substrate solution to form a colored reaction product. The reaction product intensity was photometrically determined at 405 nm (with reference wavelength at 490 nm) and is proportional to the analyte

5 concentration in the serum sample.

(B) Biotinylated anti-huCD3 –CDR antibody, test sample, Digoxigenin labelled anti-huFc antibody and anti-Digoxigenin detection antibody (POD) were added stepwise to a 96-well streptavidin-coated microtiter plate and incubated after every step for 1h at room temperature. The plate was washed three times after each step to remove unbound substances. Finally, the peroxidase-bound complex was visualized by adding ABTS substrate solution to form a colored reaction product. The reaction product intensity was photometrically determined at 405 nm (with reference wavelength at 490 nm) and is proportional to the analyte concentration in the serum sample.

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#### 4.6 Cytokine analysis of tumor, spleen and serum samples

Serum was collected, and subcutaneous tumors and spleen were harvested from animals at termination (day 50), 2 days after last Ab administration. 20-30 mg of snap-frozen spleen and tumor tissues were processed for whole protein isolation at study termination. Briefly, tissue samples were meshed by using the Tissue Lyser system and stainless steel beads in a total volume of 150 µl of lysis buffer. Meshed samples were cleared by centrifugation and whole protein content was analysis by BCA protein assay kit (Fischer Thermo Scientific) in the supernatant according to manufacturer's instructions. At total of 200 µg of whole protein of tumor and spleen lysates as well as a 1:10 dilution of serum samples was used for the analysis of different cytokines/chemokines by the Bio-Plex system following instructions of manufacturer (Bio-Plex Pro™ Human Cytokine 17-plex Assay, BioRad).

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#### 4.7 Immunohistochemistry

Immunohistochemical analysis was performed of human MKN45 gastric subcutaneous tumors cografed with 3T3 murine fibroblasts derived from the indicated treatment groups in humanized NOG mice. Subcutaneous tumors were harvested from animals at termination day, 2 days after last Ab administration, were fixed in formalin 10% (Sigma, Germany) and later processed for FFPET (Leica 1020, Germany). 4 µm paraffin sections were subsequently cut in a microtome (Leica RM2235, Germany). HuCD8 and HuCD3 immunohistochemistry was performed using anti-human CD8 (Cell Marque Corporation, California) and anti-human CD3 (ThermoFischer Scientific, USA) in the Leica autostainer (Leica ST5010, Germany) following the manufacture's protocols. Quantification of huCD3 and huCD8 positive T cells

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was performed with Definiens software (Definiens, Germany). Statistics were analyzed by one way ANOVA with multiple comparison tests.

#### 4.8 Results of Experiment 1

It could already be shown in *in vitro* experiments that FAP OX40 iMAb can change T cell activation status and cytokine release. It was also confirmed that the influence of OX40 seems to be stronger on CD4 positive T cells than on CD8 positive T cells.

To test if FAP OX40 iMAb could also *in vivo* change the immune status to a more beneficial outcome we used a humanized mouse model transferring human stem cells into immunodeficient mice and therefore generating a partially human immune system consisting mainly of T and B cells. We coinjected MKN45, a CEA expressing human gastric cancer cell line, and 3T3 fibroblasts which improve the stroma component and FAP expression in the tumor. CEA is targeted by the CEACAM5 CD3 TCB, crosslinking T cells with tumor cells and inducing T cell mediated killing of tumors cells and T cell activation. Upon T cell activation OX40 is upregulated. FAP OX40 iMAb crosslinks FAP expressing fibroblasts and OX40 expressing T cells and is therefore inducing OX40 signaling. This leads to improved T cell survival and cytokine release.

We could prove in this study that combination therapy of FAP OX40 iMAb and CEACAM5 CD3 TCB leads to improved efficacy compared to monotherapies. Also FAP OX40 iMAb monotherapy showed significant improved efficacy compared to vehicle.

We evaluated the serum concentration of CEACAM5 CD3 TCB as well as FAPOx40iMAB upon the 1rst treatment in the respective monotherapies and in the combination group to rule out differences in exposure as cause of differences in efficacy. As shown in Error! Reference source not found. **A and 18B** the exposure for all constructs was comparable in mono and combination therapy.

As shown in Figure **19**, FAP OX40 iMAb monotherapy treated animals showed a slightly delayed progression of the tumor, CEACAM5 CD3 TCB a more pronounced one. However, only in the combination therapy a regression of the subcutaneous tumor was achieved (see Table 3).

**Table 3: Tumor growth inhibition (TGI) at study day 41 and 43**

| Group                              | TGI day 41 [%] | TGI day 43 [%] |
|------------------------------------|----------------|----------------|
| CEACAM5 CD3 TCB                    | 93.6           | 92.6           |
| FAP OX40 iMab                      | 55.2           | 35.9           |
| CEACAM5 CD3 TCB +<br>FAP OX40 iMab | 103.8          | 103.4          |

#### 4.9 Results of Experiment 2

In a second study we tested different doses of FAPOX40iMAB as monotherapy and in combination with CEACAM5 CD3 TCB (CEA CD3 TCB (2)). Here, we also delayed the start of treatment until we reached a median tumor size of 490 mm<sup>3</sup> compared to 170 mm<sup>3</sup> in the first study.

All groups injected with compounds showed comparable maximum concentrations of the molecules between the different groups, either OX40 targeted compounds or TCB. In **Figures 20A and 20B** the pharmacokinetic profile of the injected compounds during the first week is shown.

As plotted in **Figures 21A-21C**, we could again confirm the superior anti-tumor efficacy of the combination versus the monotherapies. Neither FAP OX40 iMab in any of the tested doses nor CEACAM5 CD3 TCB as monotherapy was able to slow down progression of the tumor growth, which was most likely due to the considerable tumor burden already at the beginning of treatment. Only the combination treatment significantly prevented the progression of tumor growth over the whole study time (Table 4). Strong prolonged efficacy was observed at doses of 12.5 mg/kg of FAP OX40 iMAB, however, lower doses (4.2 and 1.4 mg/kg) were only temporally able to reduce progression compared to CEACAM5 CD3 TCB monotherapy (**Figure 22**). A clear dose dependency was observed. As shown in Figures 20A and 20B exposure for all constructs was comparable in mono and combination therapy.

Tumor growth inhibition based on medians was calculated at study day 40 and 49. The values can be found in Table 4 below.

**Table 4: Tumor growth inhibition (TGI) at study day 40 and 49**

| Group                      | TGI day 40 [%] | TGI day 49 [%] |
|----------------------------|----------------|----------------|
| CEACAM5 CD3 TCB            | 67.3           | 36.7           |
| FAP OX40 iMab<br>1.4 mg/kg | 11.2           | -6.3           |
| FAP OX40 iMab              | 16.2           | 20.5           |

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|                                                  |       |       |
|--------------------------------------------------|-------|-------|
| 4.2 mg/kg                                        |       |       |
| FAP OX40 iMab<br>12.5 mg/kg                      | 38.1  | 23.9  |
| CEACAM5 CD3 TCB +<br>FAP OX40 iMab 1.4 mg/kg     | 55.2  | 26.7  |
| CEACAM5 CD3 TCB +<br>FAP OX40 iMab 4.2 mg/kg     | 55.0  | 61.9  |
| CEACAM5 CD3 TCB +<br>FAP OX40 iMab 12.5<br>mg/kg | 108.5 | 102.3 |

To test for significant differences in group means for multiple comparisons, the standard analysis of variance (ANOVA) is automatically produced, using the Dunnett's method. Dunnett's method tests whether means are different from the mean of a control group.

5 **Table 5: p-values: Comparison with a control using Dunnett's method (AUC=area under the curve)**

| Group                                           | p-value day<br>49 vs vehicle | p-value AUC<br>until day 49 vs<br>vehicle | p-value day<br>49 vs CEA<br>CD3 TCB (2) | p-value AUC<br>until day 49<br>vs CEA CD3<br>TCB (2) |
|-------------------------------------------------|------------------------------|-------------------------------------------|-----------------------------------------|------------------------------------------------------|
| Vehicle                                         | 1                            | 1                                         | 0.1051                                  | 0.2158                                               |
| CEACAM5 CD3 TCB                                 | 0.1088                       | 0.2158                                    | 1                                       | 1                                                    |
| FAP OX40iMab<br>12.5mg/kg                       | 0.5990                       | 0.7234                                    | 0.7956                                  | 0.9171                                               |
| FAP OX40iMab<br>4.2mg/kg                        | 0.8848                       | 0.9178                                    | 0.5394                                  | 0.7588                                               |
| FAP OX40iMab<br>1.4mg/kg                        | 0.9986                       | 0.7666                                    | 0.2130                                  | 0.8886                                               |
| CEACAM5 CD3 TCB<br>+ FAP OX40 iMab<br>12.5mg/kg | <0.0001*                     | <0.0001*                                  | 0.0032*                                 | 0.0099*                                              |
| CEACAM5 CD3 TCB<br>+ FAP OX40 iMab<br>4.2mg/kg  | 0.0234*                      | 0.0151*                                   | 0.9924                                  | 0.8131                                               |
| CEACAM5 CD3 TCB<br>+ FAP OX40 iMab<br>1.4mg/kg  | 0.0449*                      | 0.0803                                    | 0.9998                                  | 0.9970                                               |

Flow cytometric (**Figures 23A-23D**) and histopathological (**Figures 25A and 25B**) evaluation showed an increased infiltration of the tumor mass with human leukocytes. This

was already observed for CEACAM5 CD3 TCB monotherapy, but strongly enhanced in the combination of CEACAM5 CD3 TCB with 4.2 or 12.5mg/kg FAP OX40 iMAB. FAP OX40 iMAB monotherapy per se increased intratumoral leukocyte counts only minimally. Cell types detected were human CD4 as well as CD8 T cells, but also non-T cells (e.g. B cells or myeloid derived cells). Interestingly, the fold increase in the combination therapy compared to CEACAM5 CD3 TCB monotherapy was more pronounced for CD4 T cells than for CD8 T cell counts, which is in line with the biology of OX40, being primarily expressed on CD4 T cells. In the periphery no significant alterations in cell numbers were detected, emphasizing the tumor targeted nature of both compounds (**Figures 24A and 24B**).

We also evaluated the concentrations of spleen, blood and intratumoral cytokines (Bio-Plex Pro™ Human Cytokine 17-plex Assay, BioRad). The group with the highest anti-tumor efficacy showed also the biggest overall increase in intratumoral cytokines (e.g. IL-6, IL-8, IFN- $\gamma$ , TNF- $\alpha$ , MCP-1, MIP-1 $\beta$  (**Figures 26A-26C**) and was the combination of FAP OX40 iMAB (12.5 mg/kg) and CEACAM5 CD3 TCB. No significant changes were observed in the periphery (spleen or blood). Thus, the immunological changes triggered by FAP OX40 iMAB and CEACAM5 CD3 TCB treatment were tumor-specific indicating that the cross-linking and activation of human T-cells occurs exclusively in CEA expressing tumors and not in other areas that are negative for CEA like blood and spleen.

We further found a direct negative correlation between tumor progression and the amount of intratumoral cytokine concentration, but not between the intratumoral leukocyte count for combination treated animals (**Figures 27A-27F**). The amount of cytokines present did also not strictly correlate with the number of infiltrating leukocytes for all animals. Especially, when CEACAM5 CD3 TCB monotherapy treated animals were compared with combination treated animals we observed that similar leukocyte counts did not necessarily mean the same anti-tumor efficacy or cytokine content present. This leads to the assumption, that beyond the mere increased number of intratumoral T cells, a higher per cell functionality and potential to secrete cytokines of intratumoral T cells are causative for the enhanced anti-tumor activity of the combination of FAP OX40 iMAB and CEACAM5 CD3 TCB.

An improved cytokine milieu plays a major role in mediating anti tumor efficacy. It can recruit more lymphocytes to the tumor, support proliferation and increase the survival of those T cells and prevents the establishment of suppression and exhaustion. We could show that FAP OX40 iMAB was able to modulate *in vitro* the TCB mediated secretion of cytokines for different tumor cell lines, effector populations and tumor targets towards a more inflammatory and less suppressive one. Furthermore, we could also show that this translated into improved anti-tumor efficacy in a humanized mouse model simulating the human immune system.

## Example 5

### Combination therapy of FAP OX40 iMab, CEA TCB and PD-L1 antibody *in vivo*

#### 5.1 Experimental Procedure

In the following example the human FAP targeted OX40 agonist FAP OX40 iMab  
5 (FAP binder 4B9) was tested in a concentration of 12.5 mg/kg in combination with the human  
CEA CD3 TCB and an anti-PD-L1 antibody (a-PD-L1) in a human gastric MKN45 cancer  
model. MKN45 cells were co-grafted subcutaneously with a mouse fibroblast cell line (3T3)  
in NSG humanized mice.

Human MKN45 cells (human gastric carcinoma) were originally obtained from DSMZ  
10 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<sub>2</sub>. In vitro passage  
13 was used for subcutaneous injection at a viability of 99.1%. Human fibroblasts NIH-3T3  
were originally obtained from ATCC, engineered at Hoffmann-La Roche Inc. to express  
human FAP and cultured in DMEM containing 10% Calf serum, 1x Sodium Pyruvate and 1.5  
15 µg/ml Puromycin. Clone 39 was used at in vitro passage number 8 and at a viability of 97.6%.

50 microliters cell suspension ( $1 \times 10^6$  MKN45 cells +  $1 \times 10^6$  3T3-huFAP) mixed with 50  
microliters Matrigel were injected subcutaneously in the flank of anaesthetized mice with a  
22G to 30G needle. NSG female mice (purchased from Charles River), age 5 weeks at start of  
the experiment, were maintained under specific-pathogen-free condition with daily cycles of  
20 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG).  
Experimental study protocol was reviewed and approved by local government authorities.  
After arrival, animals were maintained for one week to get accustomed to the new  
environment and for observation. Continuous health monitoring was carried out on a daily  
basis.

25 For humanization, mice were injected with Busulfan (20mg/kg) followed 24 hours later  
by injection of 100,000 human HSC (purchased from StemCell Technologies).

7-14 days before cell injection mice were bled and screened for the amount of human T  
cells in the blood. Mice were randomized for human T cells with an average T cell count/ul  
blood of 131. Mice were injected subcutaneously on study day 0 with  $1 \times 10^6$  MKN45 cells  
30 mixed with  $1 \times 10^6$  3T3 fibroblasts. Tumors were measured 2 to 3 times per week during the  
whole experiment by Caliper. On day 17, mice were randomized for tumor size with an  
average tumor size of 205 mm<sup>3</sup>. On day of randomization mice were injected weekly i.v. with  
Vehicle, CEA CD3 TCB, CEA CD3 TCB plus a-PD-L1, CEA CD3 TCB plus FAP OX40  
iMab or the triple combination of CEA CD3 TCB, a-PD-L1 and FAP OX40 iMab for up to 4



weeks. All mice were injected i.v. with 200  $\mu$ l of the appropriate solution. The mice in the vehicle group were injected with Histidine Buffer and the treatment groups with the CEA CD3 TCB and the combinations of CEA CD3 TCB and/or FAP OX40 iMAb. To obtain the proper amount of compound per 200  $\mu$ l, the stock solutions were diluted with Histidine Buffer when necessary. The dose and schedule used for CEA CD3 TCB was 2.5 mg/kg twice/week whereas FAP OX40 iMAb was given at a dose of 12.5 mg/kg and a-PD-L1 at a dose of 10 mg/kg once/week (Table 7). The experiment was terminated at study day 44. Some mice had to be sacrificed due to bad health status during the experiment.

**Table 6: Mice alive on day 44**

| Group             | Vehicle | CEA CD3 TCB | CEA CD3 TCB + FAP OX40 iMAb | CEA CD3 TCB + a-PD-L1 | CEA CD3 TCB + FAP OX40 iMAb + a-PD-L1 |
|-------------------|---------|-------------|-----------------------------|-----------------------|---------------------------------------|
| mice alive day 44 | 7/9     | 5/9         | 5/10                        | 3/9                   | 6/10                                  |

Tumors and blood were harvested in PBS, single cell suspensions were generated and stained for different immune cell markers and analysed by FACS. Plasma as well as part of tumor was frozen for Cytokine analysis via Multiplex. Parts of tumors at termination were formalin fixed and afterwards embedded in Paraffin. Samples were cut and stained for CD3 and CD8.

**Table 7: Compositions used in the *in vivo* experiment**

| Compound            | Dose (mg/kg) | Concentration (mg/mL)       | Formulation buffer                                  |
|---------------------|--------------|-----------------------------|-----------------------------------------------------|
| a-PD-L1 (iTME-0005) | 10           | 2.54 (=stock concentration) | 20 mM Histidine, 140 mM NaCl, pH 6.0                |
| CEA CD3 TCB         | 2.5          | 4.82 (=stock concentration) | 20 mM Histidine, 140 mM NaCl, 0.01% Tween20, pH 6.0 |
| FAP OX40 iMAb       | 3.2          | 4.82 (=stock concentration) | 20 mM Histidine, 140 mM NaCl, pH 6.0                |

## 5.2 Results

In this study we wanted to prove for the first time that FAP OX40 iMAb can improve efficacy mediated by the combination of CEA CD3 TCB and a-PD-L1. a-PD-L1 is an immune checkpoint inhibitor and is well established in the field of cancer immunotherapy.

5 The a-PD-L1 binder is crossreactive to mouse PD-L1 and was produced in a murine IgG format. CEA CD3 TCB is targeting CEA expressed on cancer cells and FAP OX40 iMAb binds to FAP expressing fibroblasts in the tumor stroma. FAP OX40 iMAb was given weekly at a dose of 12.5 mg/kg and a-PD-L1 at a dose of 10 mg/kg whereas the CEA CD3 TCB was given at the dose of 2.5mg/kg twice per week.

10 To test our human constructs human immune cells and specifically T cells have to be present in the mouse system. For this reason, we used humanized mice meaning mice transferred with human stem cells. These mice develop over time a partially human immune system consisting mainly of T and B cells.

We coinjected MKN45, a CEA expressing human gastric cancer cell line, and 3T3  
15 fibroblasts which improve the stroma component in the tumor. CEA is targeted by the CEA CD3 TCB, crosslinking T cells with tumor cells and inducing T cell mediated killing of tumor cells and T cell activation. Upon T cell activation OX40 is upregulated as well as PD-1. FAP OX40 iMAb crosslinks FAP expressing stroma cells and OX40 expressing T cells and is therefore inducing OX40 signaling. This leads to improved cytokine secretion, survival and  
20 proliferation of the T cells. PD-L1 is mainly expressed by tumor cells, blocking of PD-L1 prevents crosslinking with PD-1 expressing T cells and therefore prevents PD-1 dependent inactivation of T cells.

We could show in this study that CEA CD3 TCB in combination with a-PD-L1 and FAP OX40 iMAb mediates improved efficacy in terms of tumor growth inhibition compared  
25 to the vehicle group (**Figures 28A and 28B**). Tumor growth inhibition based on medians was calculated at study day 36, 38, 41 and 43. The Group treated with CEA CD3 TCB + a-PD-L1 + FAP OX40iMAb shows the strongest inhibition of tumor growth.

**Table 8: Tumor growth inhibition (TGI) on day 36, 38, 41 and 43**

| Group                                    | Day 36 | Day 38 | Day 41 | Day 43 |
|------------------------------------------|--------|--------|--------|--------|
| CEA CD3 TCB                              | 62.77  | 55.13  | 47.30  | 32.73  |
| CEA CD3 TCB + FAP OX40<br>iMab 12.5mg/kg | 36.76  | 40.63  | 32.97  | 19.48  |
| CEA CD3 TCB + a-PD-L1                    | 45.91  | 55.32  | 41.35  | 37.60  |
| CEA CD3 TCB + a-PD-L1 +<br>FAP OX40iMab  | 81.28  | 82.63  | 71.61  | 59.21  |

Considering the area under the curve (AUC) until day 43 only the combination of CEA CD3 TCB + a-PD-L1 + FAP OX40iMAb is significant different from vehicle monotherapy.

**Table 9: One Way Analysis of tumor volumes until day 43, AUC, comparison with vehicle**

| Means Comparisons with a control using Dunnett's Method (sAUC) | p-Value |
|----------------------------------------------------------------|---------|
| Control Group = vehicle                                        | -       |
| CEA CD3 TCB                                                    | 0.0563  |
| CEA CD3 TCB + FAP OX40iMAb                                     | 0.6395  |
| CEA CD3 TCB + a-PD-L1                                          | 0.1318  |
| CEA CD3 TCB + a-PD-L1 + FAP OX40iMAb                           | 0.0079* |

**Table 10: One Way Analysis of tumor volumes on day 43, AUC, comparison with vehicle**

| Comparisons with a control using Dunnett's Method (day43) | p-Value |
|-----------------------------------------------------------|---------|
| Control Group = vehicle                                   |         |
| CEA CD3 TCB                                               | 0.1311  |
| CEA CD3 TCB + FAP OX40iMAb                                | 0.7221  |
| CEA CD3 TCB + a-PD-L1                                     | 0.1186  |
| CEA CD3 TCB + a-PD-L1 + FAP OX40iMAb                      | 0.0024* |

- 10 All other groups (monotherapies as well as double therapies) could not significantly improve efficacy compared to vehicle.

The pharmacokinetic profile of the injected compounds during the first week was studied as described in Example 4. In addition, for detecting a-PD-L1 biotinylated anti human Fc, PD-L1-huFc, test sample and polyclonal anti murine IgG (HRP) are added stepwise to a 96-well streptavidin-coated microtiter plate and incubated after every step for 1h at room temperature. The plate was washed three times after each step to remove unbound substances. Finally, the peroxidase-bound complex is visualized by adding ABTS substrate solution to form a colored reaction product. The reaction product intensity, which is photometrically determined at 405 nm (with reference wavelength at 490 nm), is proportional to the analyte concentration in the serum sample. 2 mice per Group were bled 1h and 72h after 1<sup>st</sup> and 3<sup>rd</sup> therapy and the injected compounds were analysed by ELISA. All groups injected with compounds show comparable exposure of the molecules between the different groups, either FAP OX40 iMAb, CEA CD3 TCB or a-PD-L1 (see **Figures 29A, 29B and 29C**).

T-cell infiltration in the tumor at termination by IHC (Immune histochemistry) on day 44 is significantly increased in the triple combination group compared to all other groups (see **Figures 30A and 30B**).

### Example 6

#### 5      **Combination therapy of FAP OX40 iMab, CEA TCB and PD-L1 antibody *in vitro***

##### 6.1 Experimental Procedure

In this assay FAP OX40 iMab was tested for its potential to activate human PBMCs (isolated from buffy coat, frozen and stored in liquid nitrogen) in the presence or absence of CEA CD3 TCB and atezolizumab (Tecentriq, anti-human PD-L1-specific humanized human IgG1k antibody) similar as described in Example 5. To mimic the tumor environment PBMCs of six different donors were incubated with FAP-expression NIH/3T3-huFAP fibroblast cell line and with CEA-expressing MKN45-FolR1-PDL1 gastric cancer cell line for four days in the presence or absence of 2 nM FAP OX40 iMab and/or 100 nM CEA CD3 TCB and/or 80 nM atezolizumab. For determining PBMC activation CD4 and CD8 T cells were analyzed by flow cytometry for proliferation (CFSE-dilution), CD25 (IL-2R $\alpha$ ), 4-1BB (CD137), OX-40 (CD134), T-bet (T-box transcription factor), Eomes (Eomesodermin), Granzyme B, and PD-1 expression. Supernatant was analyzed by Multiplex for IFN $\gamma$ , TNF $\alpha$ , GM-CSF, Granzyme B, IL-2, IL-8 and IL-10.

##### a) Preparation of PBMCs

20      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.14190326). 50 mL Falcon centrifuge tubes (TPP, Cat.-No. 91050) were supplied with 15 mL Histopaque 1077 (SIGMA Life Science, Cat.-No. 10771, polysucrose and sodium diatrizoate, adjusted to a density of 1.077 g/mL) and the buffy coat solution was over-layered on 15 mL Histopaque 1077. The tubes were centrifuged for 30 min at 400 x g, room temperature and with low acceleration and no break. Afterwards the PBMCs were collected from the interface, washed three times with DPBS and resuspended in T cell freezing medium consisting of 90 % (v/v) Fetal Bovine Serum (FBS, Gibco by Life Technology, Cat. No. 16000-044, Lot 941273, gamma-irradiated, mycoplasma-free and heat inactivated at 56 °C for 35 min) and 10% Dimethyl sulfoxide (Sigma, Cat.-No. D2650) 10 % (v/v). 1 mL were transferred quickly to sterile Cryovials, transferred to Cryoboxes and stored for 24 h at -80°C. Afterwards vials were transferred to liquid nitrogen containers or Vapor phase containers.

Vials from 6 donors were thawed in the water bath at 37°C and washed in assay medium consisting of RPMI 1640 medium supplied with 10 % (v/v) Fetal Bovine Serum (FBS), 1 % (v/v) GlutaMAX I, 1 mM Sodium pyruvate (SIGMA, Cat. No. S8636), 1 % (v/v) MEM non-essential amino acids (SIGMA, Cat.-No. M7145) and 50 µM β-Mercaptoethanol (SIGMA, M3148). After thawing the cells were rested for 2 hours at 37 °C and 5% CO<sub>2</sub> in cell incubator. Cells were counted, washed with DPBS and resuspended in 37°C DPBS to 1 x10<sup>6</sup> cells/mL. CFDA-SE was added to a final concentration of 200 nM and incubated for 10 min at 37 °C. Afterwards FBS was added, cells were washed and set in assay medium to 2 x 10<sup>6</sup> cells/mL).

10 b) Target cell lines

T150 flasks containing NIH/3T3-huFAP clone 19 were washed with DPBS and incubated with enzyme-free PBS-based dissociation buffer for 8 min at 37 °C. Cells were collected, washed, resuspended in assay medium and irradiated with 50 Gy using X-Ray Irradiator RS 2000. Cells were set in assay medium to 1 x 10<sup>6</sup> cells/mL.

15 T150 flasks containing MKN45-FolR1-PDL1 gastric cancer cell line were washed with DPBS and incubated with enzyme-free PBS-based dissociation buffer for 8 min at 37 °C. Cells were collected, washed with DPBS and resuspended in C diluent (at least 250 µL, 8 x 10<sup>7</sup> cells/mL or lower). The same amount of C diluent was supplied with 4 µL/mL PKH-26 dye and mixed well. This dye solution was added to the cells and mixed well and immediately.  
20 Cells were incubated for 5 min at room temperature. Afterwards FBS was added, cells were washed in assay, resuspended in assay medium and irradiated with 50 Gy with the X-Ray Irradiator RS 2000 (Rad source). Cells were set in assay medium to 1 x 10<sup>6</sup> cells/mL.

c) Assay Setup

For the test compounds master solutions were prepared of each component in assay medium as follows 16 nM FAP OX40 iMAB, 800 nM CEA CD3 TCB and 640 nM Atezolizumab. Cells and components were combined in 96-well round bottom tissue culture plates (TTP, Cat.-No. 92097) in amounts of 50 µL of PKH-26 red labeled MKN45-FolR1 - PD-L1 (10<sup>4</sup>000 cells/well), 50 µL of NIH/3T3-huFAP clone 19 (10<sup>4</sup>000 cells/well), 25 µL of PBMC of one donor (50<sup>4</sup>000 cells/well), 25 µL of 16 nM FAP OX40 iMAB solution or assay medium (final concentration 2 nM), 25 µL of 800 nM CEA CD3 TCB solution or assay medium (final concentration 100 nM), and 25 µL of 640 nM Atezolizumab solution or assay medium (final concentration 80 nM). Plates were then incubated for four days at 37 °C and 5 % CO<sub>2</sub> in a humidified cell incubator.

After four days 50 µL supernatant was removed and stored at -80 °C to be later analyzed for cytokine content (see below). To perform a flow cytometry analysis of T-cell proliferation and surface expression of T cell activation markers, plates were centrifuged and washed once with cold DPBS. Samples were divided in equal volumes in two 96-welled plate  
5 for 2 individual staining panels. For staining panel 1, cells were stained for 15 min at room temperature (RT) in 50 µL/well DPBS supplied with 1:800 diluted LIVE/DEAD Fixable Aqua Dead Cell Stain. Cells were washed once with 200 µL/well FACS buffer (centrifugation 350 x g 4 min at 4°C, flick off). After, they were resuspended in 25µ L/well staining solution composed of FACS-buffer containing antibodies anti-human CD4 (clone A161A1, Biolegend,  
10 Cat.No.- 357410), CD8 (clone RPA-T8, Biolegend, Cat.-No. 301040), CD25 (clone BC96, Biolegend, Cat.-No. 302636), PD-1 (clone EH12.2H7, Biolegend, Cat.-No.329920), CD134 (clone Ber-ACT35, Biolegend, Cat.No.-350008), CD137 (clone 4B4-1, Biolegend, Cat. No.-309814) and incubated for 20 min at 4°C. Cells were washed once with 200 µL/well FACS-  
15 buffer) before they were acquired the same day using 4-laser LSRII (BD Bioscience with DIVA software).

For Staining Panel 2, cells were stained for 15 min at room temperature (RT) in 50 µL/well DPBS supplied with 1:800 diluted LIVE/DEAD Fixable Aqua Dead Cell Stain and were washed once with 200 µL/well FACS-buffer (centrifugation 350 x g 4 min 4°C, flick  
20 off). Cells were resuspended in 25 µL/well staining solution composed of FACS-buffer containing antibodies anti-human CD4 (clone RPA-T4, Biolegend, Cat.-No. 300558 ), CD8 (SK-1, Biolegend, Cat.-No. 344710), CCR7 (clone G043H7, Biolegend, Cat.-No.353204), CD45RO (clone BC96, Biolegend, Cat.-No. 304236) and incubated for 20 min at 4°C. Cells were washed once with 200 µL/well FACS-buffer (centrifugation 350 x g 4 min 4°C, flick off)  
25 and resuspended in 100 µL/well of Foxp3 Fixation/Permeabilization working solution by mixing 1 part of Foxp3 Fixation/Permeabilization Concentrate with 3 parts of Foxp3 Fixation/Permeabilization Diluent (FoxP3/Transcription Factor Staining Buffer Set, eBiosciences, Cat.No.-005523-00) for 60 min at RT. Cells were then washed once with  
30 Permeabilization Buffer working solution by mixing 1 part Permeabilization buffer with 9 parts of water (FoxP3/Transcription Factor Staining Buffer Set, eBiosciences, Cat.No.-005523-00) and were resuspended in 50µL/well staining solution composed of Permeabilization buffer working solution containing antibodies anti-human EOMES (clone Dan11mag, eBiosciences, Cat. No.-25-4857-80), T-bet (clone 4B10, Biolegend, Cat.No.-644815) and Granzyme B (clone GB11, Biolegend, Cat. No.515406) for 40 mins at RT.Cells  
35 were then washed twice with 200µL/well Permeabilization Buffer working solution and resuspended in 120 µL/well FACS buffer) before they were acquired the same day using 4-laser LSRII (BD Bioscience with DIVA software). Data was analyzed using FlowJo v10.3 for

PC (FlowJo LLC), Microsoft Excel (professional Plus 2010) and GraphPad Prism v6.07 (GraphPad Software, Inc). Living CD4 and CD8 T cells were gated (Zombie Aqua-, CD4 or CD8+) and counts, the mean fluorescence intensity (MFI) of activation marker (CD134, CD137, CD25, PD-1) or maturation marker (CCR7, CD45RO) or Transcription factors (T-bet, Eomes) or cytokine (Granzyme B) and percentage of positive cells or mean fluorescent intensity (MFI) were plotted for each condition.

To analyze the released cytokines in the supernatant, the previous frozen samples were taken and analyzed for IFN $\gamma$ , GM-CSF, TNF $\alpha$ , IL-2, Granzyme B, IL-8 and IL-10 using the cytometric bead array according to manufacturer's instructions. Evaluated cytokines were IL-2 (Human IL-2 CBA Flex- set (Bead A4), BD Bioscience, Ca.No. 558270), TNF- $\alpha$  (Human TNF- $\alpha$  CBA Flex- set (Bead C4), BD Bioscience, Ca.No. 560112), IFN- $\gamma$  (IFN- $\gamma$  CBA Flex- set (Bead E7), BD Bioscience, Ca.No. 558269), IL-10 (Human IL-10 CBA Flex- set (Bead B7), BD Bioscience, Ca.No. 558274), TNF (Human TNF CBA Flex- set (Bead C4), BD Bioscience, Ca.No. 560112), IL-8 (Human IL-8 CBA Flex- set (Bead A9), BD Bioscience, Ca.No. 558277), Granzyme B (Human Granzyme B CBA Flex- set (Bead D7), BD Bioscience, Ca.No. 560304).

## 6.2 Results

### 6.2.1 Combination of CEA CD3 TCB and FAP OX40 iMAb was superior to combination with a-PD-L1

As shown in **Figures 31A** and **31B**, the addition of 100nM CEA CD3 TCB (dotted filled bars, filled triangles) but not 2nM FAP OX40 iMAb alone (open bars, open circles) could increase the expression of activation markers CD25, and proliferation of CD4 and CD8 T cells. Combination of FAP OX40 iMAb with CEA CD3 TCB (grey bars, open squares) and/or aPD-L1 (black bars, grey filled squares) led to highest activation and proliferation of CD4 and CD8 T cells as compared to combination treatment with CEA CD3 TCB and aPD-L1 (open bars, filled black circles) as shown in Figures 31A and 31B. Additionally, FAP OX40 iMAB and CEA CD3 TCB combination treatment led to higher percentages of T cell transcription factor (T-bet) on CD4 T cells and higher expression of T-bet on CD8 T cells (**Figures 32A** and **32B**). T-bet expression regulates T helper 1 cell lineage commitment and these results show FAP OX40 iMAb treatment results in driving a Th1 T cell response. Further as shown in **Figures 33A** to **33D**, combination of FAP OX40 iMAB and CEA CD3 TCB treatment leads to higher percentages of Granzyme B expressing CD4 and CD4 T cells, suggesting higher cytotoxic potential of T cells. Analysis of cytokine in the supernatant at the endpoint of the experiment showed higher amounts of pro-inflammatory cytokines IFN- $\gamma$  and Granzyme B in CEA CD3 TCB and FAP OX40 iMAb as compared to CEA CD3 TCB and aPD-L1 treatment, however due to high donor to donor variability the differences were not

statistically significant. Taken together, combination of CEA CD3 TCB with FAP OX40 iMAb resulted in superior activation, proliferation and Th1 differentiation of both CD4 and CD8 T cells.

6.2.2 Triple combination of CEA CD3 TCB, FAP OX40 iMAb and PD-L1 leads to highest cytokine secretion

As shown in **Figures 34A to 34C**, triple combination of CEA CD3 TCB, FAP OX40 iMAb and PD-L1 (black bars, grey filled squares) was the most effective in release of immune cell-activating proinflammatory cytokines such as IFN- $\gamma$ , Granzyme B and IL-8 as compared to all other treatment groups. As shown in **Figure 34C**, triple combination treatment also led to highest intracellular expression of cytolytic enzyme Granzyme B on both CD4 and CD8 T cells, in concordance with our results measuring secreted cytokines. Fold increase of cytokines comparing the triple combination with the combination of CEA CD3 TCB and aPD-L1 is shown in **Figures 35A to 35C**. Despite the strong differences in level of cytokine secretion between different donors, triple combination led to higher than 2-fold difference in majority of the tested donors. Highest fold changes were observed for IL-8 and IFN $\gamma$ . As shown in Figures 31A and 31B, triple combination (black bars, grey filled circles) did not lead to changes in proliferation and activation of CD4 and CD8 T cells as compared to CEA CD3 TCB and FAP OX40 iMAb combination treatment. Taken together, FAP OX40 iMAb co-stimulation when combined with CEA CD3 TCB leads to strong effects in stimulating T cell activation, proliferation and intracellular expression of Th1 lineage promoting, transcription factor T-bet and Granzyme B expression. Addition of PD-L1 to this combination further enhances the cytotoxic potential of both CD4 and CD8 T cells as seen, by increased expression of intracellular and secreted granzyme B and pro-inflammatory cytokine IFN- $\gamma$ .

\*\*\*



Claims

1. A bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method for treating or delaying progression of cancer, wherein the bispecific OX40 antibody specific for a tumor-associated antigen is used in combination with a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen.

2. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of claim 1, wherein the T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen is an anti-CEA/anti-CD3 bispecific antibody or an anti-FolR1/anti-CD3 bispecific antibody.

3. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of claims 1 or 2, wherein the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen and the T-cell activating anti-CD3 bispecific antibody are administered together in a single composition or administered separately in two or more different compositions.

4. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of claims 1 to 3, wherein the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen acts synergistically with the T-cell activating anti-CD3 bispecific antibody.

5. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 4, wherein the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen is an anti-Fibroblast activation protein (FAP)/anti-OX40 bispecific antibody.

6. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 5, wherein the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to FAP comprising  
(a) a heavy chain variable region ( $V_H$ FAP) comprising (i) CDR-H1 comprising the amino acid

sequence of SEQ ID NO:1, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:3, and a light chain variable region (V<sub>L</sub>FAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:4, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:5, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:6, or  
(b) a heavy chain variable region (V<sub>H</sub>FAP) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:9, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:10, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:11, and a light chain variable region (V<sub>L</sub>FAP) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:12, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:13, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:14.

7. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 6, wherein the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to FAP comprising a heavy chain variable region (V<sub>H</sub>FAP) comprising an amino acid sequence of SEQ ID NO:7 and a light chain variable region (V<sub>L</sub>FAP) comprising an amino acid sequence of SEQ ID NO:8 or wherein the antigen binding domain capable of specific binding to FAP comprises a heavy chain variable region (V<sub>H</sub>FAP) comprising an amino acid sequence of SEQ ID NO:15 and a light chain variable region (V<sub>L</sub>FAP) comprising an amino acid sequence of SEQ ID NO:16.

8. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 7, wherein the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to OX40 comprising  
(a) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:22, and a light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:35, or  
(b) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:21, and a light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:34, or

- (c) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:23, and a light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:36, or
- (d) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:19, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:24, and a light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:28, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:31, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:37, or
- (e) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:18, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:20, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:25, and a light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:29, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:32, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:38, or
- (f) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:18, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:20, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:26, and a light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:29, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:32, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:38, or
- (g) a heavy chain variable region (V<sub>H</sub>OX40) comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:18, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:20, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:27, and a light chain variable region (V<sub>L</sub>OX40) comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:30, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:33, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:39.

9. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 8, wherein the bispecific OX40 antibody comprises at least one antigen binding domain capable of specific binding to OX40 comprising

- (a) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:40 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:41, or

- (b) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:42 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:43, or
- (c) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:44 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:45, or
- (d) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:46 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:47, or
- (a) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:48 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:49, or
- (a) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:50 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:51, or
- (a) a heavy chain variable region (V<sub>H</sub>OX40) comprising an amino acid sequence of SEQ ID NO:52 and a light chain variable region (V<sub>L</sub>OX40) comprising an amino acid sequence of SEQ ID NO:53.

10. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 9, wherein the bispecific OX40 antibody comprises an IgG Fc domain, specifically an IgG1 Fc domain or an IgG4 Fc domain.

11. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 10, wherein the bispecific OX40 antibody comprises a Fc domain that comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

12. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 11, wherein the bispecific OX40 antibody comprises monovalent binding to a tumor associated target and tetravalent binding to OX40.

13. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 12, wherein the bispecific OX40 antibody comprises a first Fab fragment capable

of specific binding to OX40 fused at the C-terminus of the CH1 domain to the VH domain of a second Fab fragment capable of specific binding to OX40 and a third Fab fragment capable of specific binding to OX40 fused at the C-terminus of the CH1 domain to the VH domain of a fourth Fab fragment capable of specific binding to OX40.

- 5           14. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 13, wherein the bispecific OX40 antibody comprises
- 10           (i) a first heavy chain comprising an amino acid sequence of SEQ ID NO:54, a second heavy chain comprising an amino acid sequence of SEQ ID NO:55, and four light chains comprising an amino acid sequence of SEQ ID NO:56, or
- (ii) a first heavy chain comprising an amino acid sequence of SEQ ID NO:57, a second heavy chain comprising an amino acid sequence of SEQ ID NO:58, and four light chains comprising an amino acid sequence of SEQ ID NO:56, or
- 15           (i) a first heavy chain comprising an amino acid sequence of SEQ ID NO:59, a second heavy chain comprising an amino acid sequence of SEQ ID NO:60, and four light chains comprising an amino acid sequence of SEQ ID NO:56, or
- (ii) a first heavy chain comprising an amino acid sequence of SEQ ID NO:61, a second heavy chain comprising an amino acid sequence of SEQ ID NO:62, and four light chains comprising an amino acid sequence of SEQ ID NO:56.
- 20           15. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 14, wherein the T-cell activating anti-CD3 bispecific antibody is an anti-CEA/anti-CD3 bispecific antibody.
16. The bispecific OX40 antibody comprising at least one antigen binding domain
- 25           capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 15, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CD3) and a light chain variable region (V<sub>L</sub>CD3), and a second antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CEA) and a light chain variable region (V<sub>L</sub>CEA).
- 30           17. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 16, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CD3) comprising CDR-H1 sequence of SEQ ID NO:63, CDR-H2 sequence of SEQ ID NO:64, and CDR-H3

sequence of SEQ ID NO:65; and/or a light chain variable region (V<sub>L</sub>CD3) comprising CDR-L1 sequence of SEQ ID NO:66, CDR-L2 sequence of SEQ ID NO:67, and CDR-L3 sequence of SEQ ID NO:68.

18. The bispecific OX40 antibody comprising at least one antigen binding domain  
5 capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 17, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CD3) comprising the amino acid sequence of SEQ ID NO:69 and/or a light chain variable region (V<sub>L</sub>CD3) comprising the amino acid sequence of SEQ ID NO:70.

10 19. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 18, wherein the T-cell activating anti-CD3 bispecific antibody comprises a second antigen binding domain comprising  
(a) a heavy chain variable region (V<sub>H</sub>CEA) comprising CDR-H1 sequence of SEQ ID NO:71, CDR-H2 sequence of SEQ ID NO:72, and CDR-H3 sequence of SEQ ID NO:73, and/or a  
15 light chain variable region (V<sub>L</sub>CEA) comprising CDR-L1 sequence of SEQ ID NO:74, CDR-L2 sequence of SEQ ID NO:75, and CDR-L3 sequence of SEQ ID NO:76, or  
(b) a heavy chain variable region (V<sub>H</sub>CEA) comprising CDR-H1 sequence of SEQ ID NO:79, CDR-H2 sequence of SEQ ID NO:80, and CDR-H3 sequence of SEQ ID NO:81, and/or a  
20 light chain variable region (V<sub>L</sub>CEA) comprising CDR-L1 sequence of SEQ ID NO:82, CDR-L2 sequence of SEQ ID NO:83, and CDR-L3 sequence of SEQ ID NO:84.

20. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 19, wherein the T-cell activating anti-CD3 bispecific antibody comprises a second  
25 antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CEA) comprising the amino acid sequence of SEQ ID NO:77 and/or a light chain variable region (V<sub>L</sub>CEA) comprising the amino acid sequence of SEQ ID NO:78 or a second antigen binding domain comprising a heavy chain variable region (V<sub>H</sub>CEA) comprising the amino acid sequence of SEQ ID NO:85 and/or a light chain variable region (V<sub>L</sub>CEA) comprising the amino acid  
30 sequence of SEQ ID NO:86.

21. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 20, wherein the anti-CEA/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to CEA.

22. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 21, wherein the T-cell activating anti-CD3 bispecific antibody comprises an Fc domain comprising one or more amino acid substitutions that reduce binding to an Fc receptor and/or effector function.

23. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 14, wherein the T-cell activating anti-CD3 bispecific antibody is an anti-FolR1/anti-CD3 bispecific antibody.

24. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 14 or 23, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region ( $V_H$ CD3), a second antigen binding domain comprising a heavy chain variable region ( $V_H$ FolR1) and a common light chain variable region.

25. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 14 or 23 or 24, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region ( $V_H$ CD3) comprising CDR-H1 sequence of SEQ ID NO:95, CDR-H2 sequence of SEQ ID NO:96, and CDR-H3 sequence of SEQ ID NO:97; the second antigen binding domain comprising a heavy chain variable region ( $V_H$ FolR1) comprising CDR-H1 sequence of SEQ ID NO:98, CDR-H2 sequence of SEQ ID NO:99, and CDR-H3 sequence of SEQ ID NO:100; and a common light chain comprising a CDR-L1 sequence of SEQ ID NO:101, CDR-L2 sequence of SEQ ID NO:102, and CDR-L3 sequence of SEQ ID NO:103.

26. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 14 or 23 to 25, wherein the T-cell activating anti-CD3 bispecific antibody comprises a first antigen binding domain comprising a heavy chain variable region ( $V_H$ CD3) comprising the sequence of SEQ ID NO:104; a second antigen binding domain comprises a heavy chain variable region ( $V_H$ FolR1) comprising the sequence of SEQ ID NO:105; and a common light chain comprising the sequence of SEQ ID NO:106.

27. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 14 or 23 to 26, wherein the anti-FolR1/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to FolR1.

5        28. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 14 or 23 to 27, wherein the anti-FolR1/anti-CD3 bispecific antibody comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO:107, a second heavy chain comprising the amino acid sequence of SEQ ID NO:108 and a common light chain of  
10    SEQ ID NO: 109.

29. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of any one of claims 1 to 28, wherein the bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen specific for a tumor-  
15    associated antigen is used in combination with a T-cell activating anti-CD3 bispecific antibody specific for a tumor-associated antigen and in combination with an agent blocking PD-L1/PD-1 interaction.

30. The bispecific OX40 antibody comprising at least one antigen binding domain capable of specific binding to a tumor-associated antigen for use in a method of claim 29,  
20    wherein the agent blocking PD-L1/PD-1 interaction is a anti-PD-L1 antibody or an anti-PD1 antibody.

31. A pharmaceutical product comprising (A) a first composition comprising as active ingredient an anti-FAP/anti-OX40 bispecific antibody and a pharmaceutically acceptable excipient; and (B) a second composition comprising an anti-CEA/anti-CD3 bispecific  
25    antibody or anti-FolR1/anti-CD3 bispecific antibody and a pharmaceutically acceptable excipient, for use in the combined, sequential or simultaneous, treatment of a disease, in particular cancer.

32. A pharmaceutical composition comprising anti-FAP/anti-OX40 bispecific antibody and an anti-CEA/anti-CD3 bispecific antibody or anti-FolR1/anti-CD3 bispecific antibody.

30        33. The pharmaceutical composition of claim 32, further comprising an agent blocking PD-L1/PD-1 interaction.



34. The pharmaceutical composition of claims 32 or 33 for use in the treatment of solid tumors.

35. An anti-FAP/anti-OX40 bispecific antibody for use in a method for treating or delaying progression of cancer, wherein the anti-FAP/anti-OX40 bispecific antibody is used  
5 in combination with an agent blocking PD-L1/PD-1 interaction.

36. The anti-FAP/anti-OX40 bispecific antibody for use in a method of claim 35, wherein the agent blocking PD-L1/PD-1 interaction is an anti-PD-L1 antibody or an anti-PD1 antibody.

37. The anti-FAP/anti-OX40 bispecific antibody for use in a method of claims 35 or 36,  
10 wherein the agent blocking PD-L1/PD-1 interaction is an anti-PD-L1 antibody.

38. The anti-FAP/anti-OX40 bispecific antibody for use in a method of any one of claims 35 to 37, wherein the agent blocking PD-L1/PD-1 interaction is atezolizumab.

\*\*\*

Fig. 1

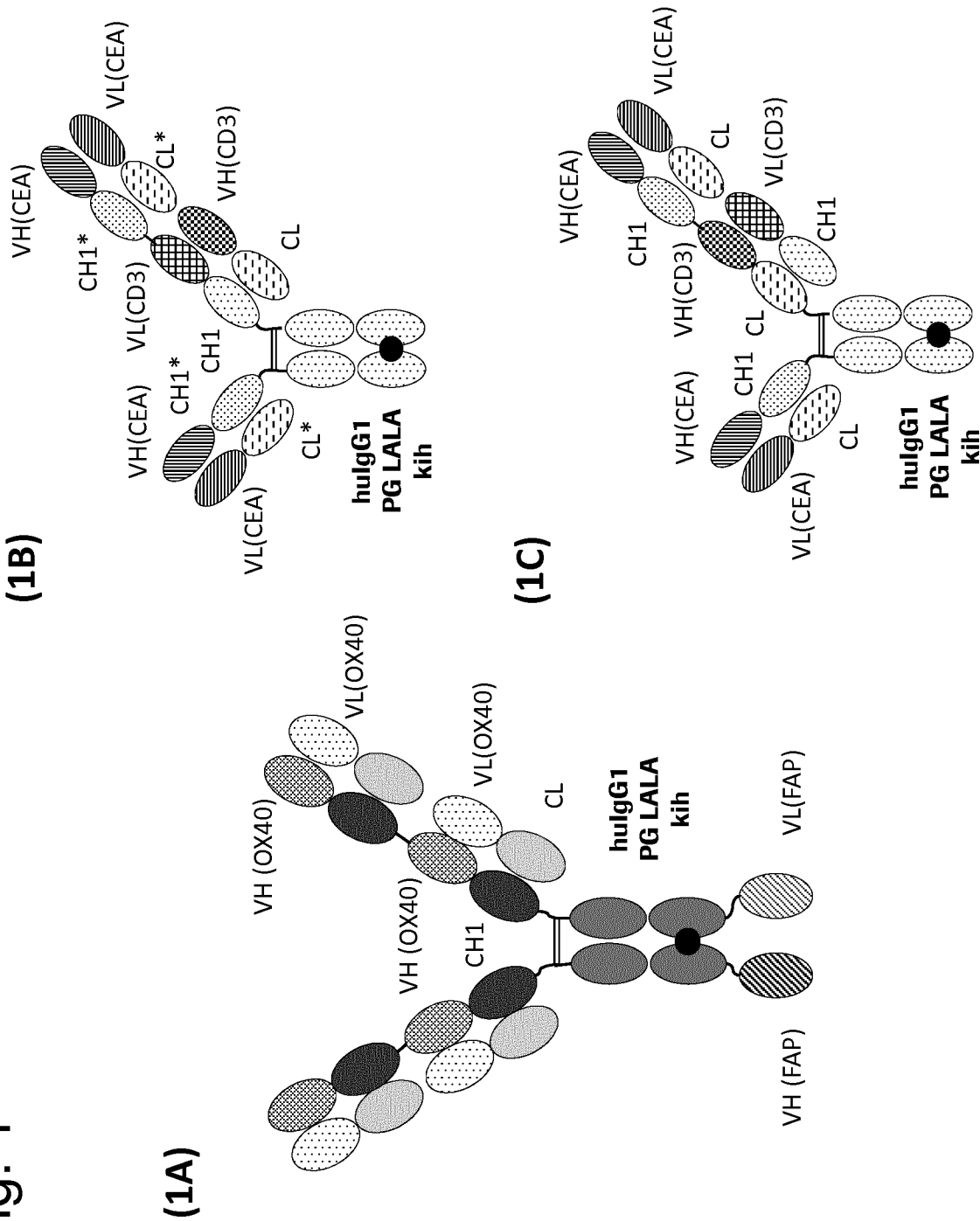


Fig. 2

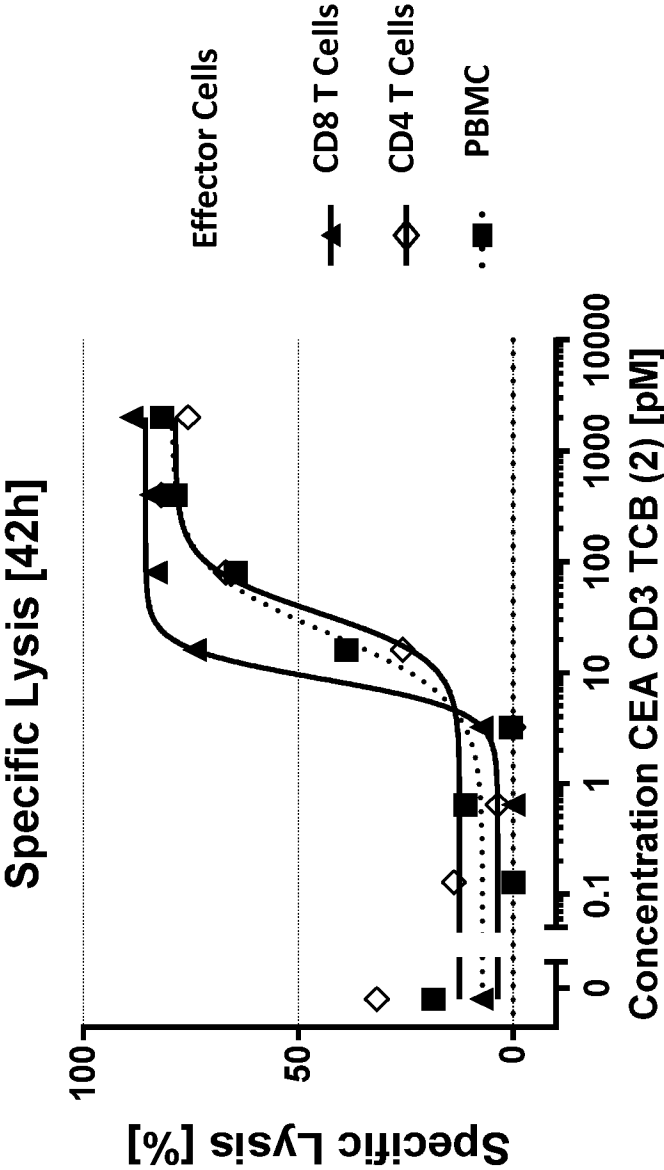
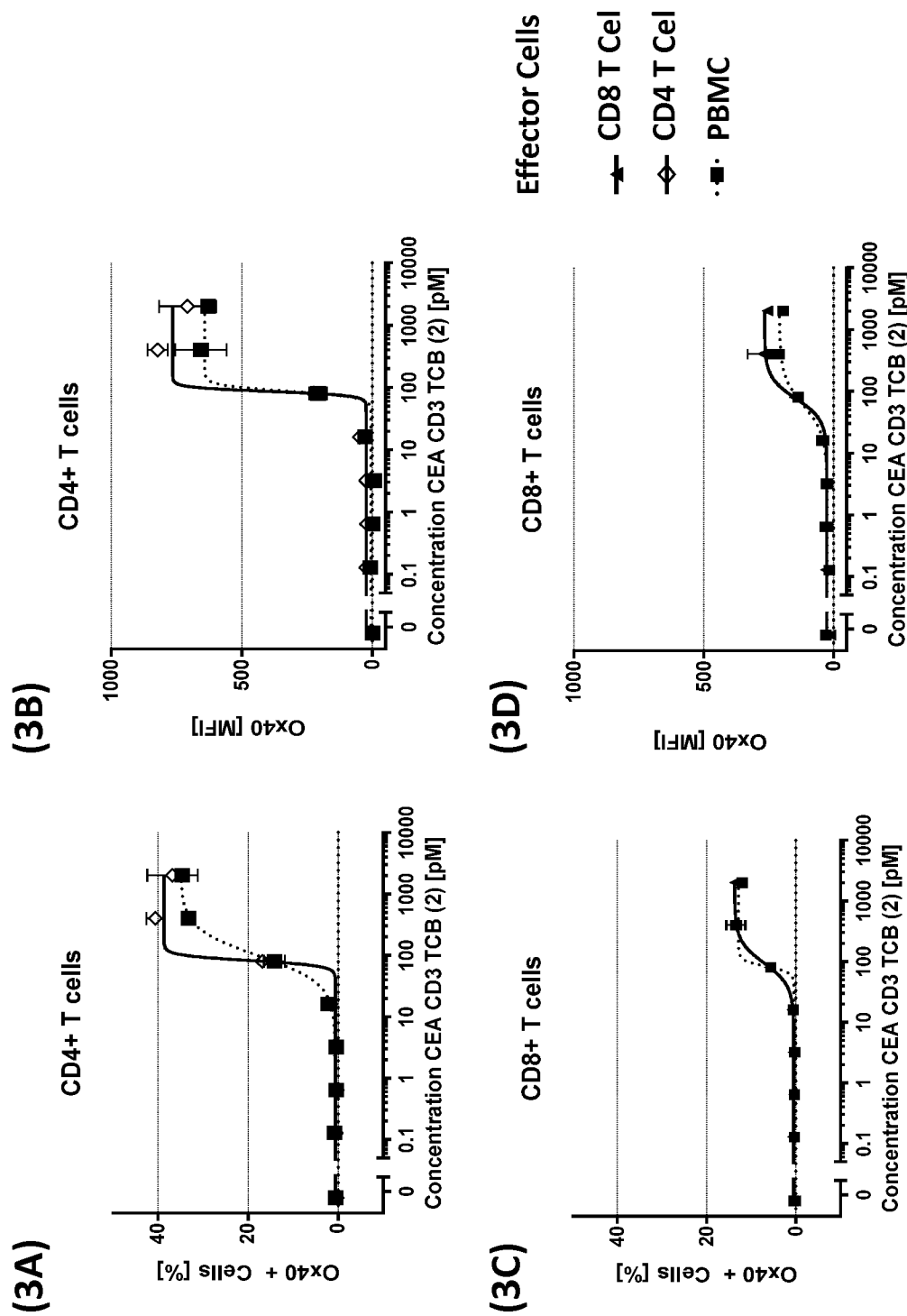


Fig. 3



**(4A)**

HeLa NucLight red  
FoIR CD3 TCB  
wo Costimulation

**(4B)**

HeLa NucLight red  
FoIR CD3 TCB  
FAP Ox40iMab

**(4C)**

AUC summary

- w/ FAPOx40iMAB
- w/o costimulation

| Time point (hrs) | w/ FAPOx40iMAB     | w/o costimulation  |
|------------------|--------------------|--------------------|
| 0                | ~10 <sup>7.0</sup> | ~10 <sup>7.0</sup> |
| 6                | ~10 <sup>7.0</sup> | ~10 <sup>7.0</sup> |
| 12               | ~10 <sup>7.0</sup> | ~10 <sup>7.0</sup> |
| 15               | ~10 <sup>7.1</sup> | ~10 <sup>7.1</sup> |
| 18               | ~10 <sup>7.2</sup> | ~10 <sup>7.2</sup> |
| 21               | ~10 <sup>7.3</sup> | ~10 <sup>7.3</sup> |
| 24               | ~10 <sup>7.4</sup> | ~10 <sup>7.4</sup> |
| 27               | ~10 <sup>7.4</sup> | ~10 <sup>7.4</sup> |
| 30               | ~10 <sup>7.5</sup> | ~10 <sup>7.5</sup> |
| 33               | ~10 <sup>7.5</sup> | ~10 <sup>7.5</sup> |
| 36               | ~10 <sup>7.5</sup> | ~10 <sup>7.5</sup> |

Fig. 5

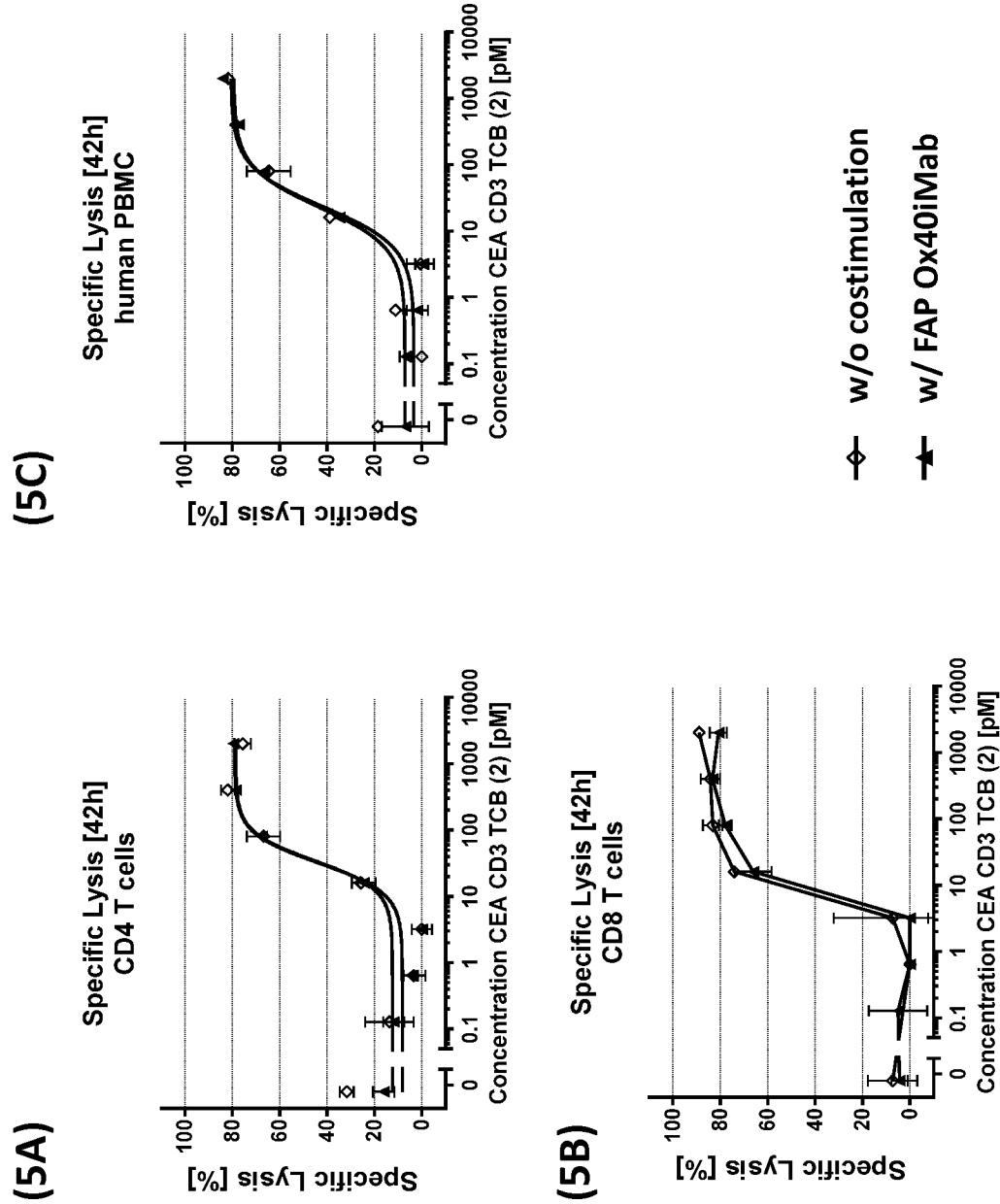
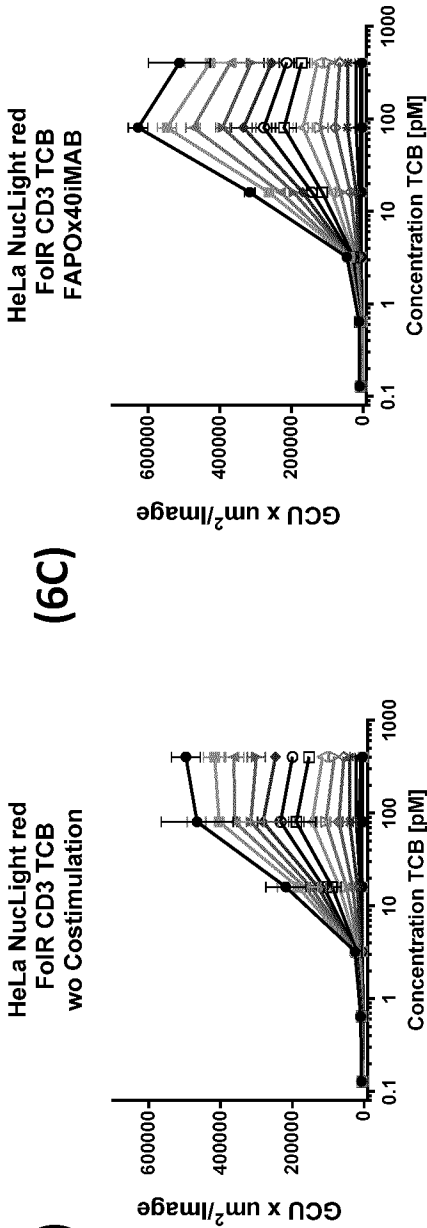
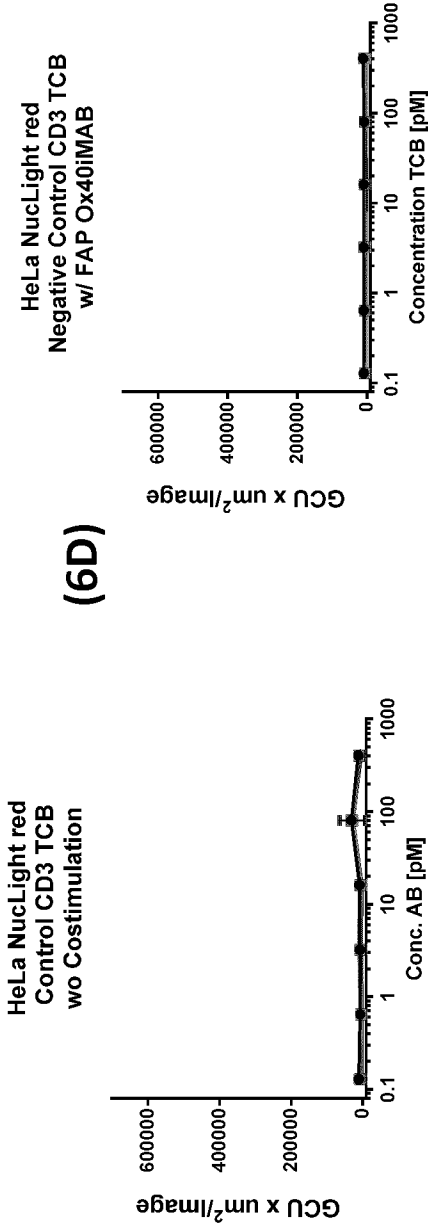


Fig. 6  
(6A)



(6B)



Time point (hrs)

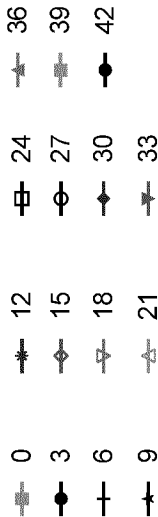
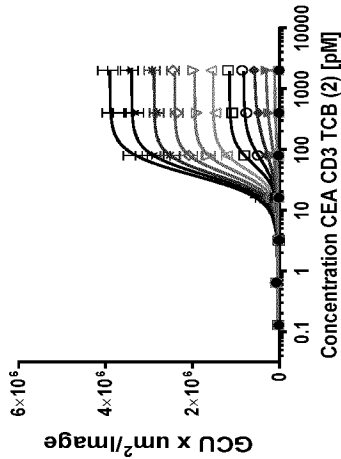


Fig. 7

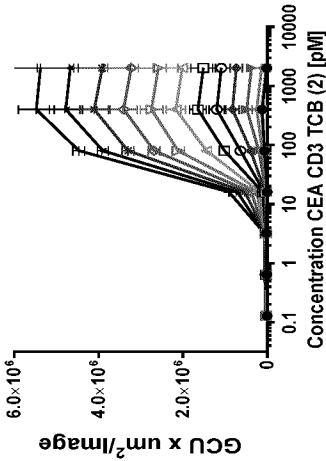
(7A)

MKN-45 NucLight red  
CEA CD3 TCB (2)  
w/o costimulation



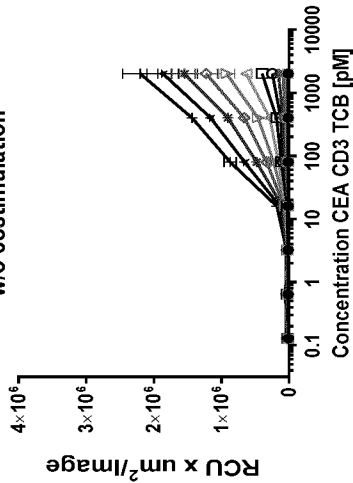
(7C)

MKN-45 NucLight red  
CEA CD3 TCB (2)  
w/ FAP Ox40iMAB



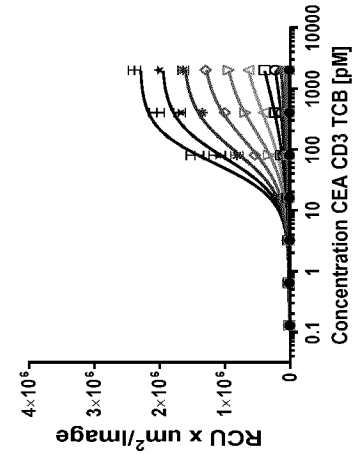
(7B)

MKN-45 NucLight red  
CEA CD3 TCB  
w/o costimulation



(7D)

MKN-45 NucLight red  
CEA CD3 TCB  
w/ FAP Ox40iMab



Time point (hrs)





Fig. 8

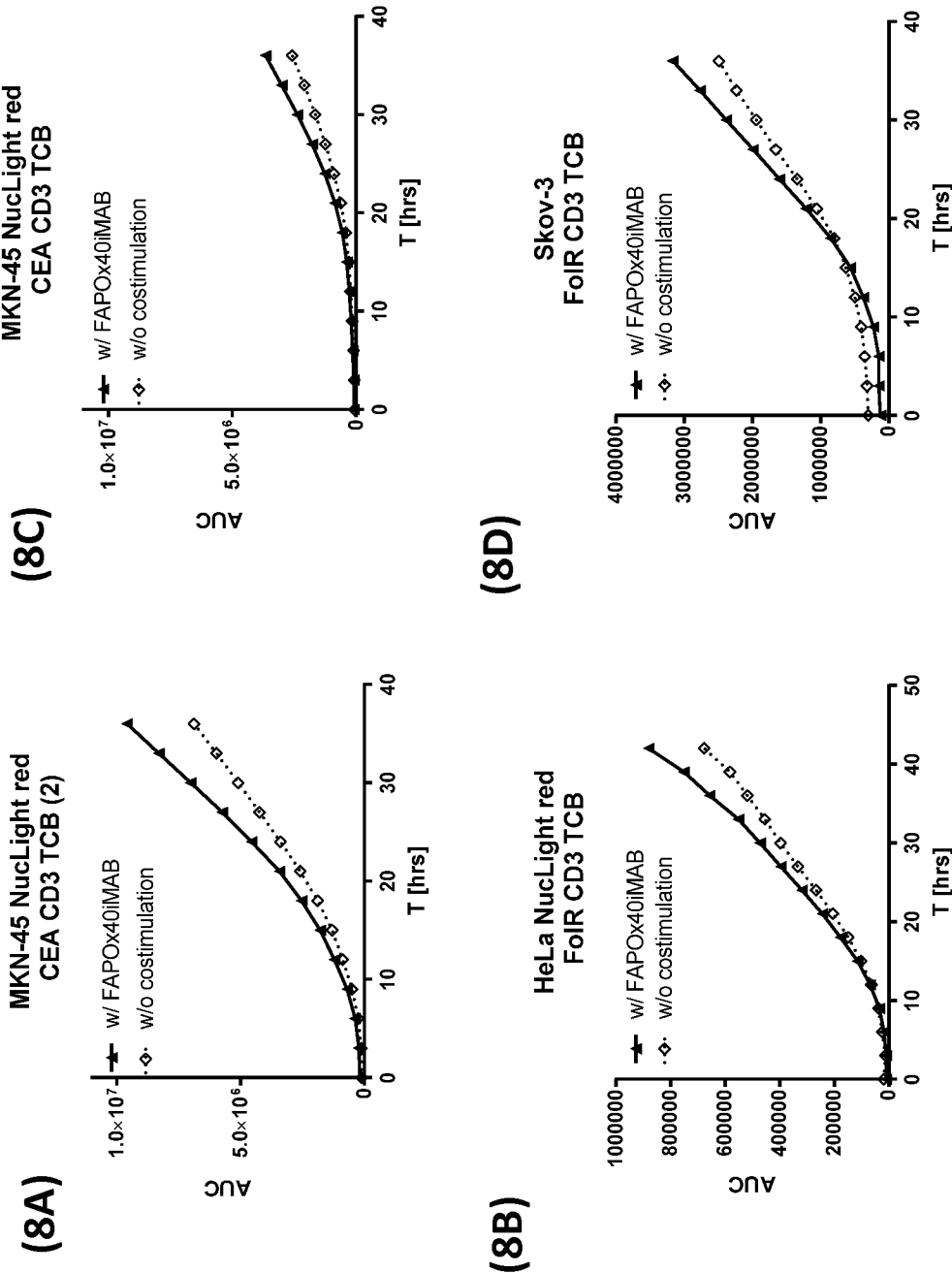


Fig. 9

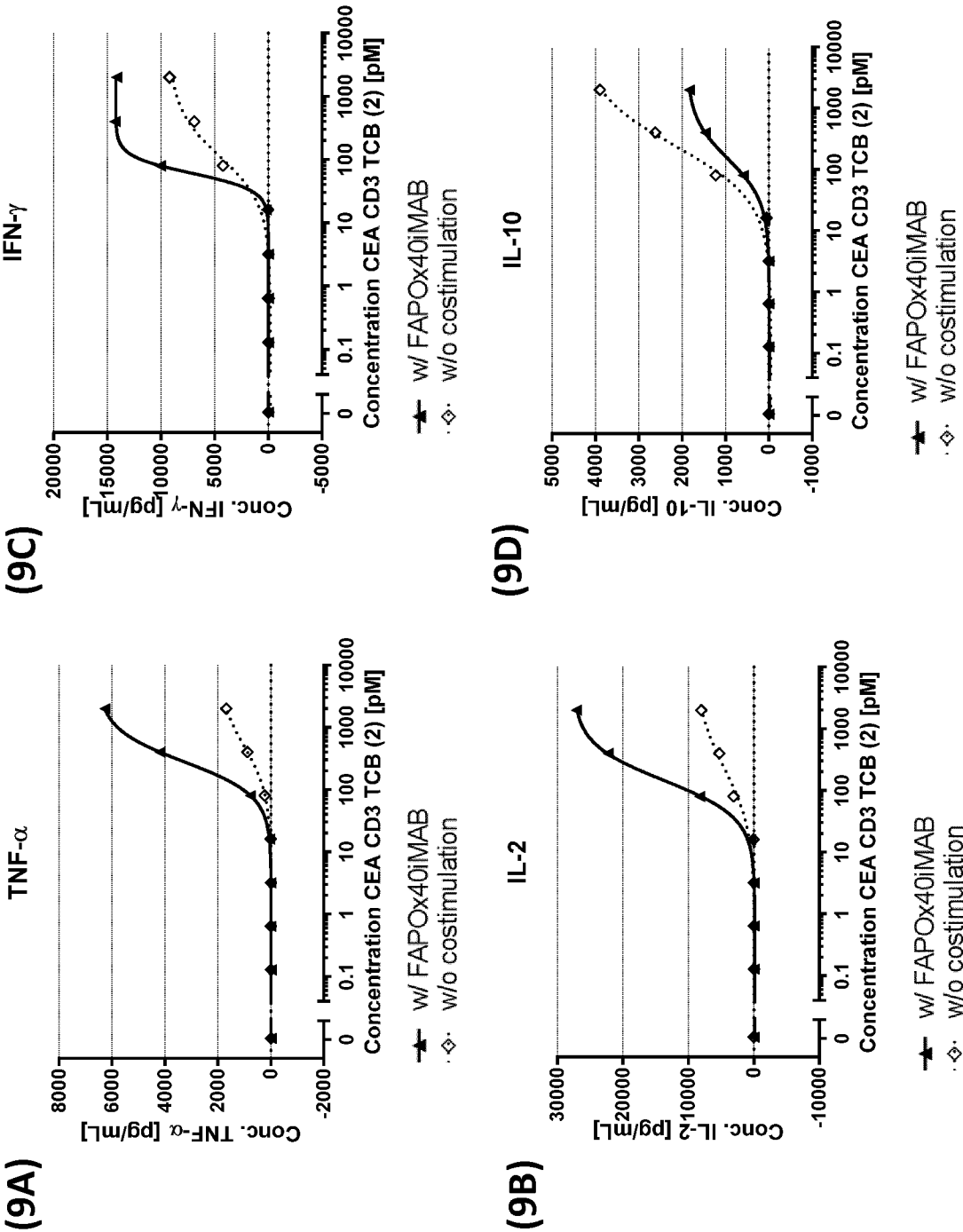


Fig. 10

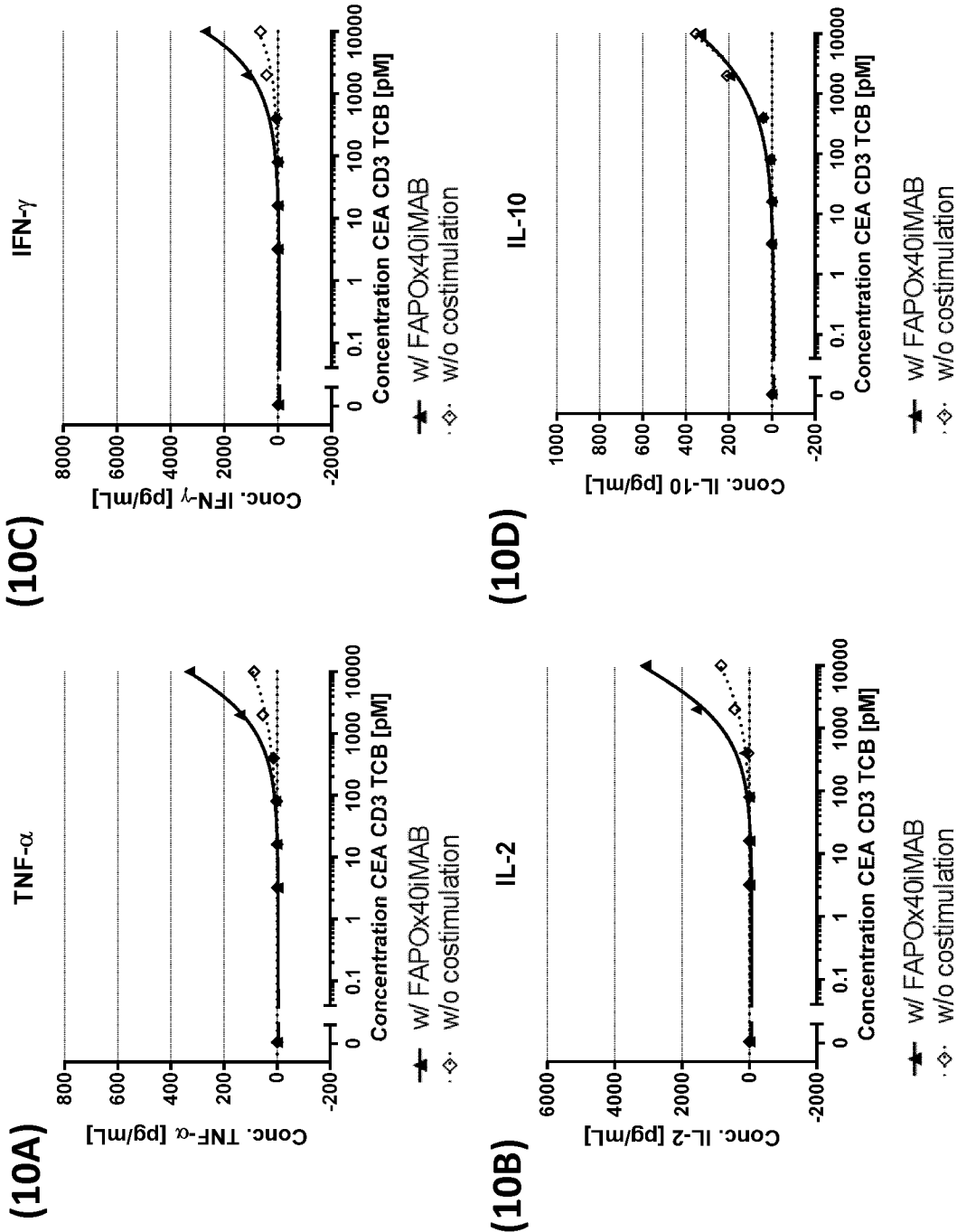


Fig. 11

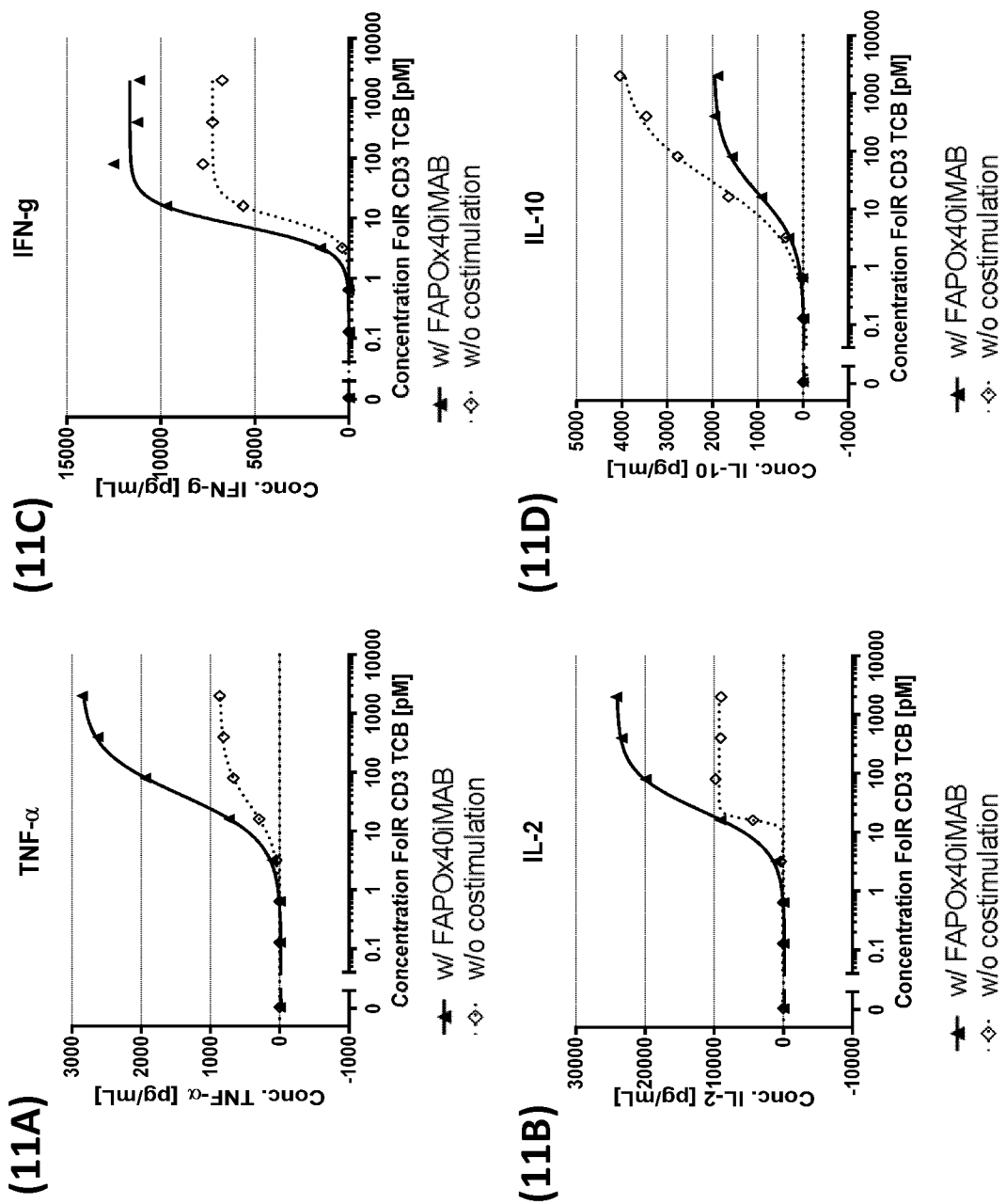


Fig. 12

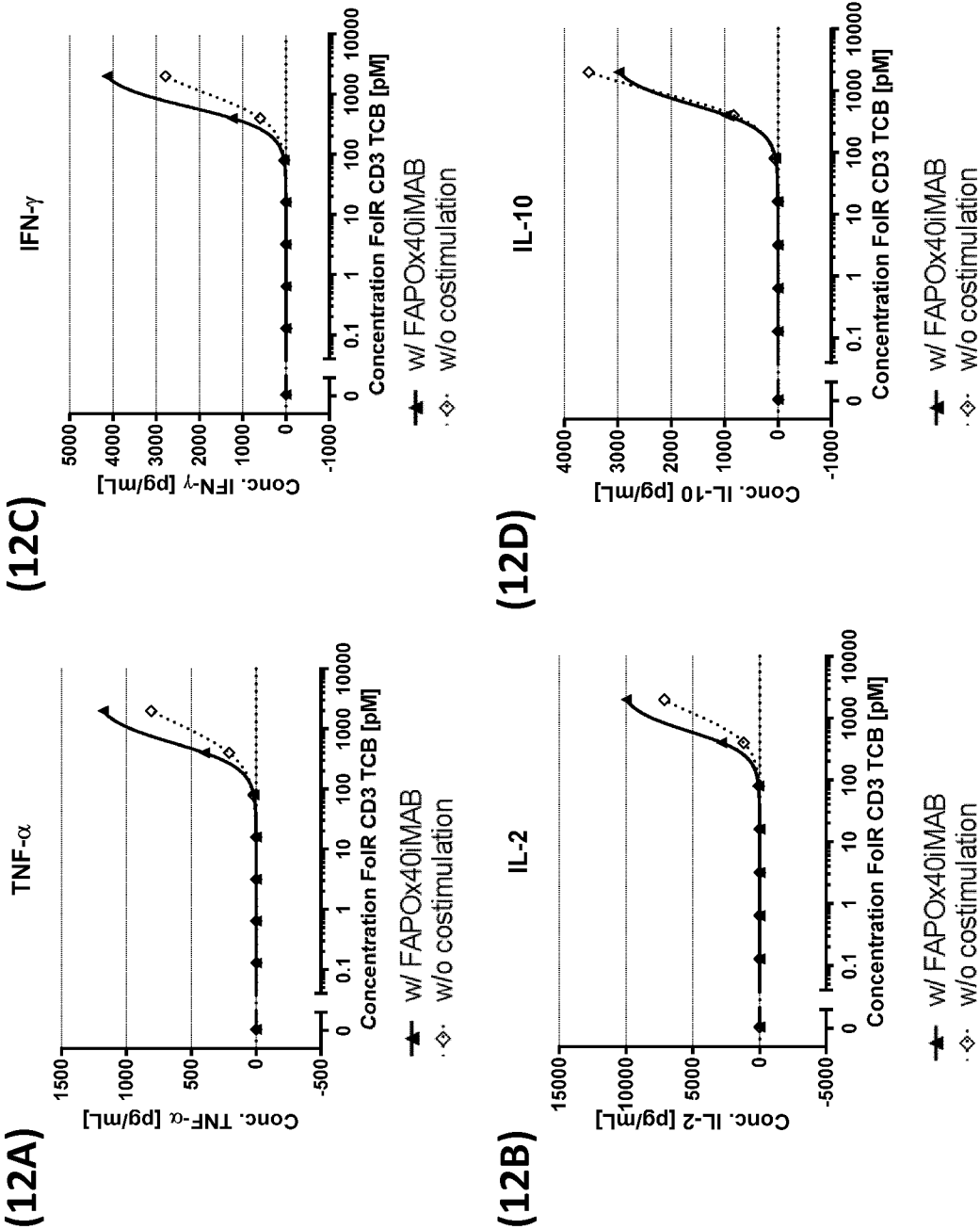


Fig. 13

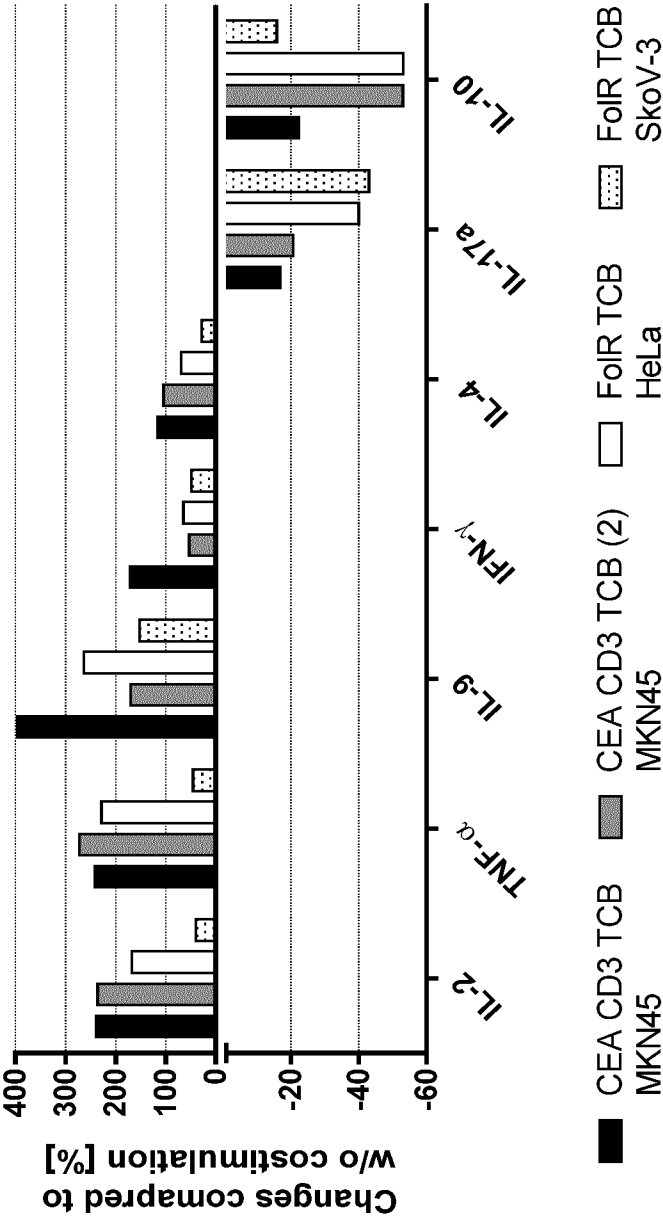


Fig. 14

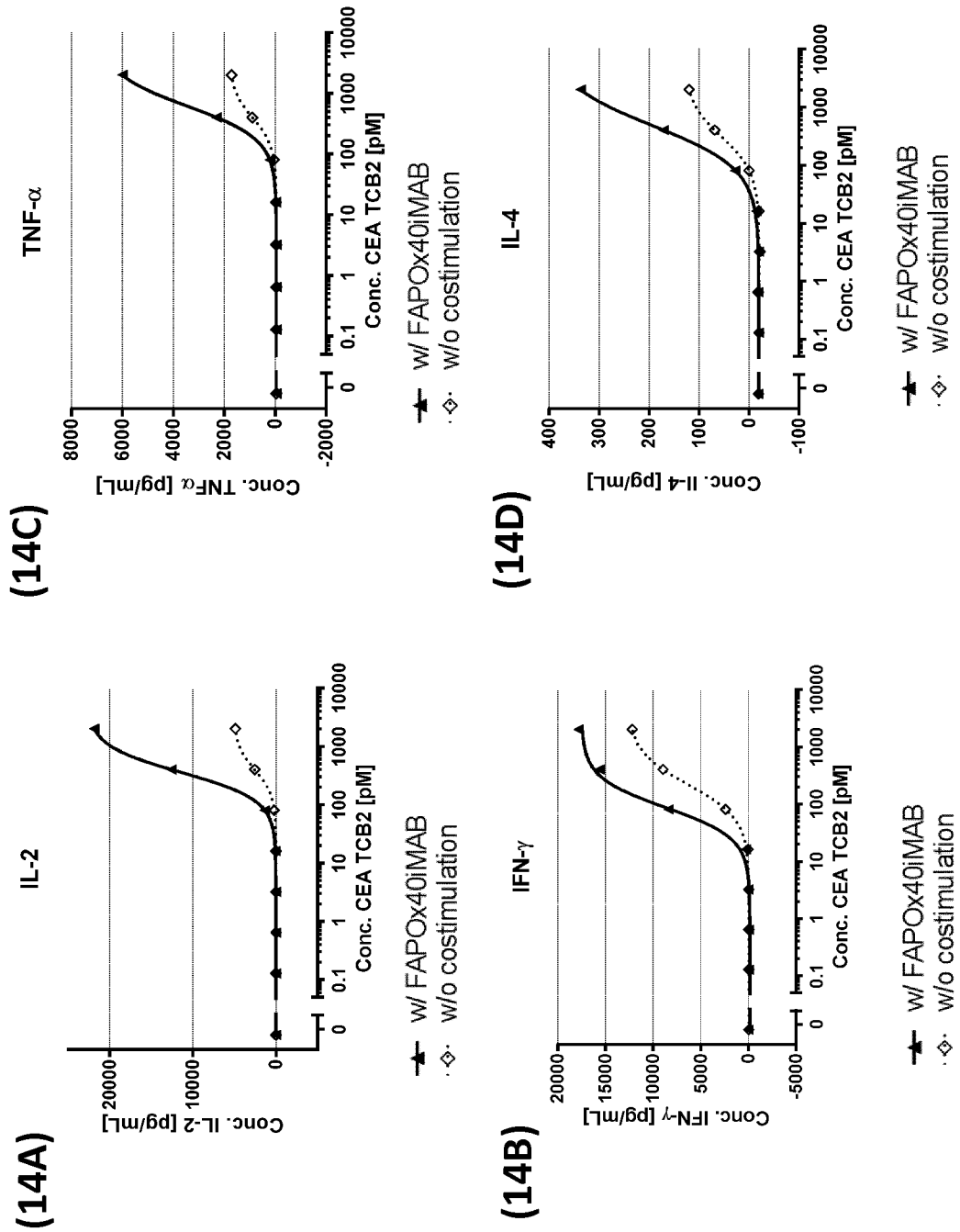


Fig. 14

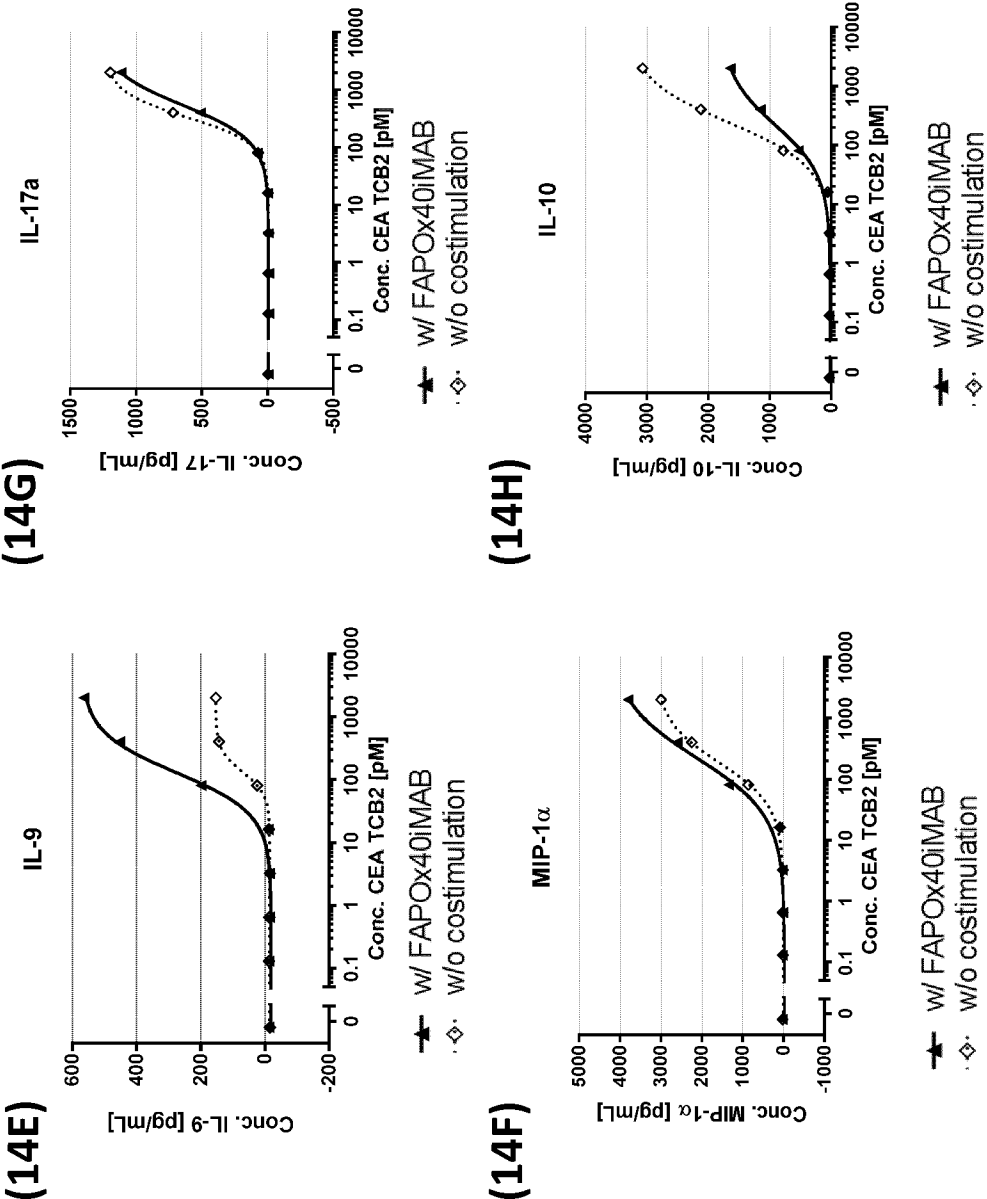




Fig. 15

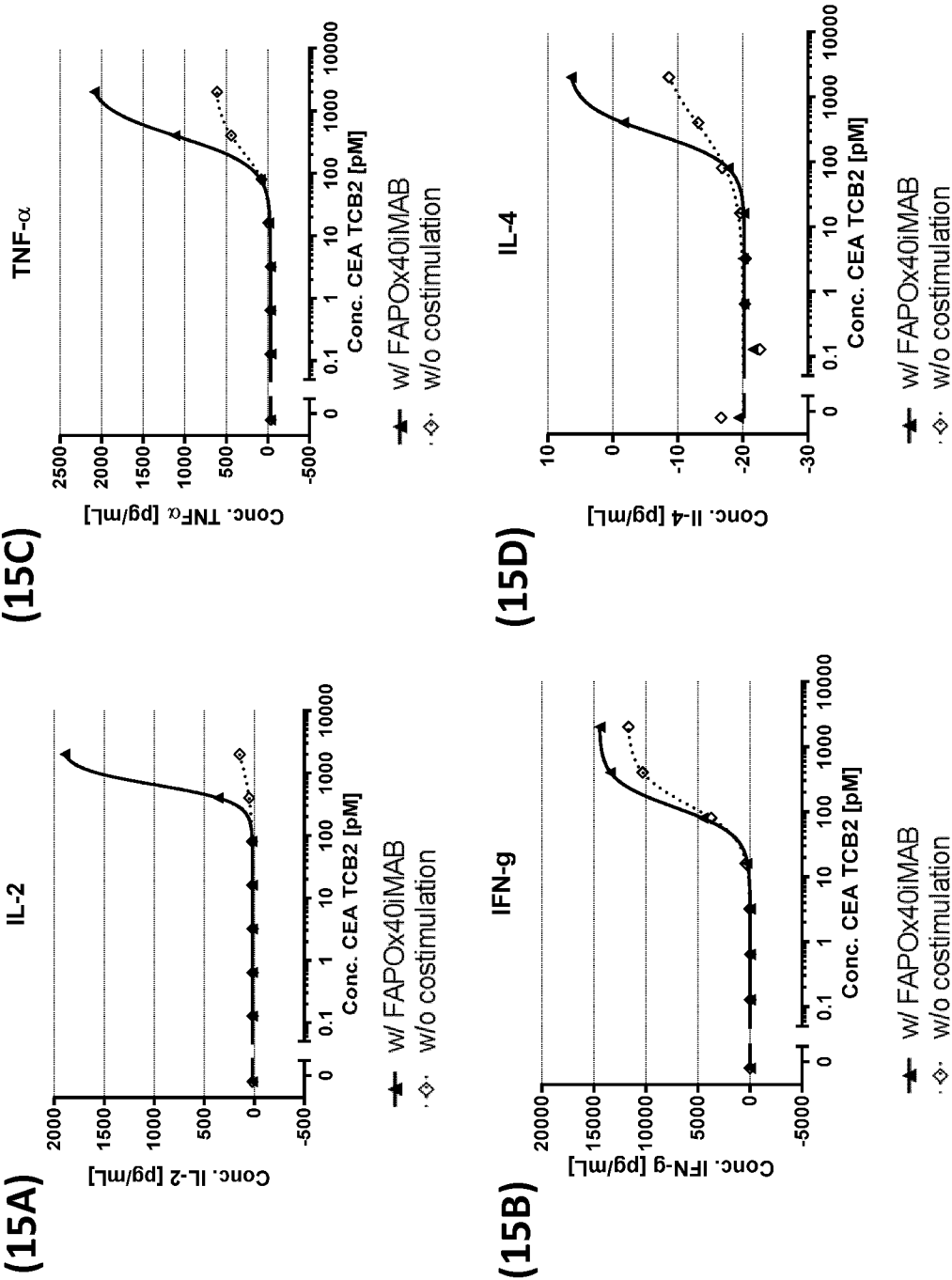
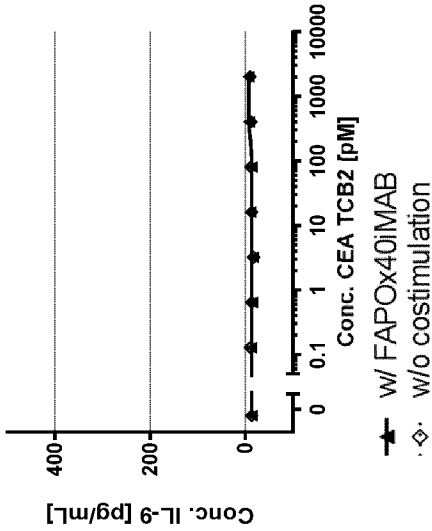
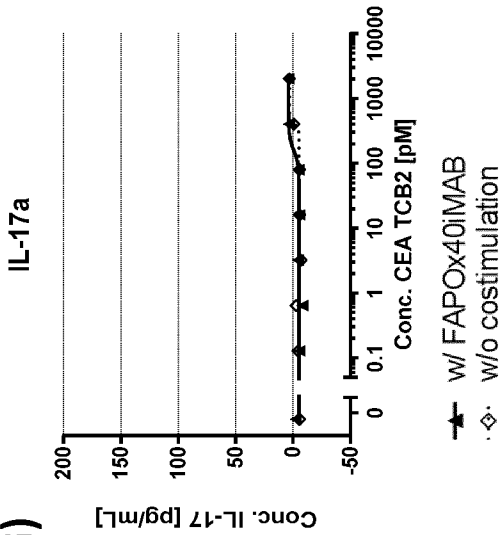


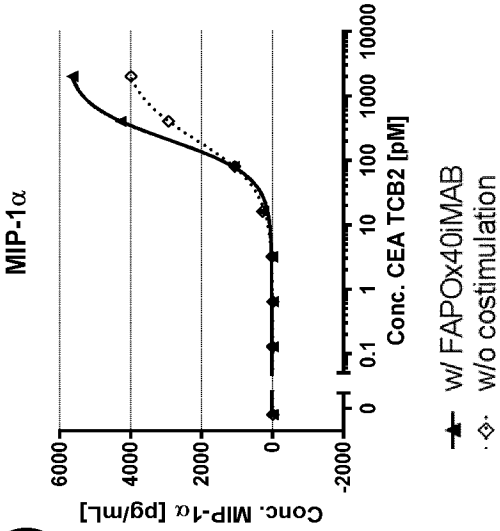
Fig. 15  
(15E)



(15G)



(15F)



(15H)

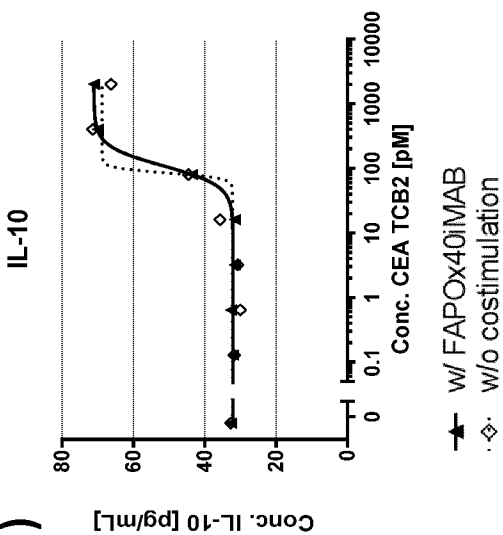


Fig. 16

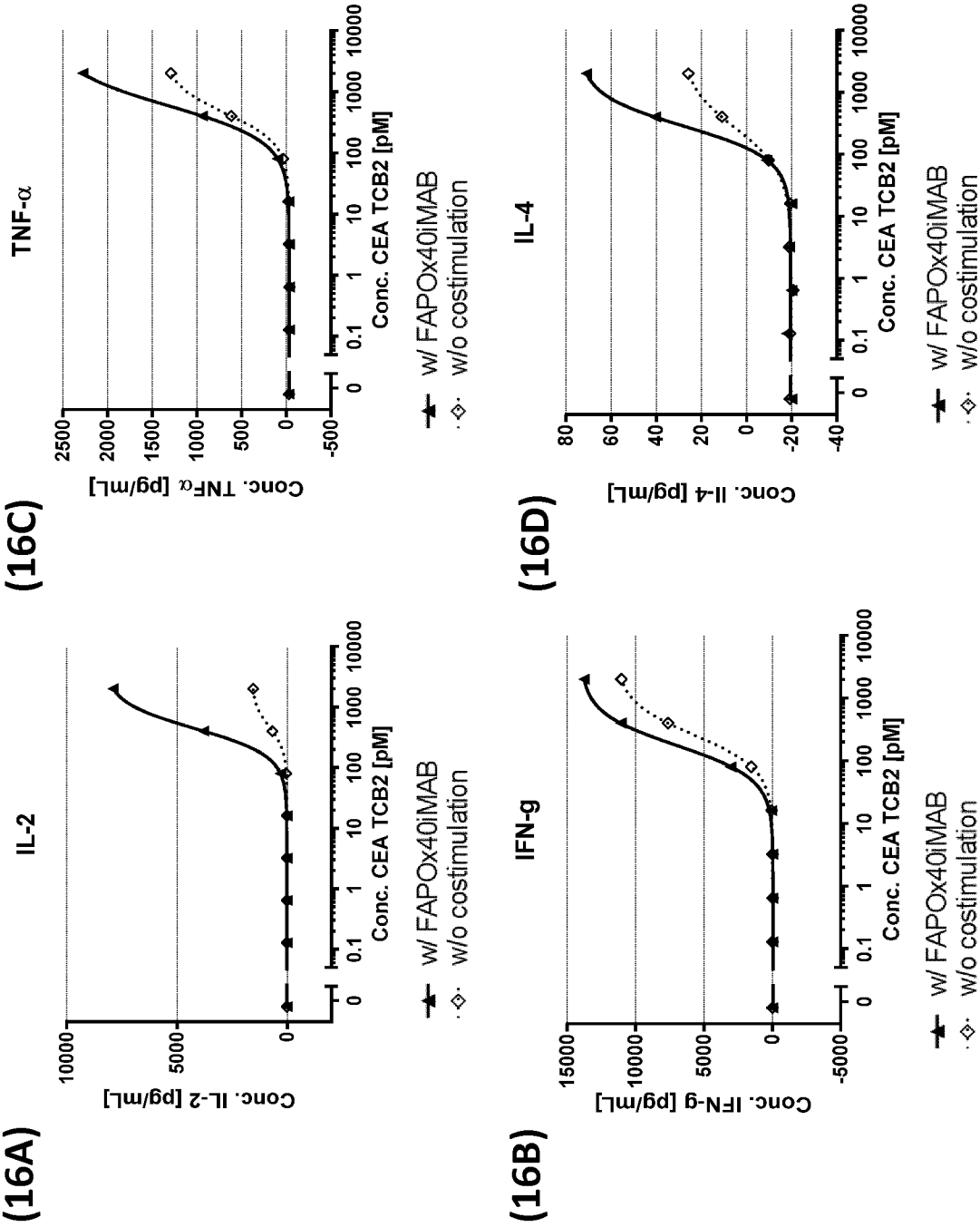
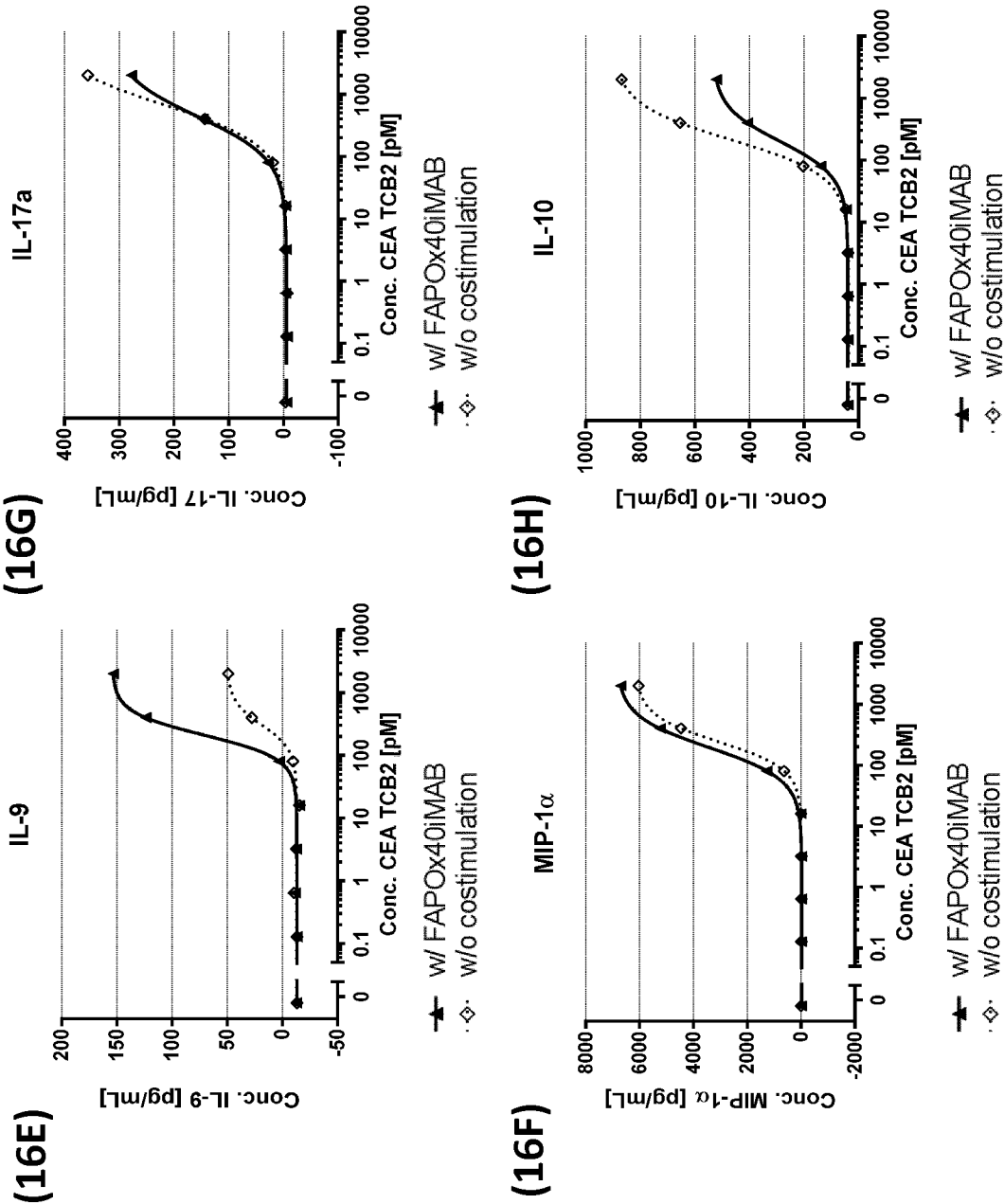


Fig. 16



[illegible]

Fig. 18  
(18A)

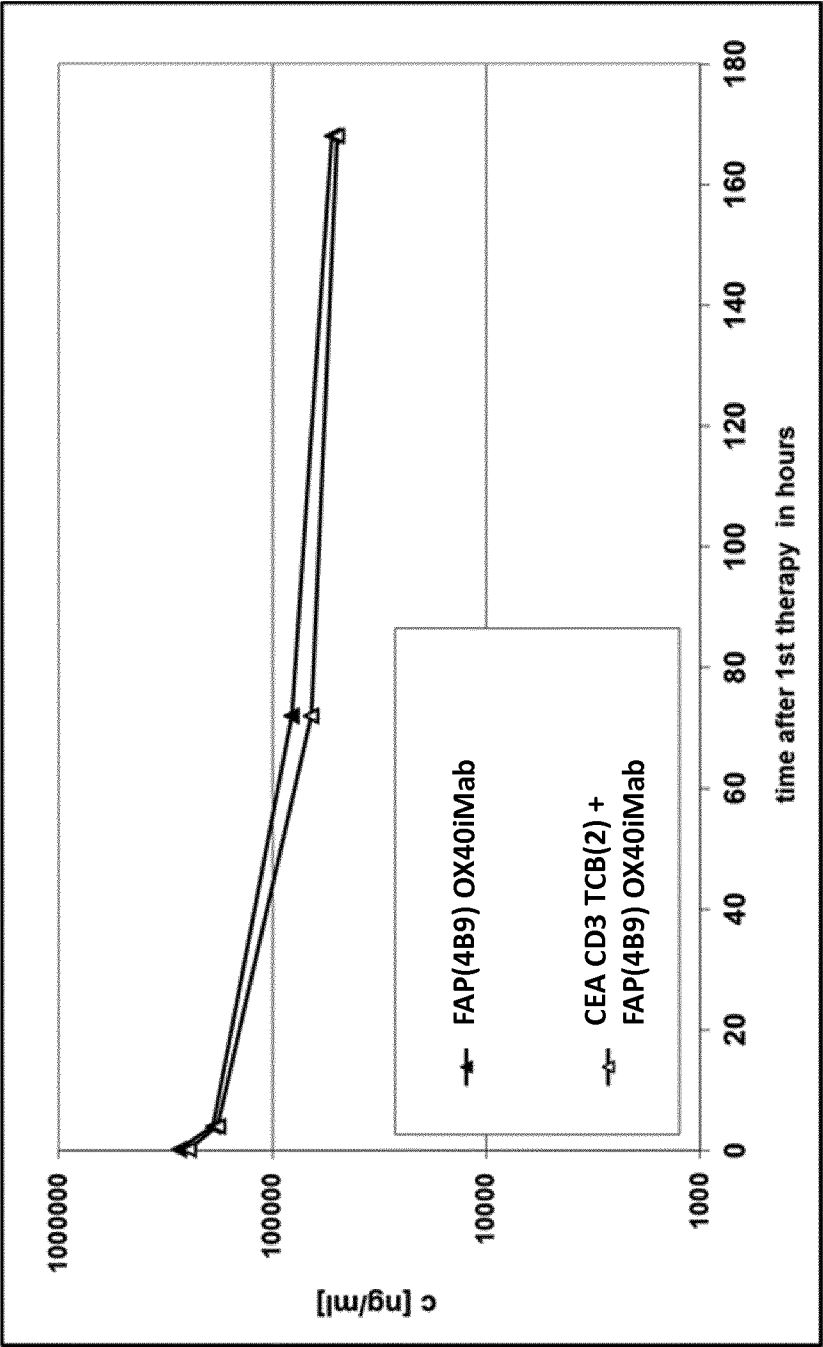


Fig. 18  
(18B)

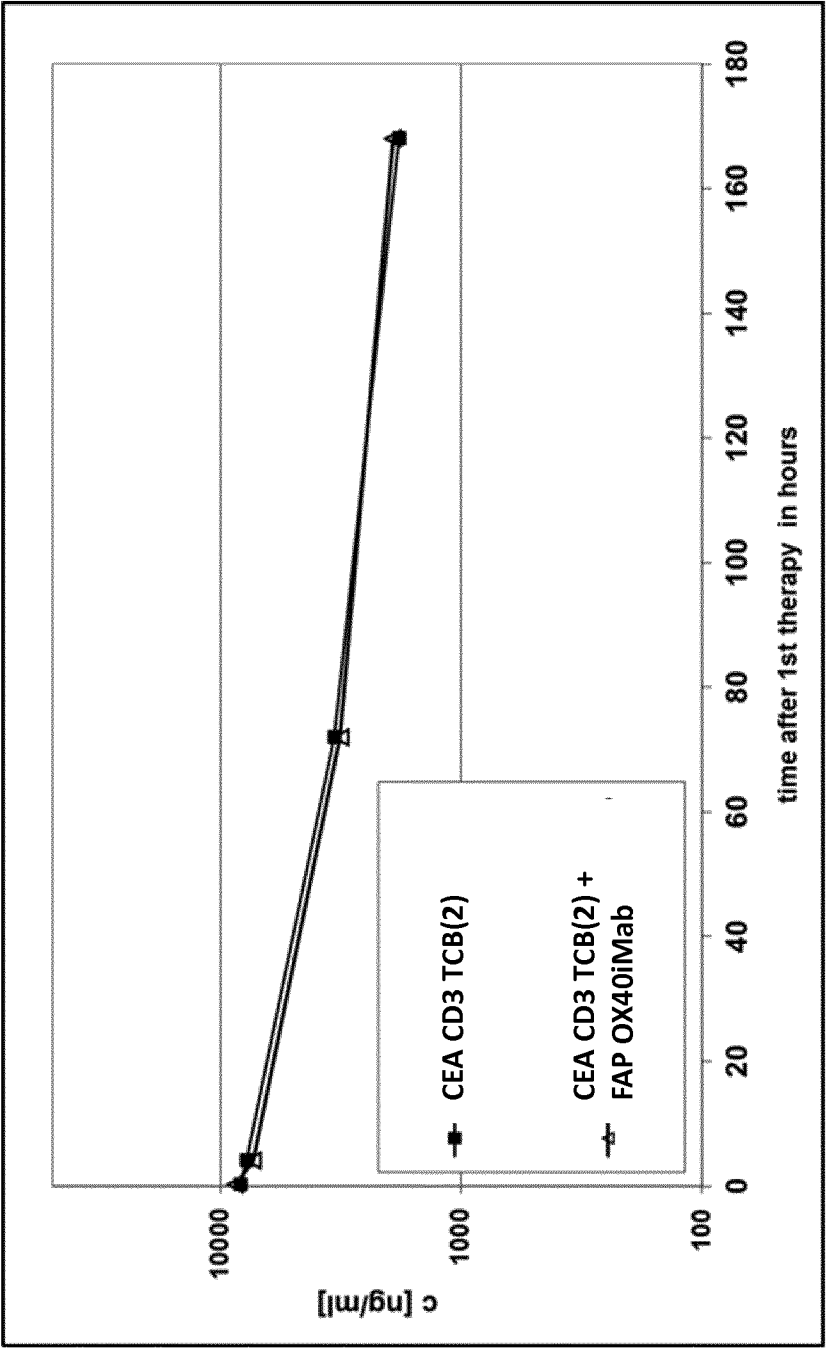


Fig. 19

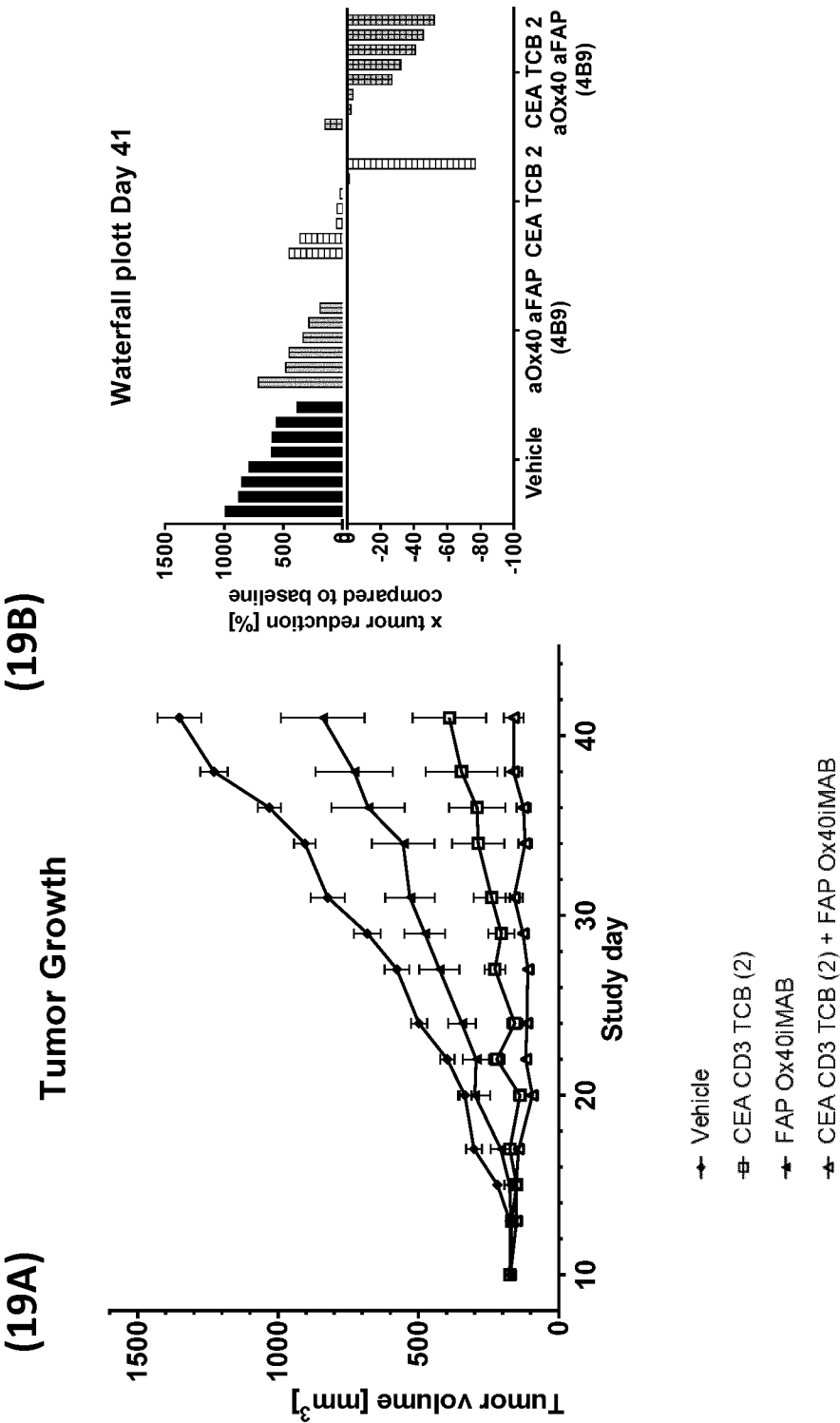




Fig. 20  
(20A)

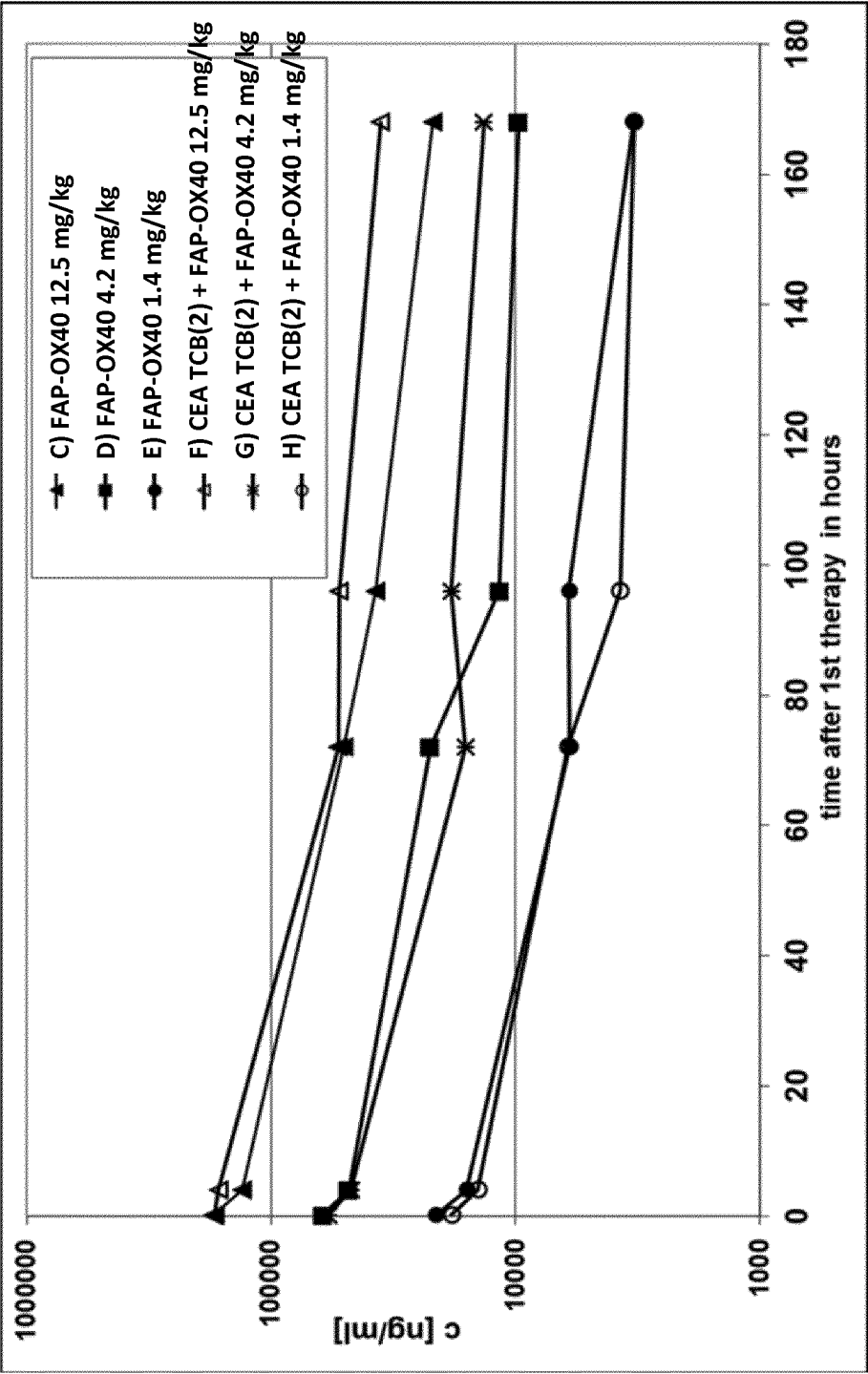


Fig. 20  
(20B)

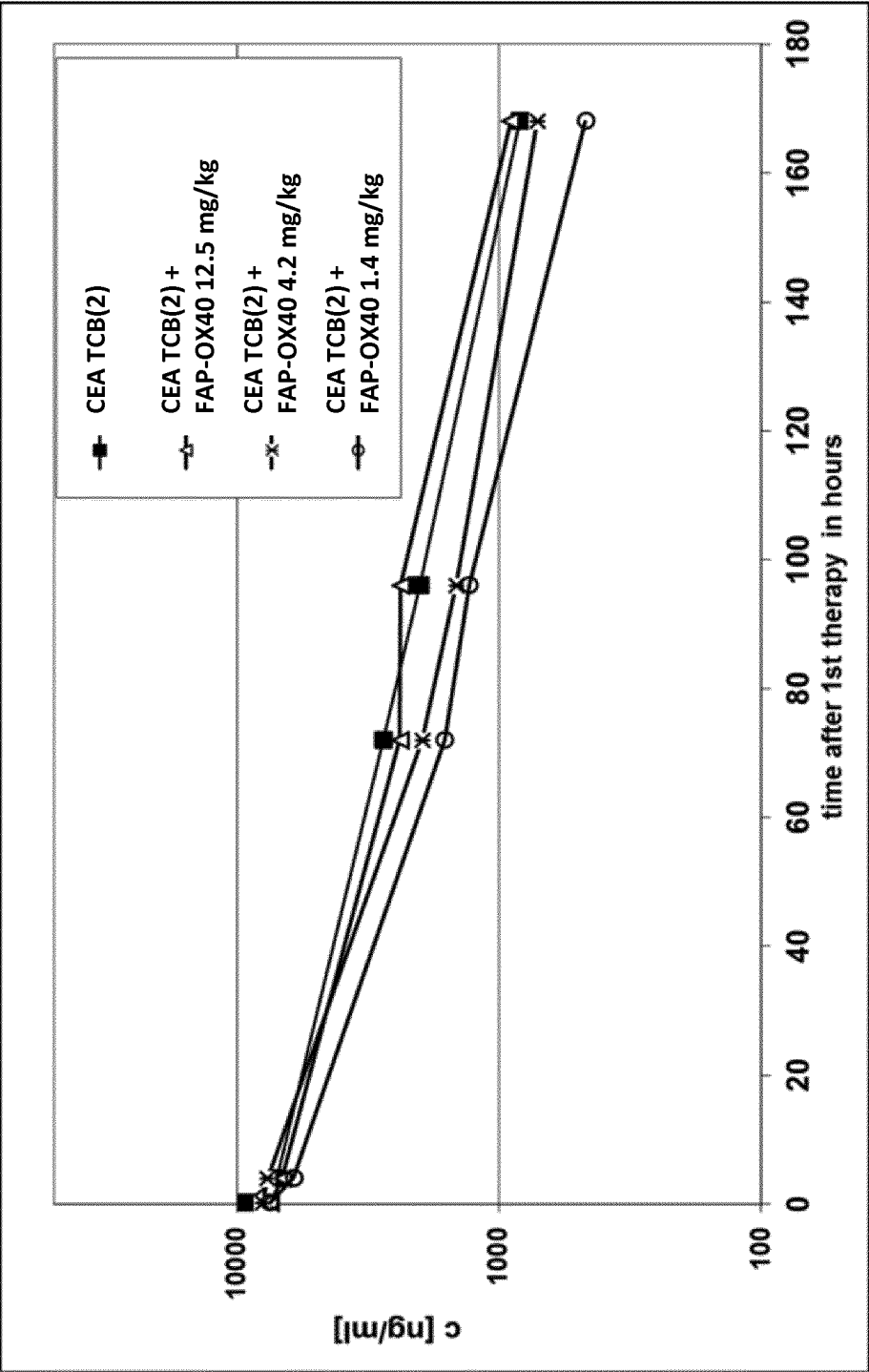


Fig. 21

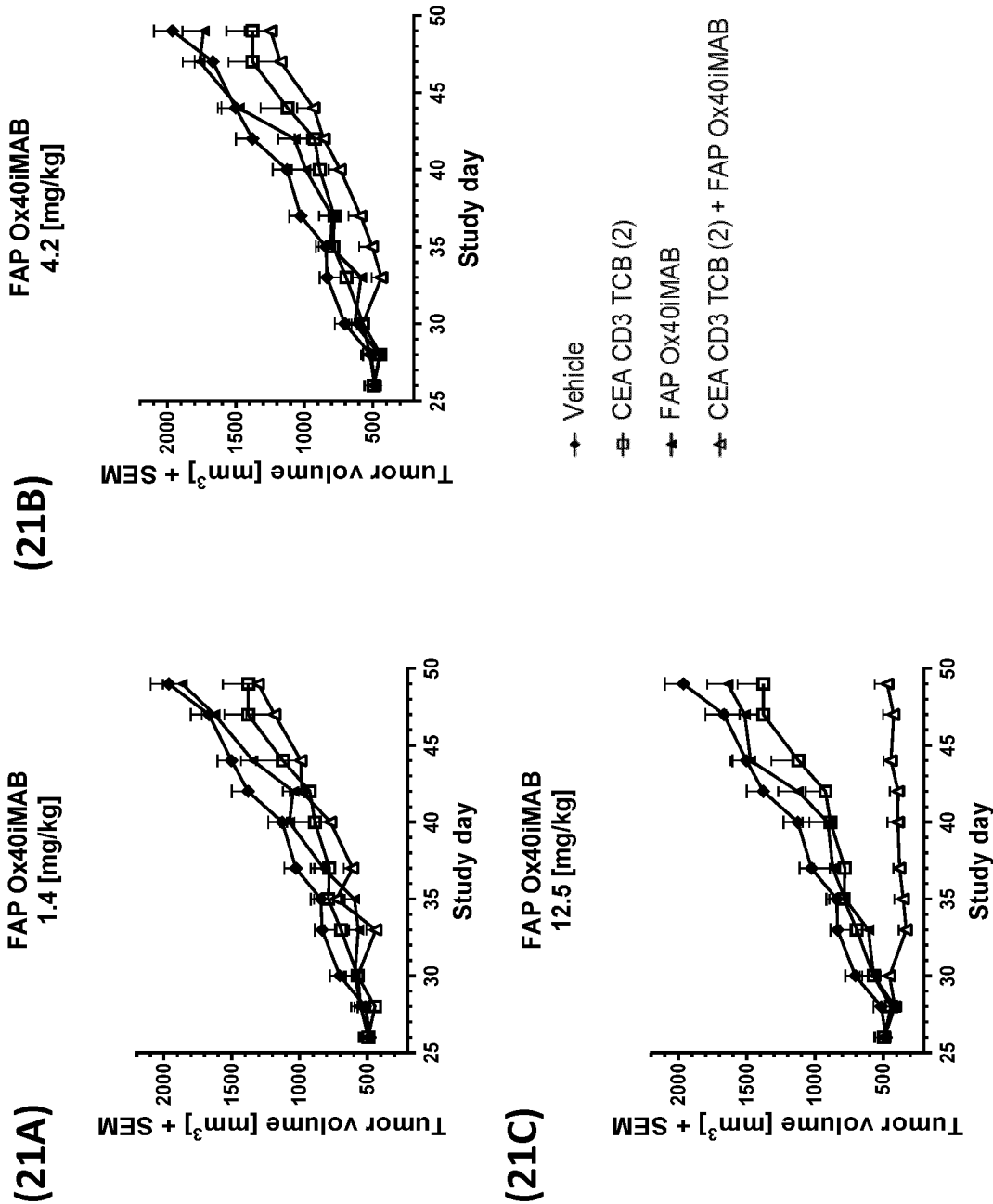


Fig. 22

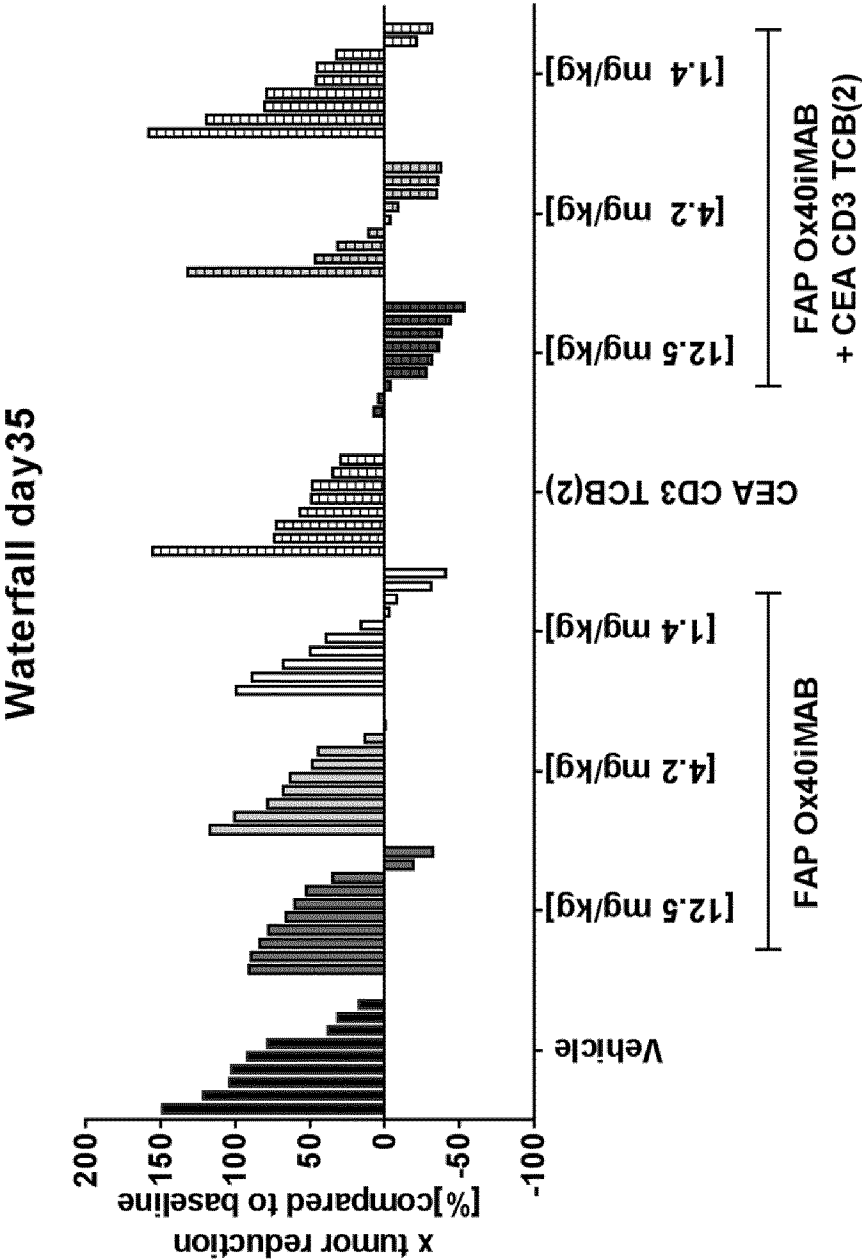
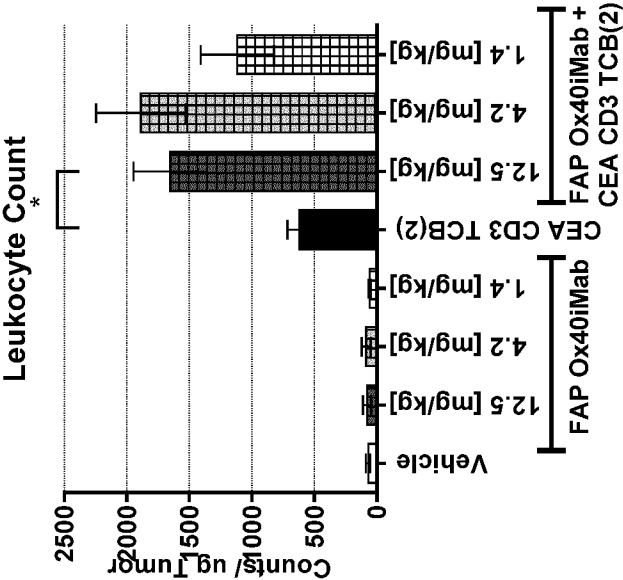


Fig. 23

(23A)



(23B)

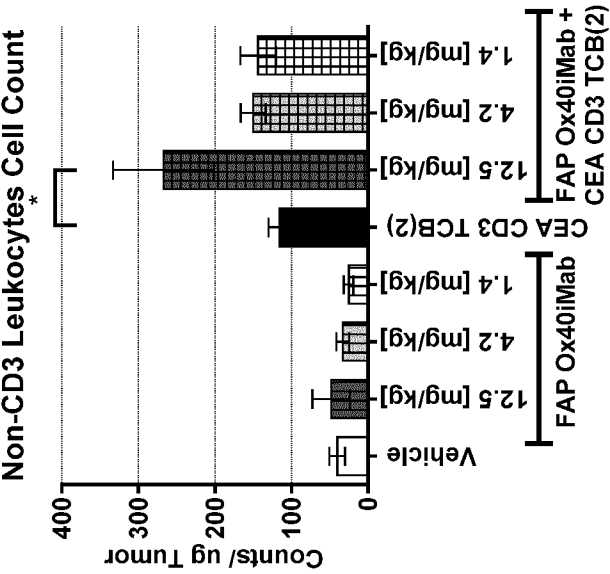
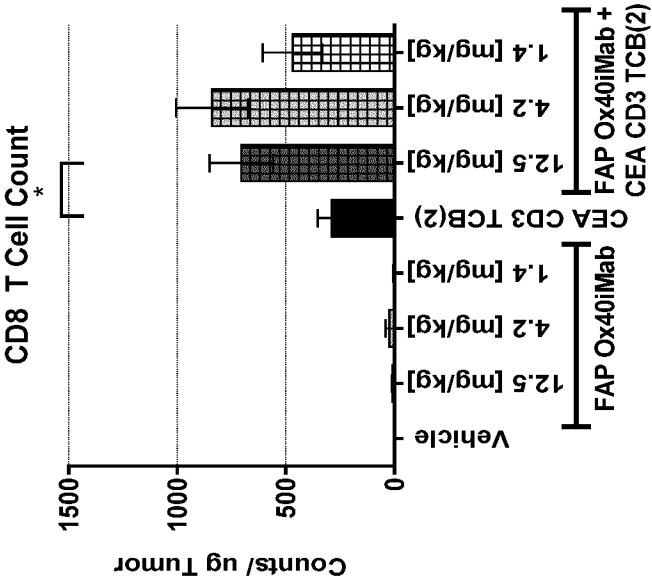


Fig. 23

(23C)



(23D)

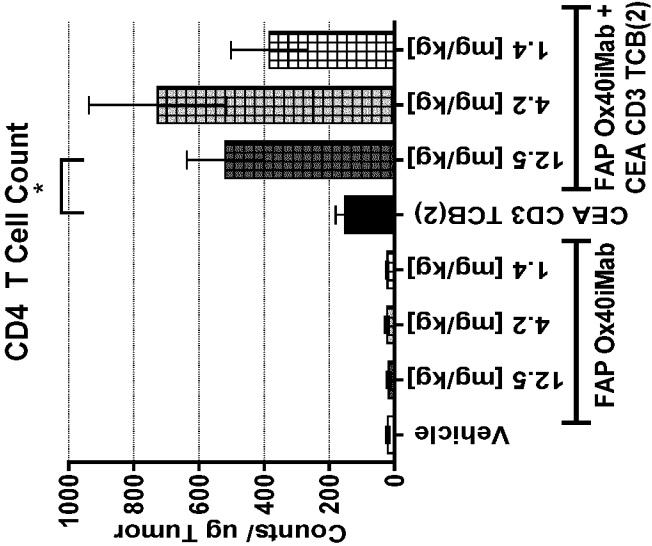


Fig. 24

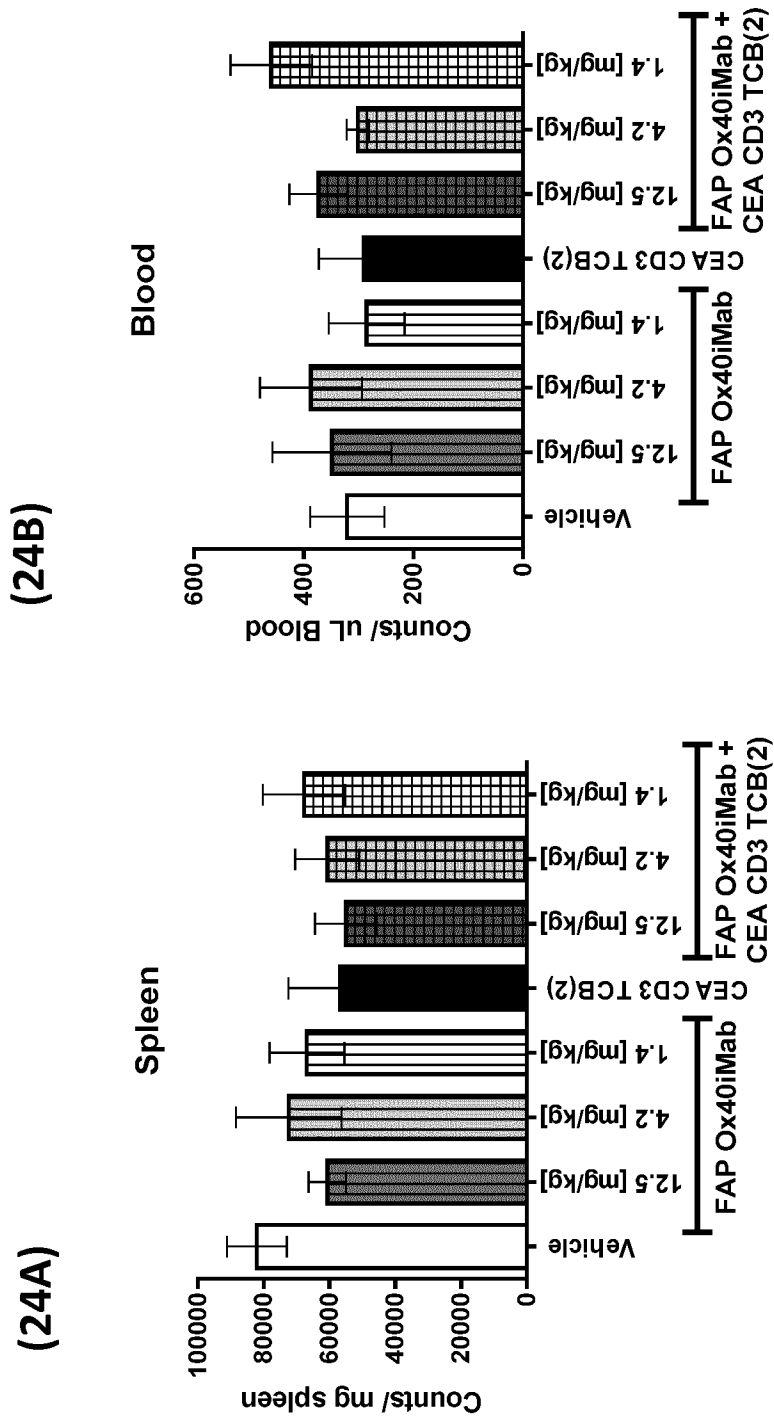


Figure 1 is a dot plot with error bars showing the number of CD3 positive T cells per area (mm²) in the mesenteric lymph node. The y-axis ranges from 0 to 3000. The x-axis shows the treatment groups: Vehicle, CEA CD3 TCB (2), and three groups of mg/kg FAPOx40iMAB (12.5, 4.2, 1.4) with and without CEA CD3 TCB (2). The data is summarized in the following table:

| Treatment Group                                | CD3 positive T cells per area (mm²) | Significance |
|------------------------------------------------|-------------------------------------|--------------|
| Vehicle                                        | ~100                                |              |
| CEA CD3 TCB (2)                                | ~250                                |              |
| mg/kg FAPOx40iMAB 12.5                         | ~250                                |              |
| mg/kg FAPOx40iMAB 4.2                          | ~250                                |              |
| mg/kg FAPOx40iMAB 1.4                          | ~250                                |              |
| mg/kg FAPOx40iMAB 12.5 + CEA CD3 TCB (2)       | ~250                                |              |
| mg/kg FAPOx40iMAB 4.2 + CEA CD3 TCB (2)        | ~250                                |              |
| mg/kg FAPOx40iMAB 1.4 + CEA CD3 TCB (2)        | ~250                                |              |
| mg/kg FAPOx40iMAB 12.5 + CEA CD3 TCB (2) (n=1) | ~1300                               | *            |
| mg/kg FAPOx40iMAB 4.2 + CEA CD3 TCB (2)        | ~250                                |              |
| mg/kg FAPOx40iMAB 1.4 + CEA CD3 TCB (2)        | ~250                                |              |
| mg/kg FAPOx40iMAB 1.4 + CEA CD3 TCB (2) (n=1)  | ~450                                |              |



Fig. 25B

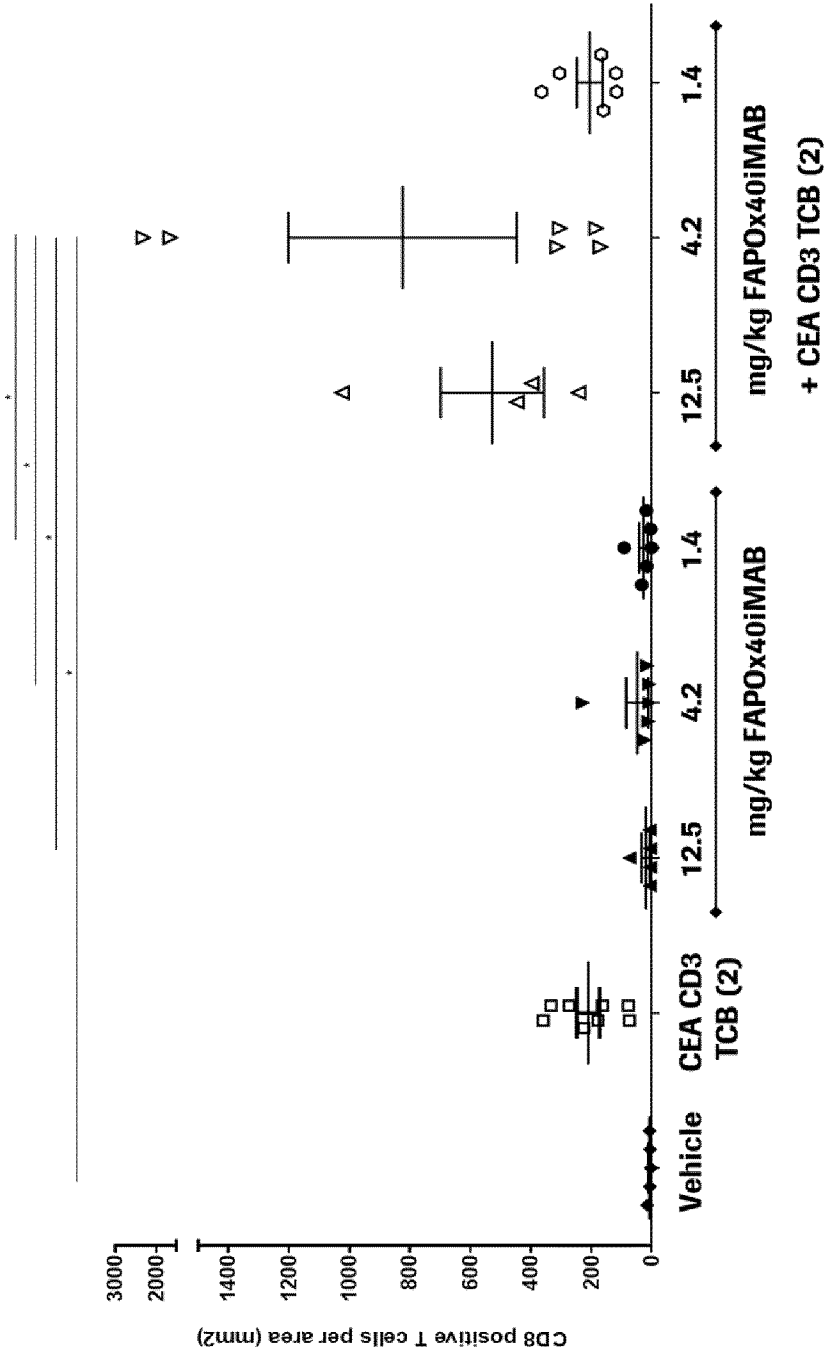


Fig. 26

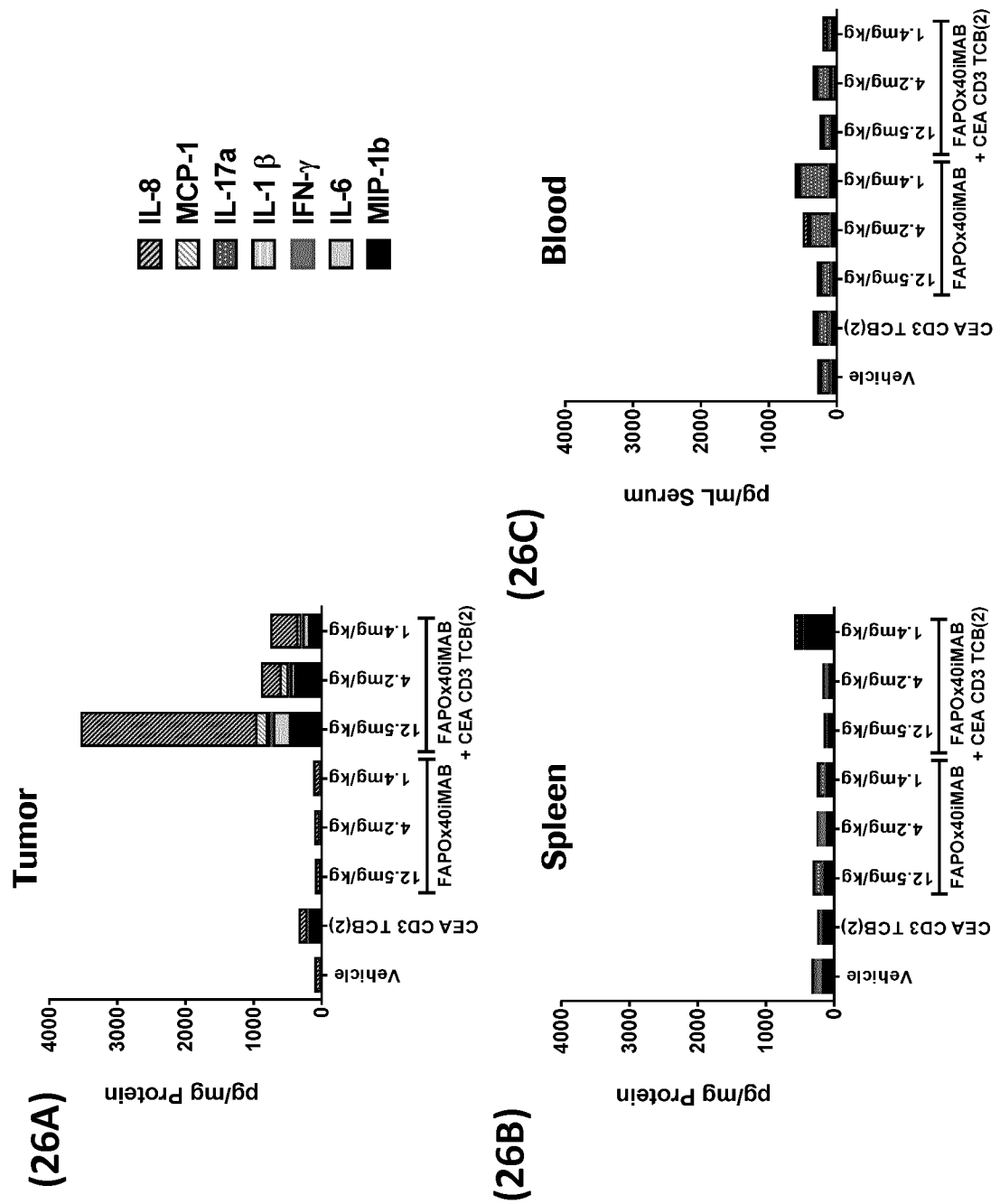


Fig. 27

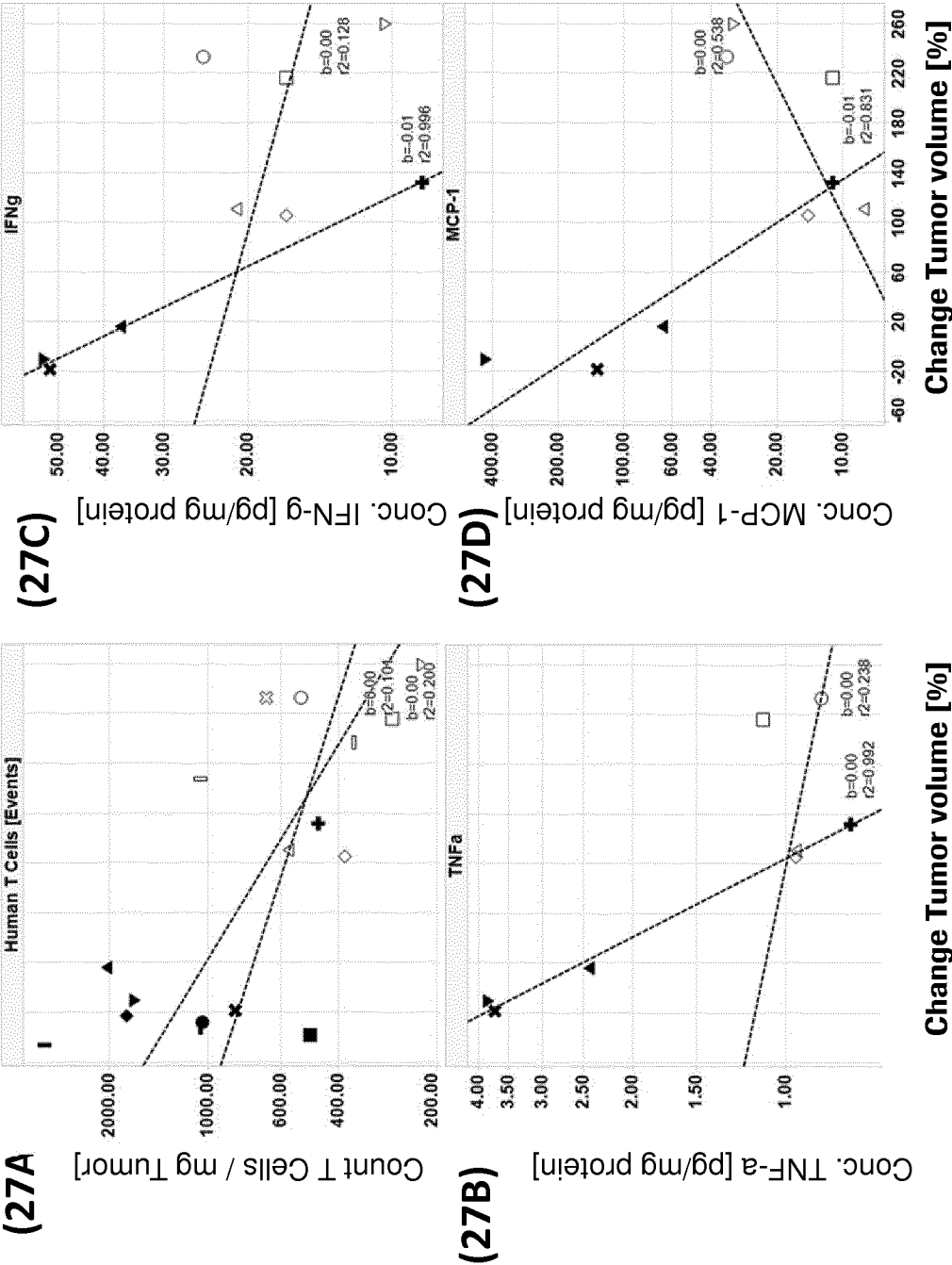
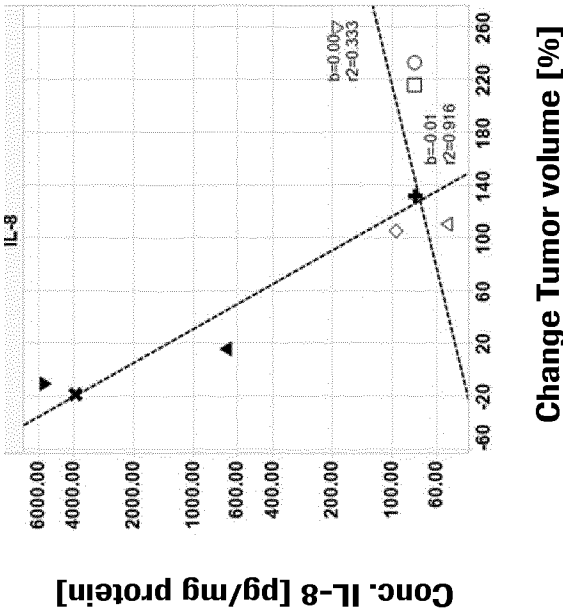


Fig. 27

(27E)



(27F)

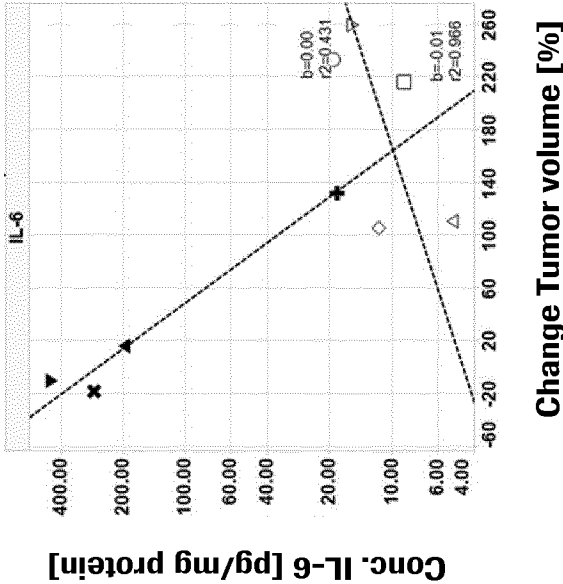
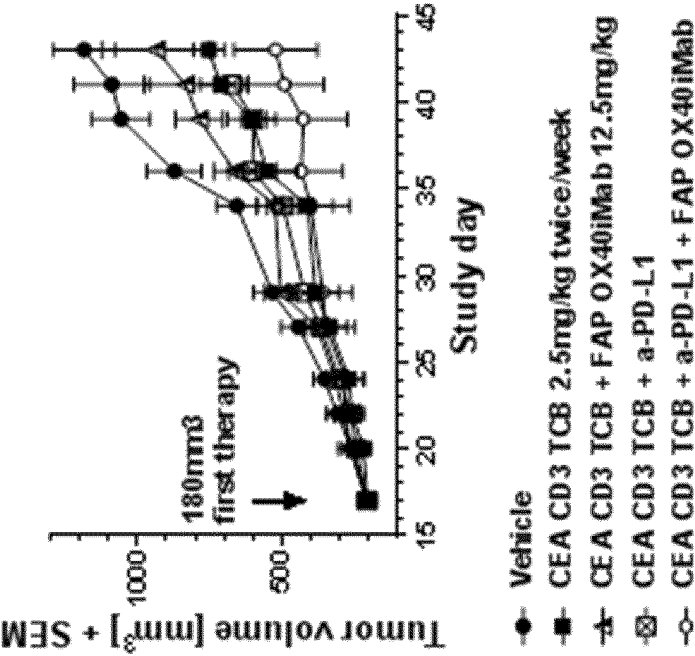


Fig. 28

(28A)



(28B)

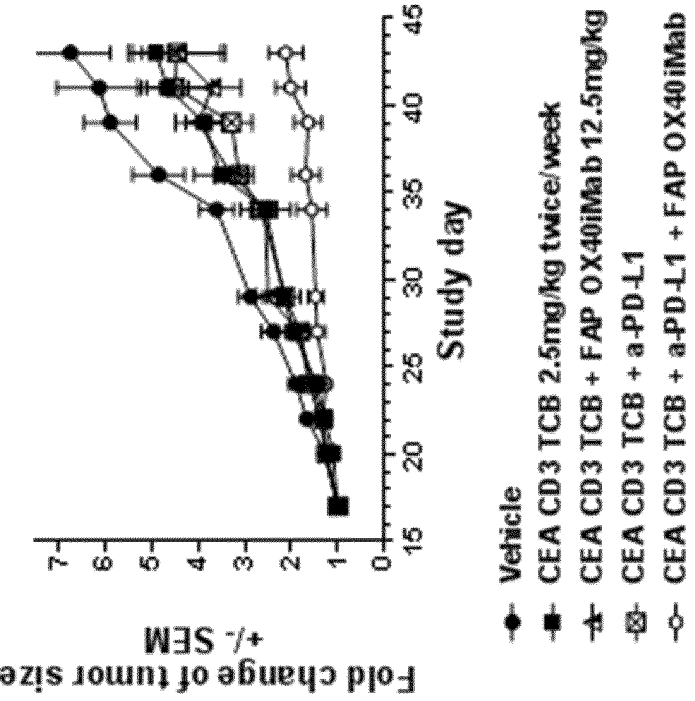


Fig. 29  
(29A)

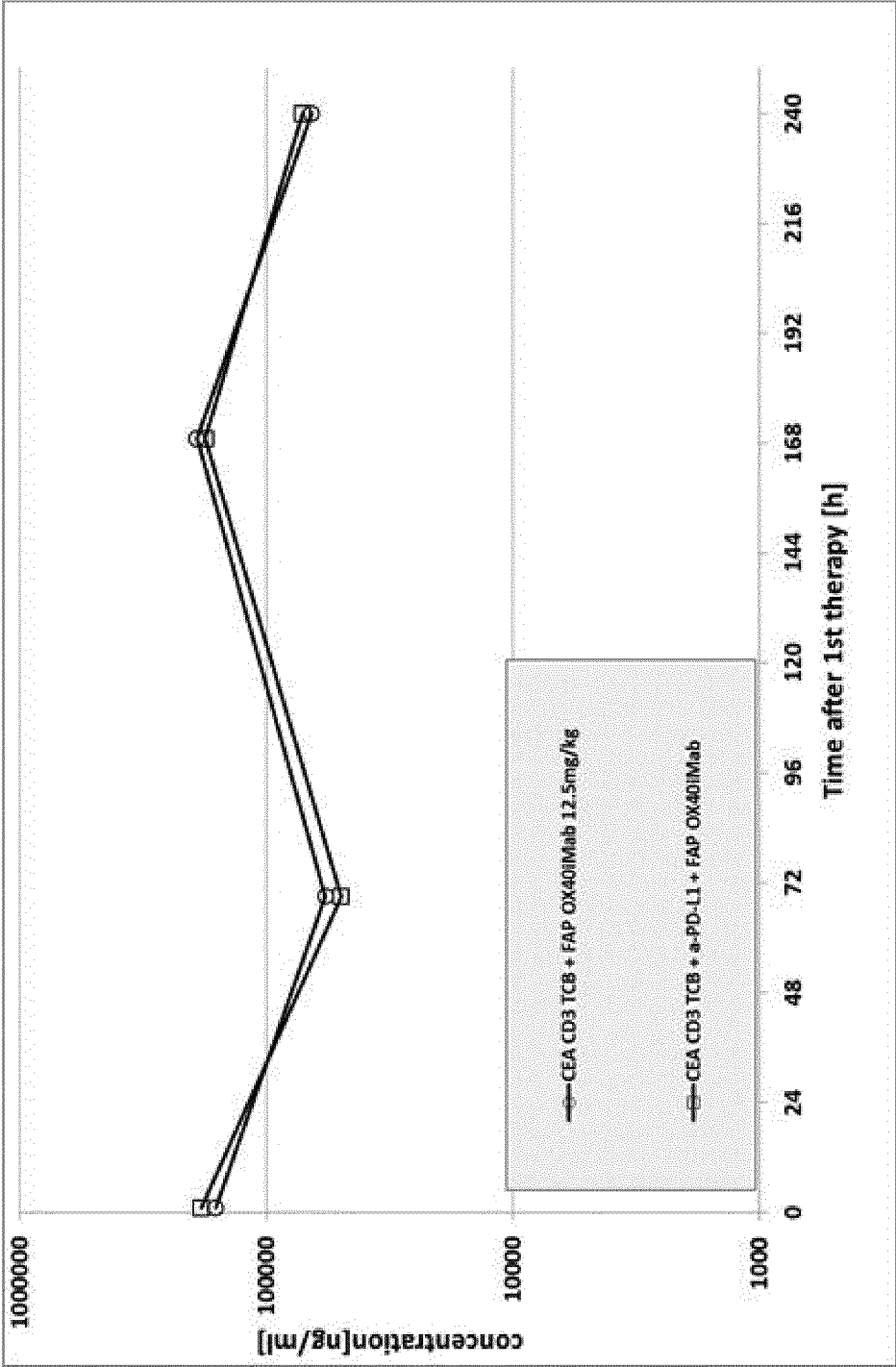


Fig. 29  
(29B)

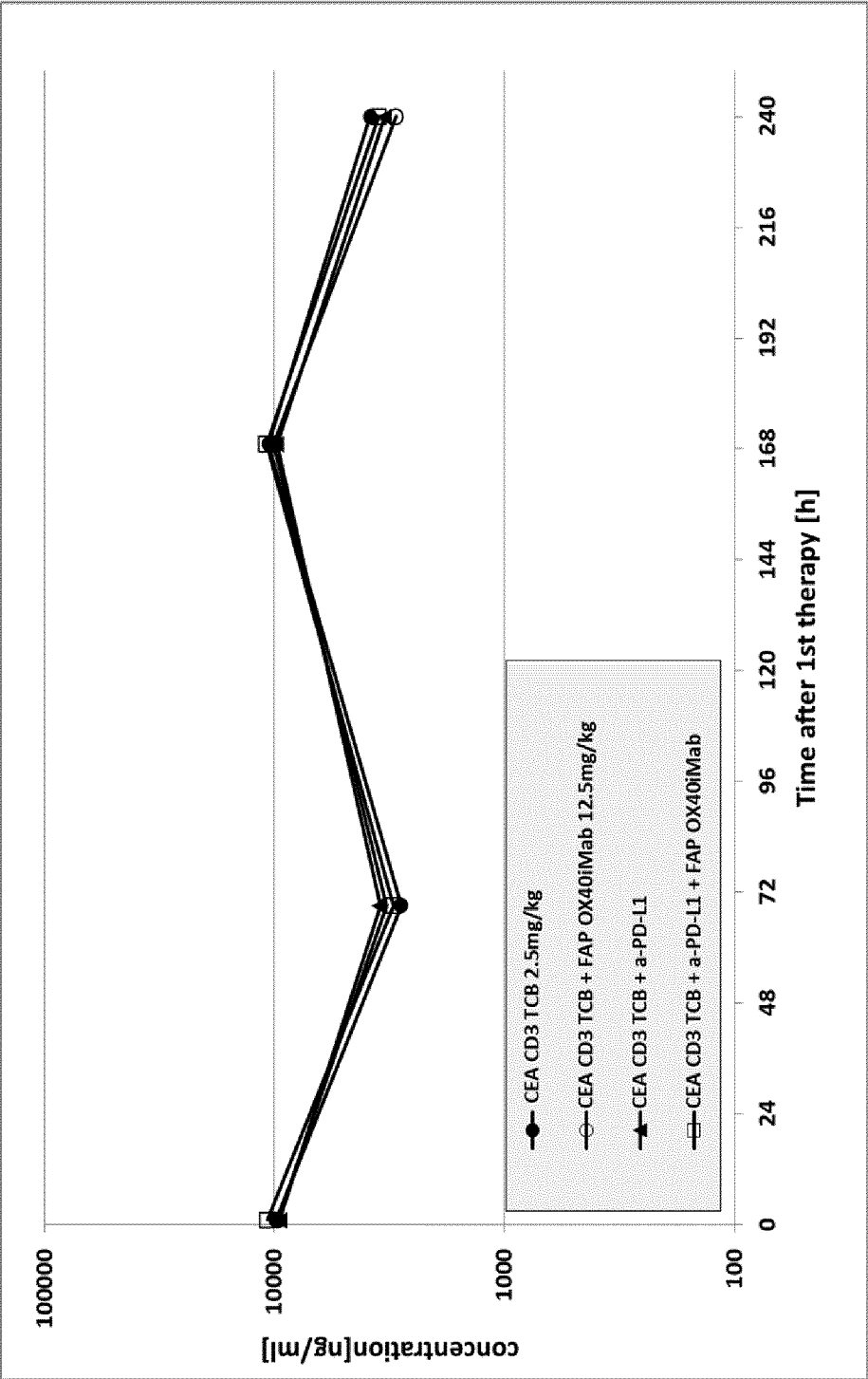


Fig. 29

(29C)

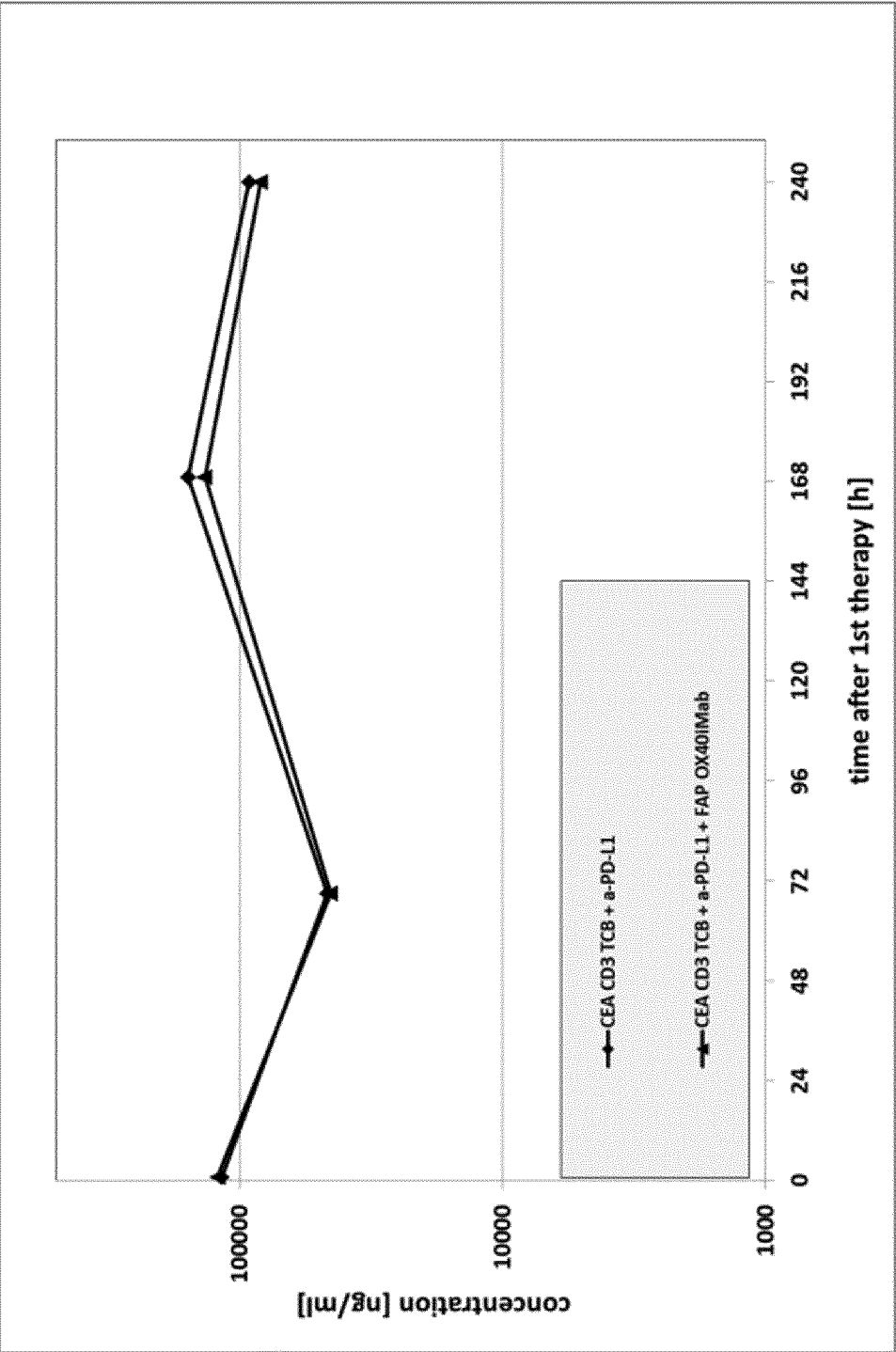




Fig. 30

(30A)

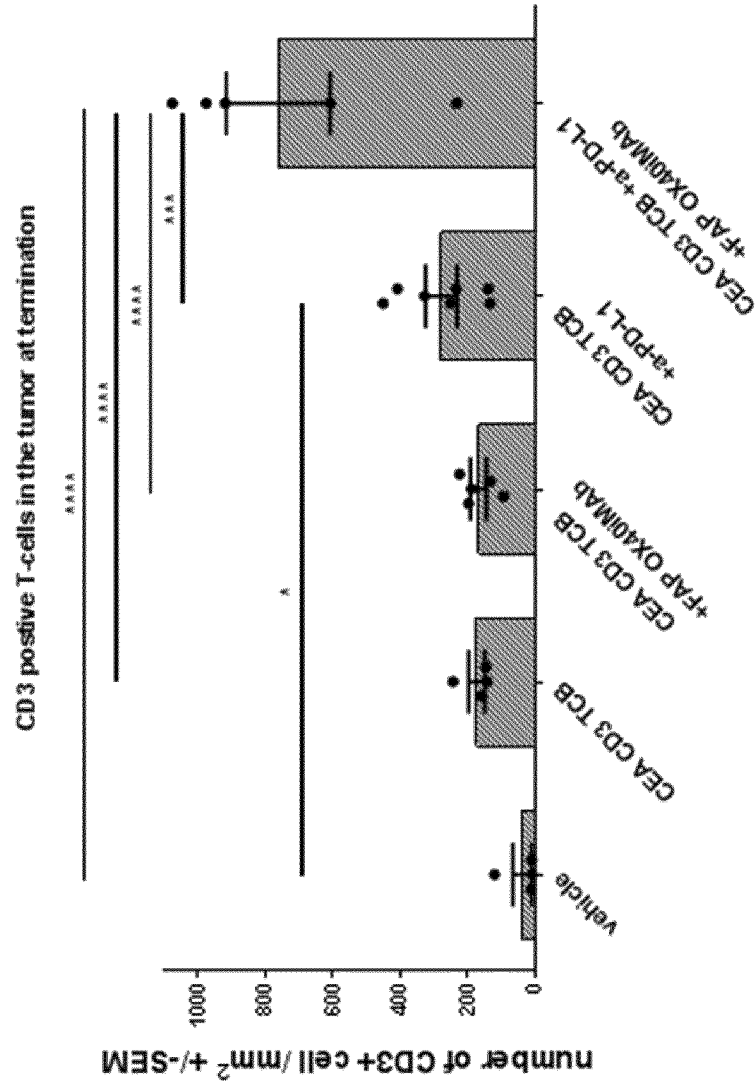


Fig. 30

(30B)

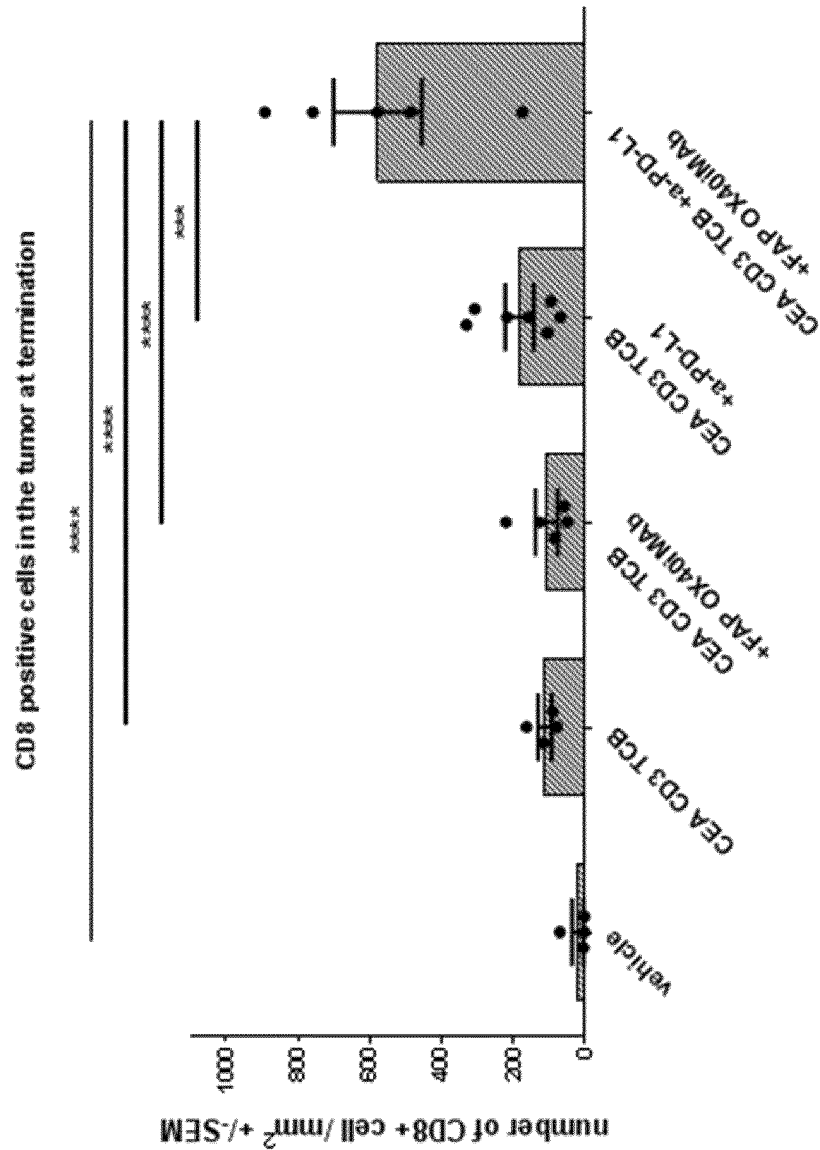


Fig. 31

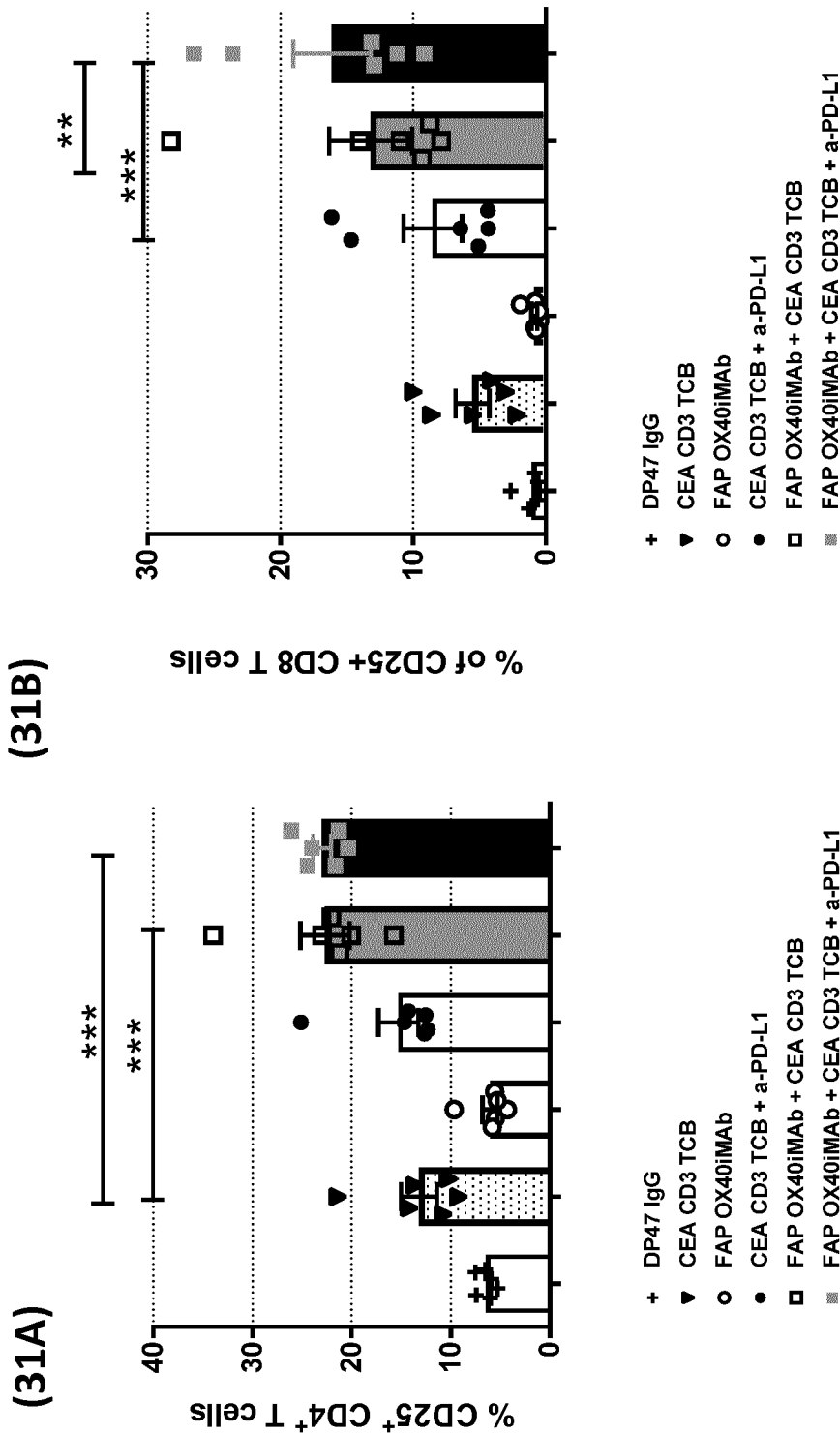


Fig. 32

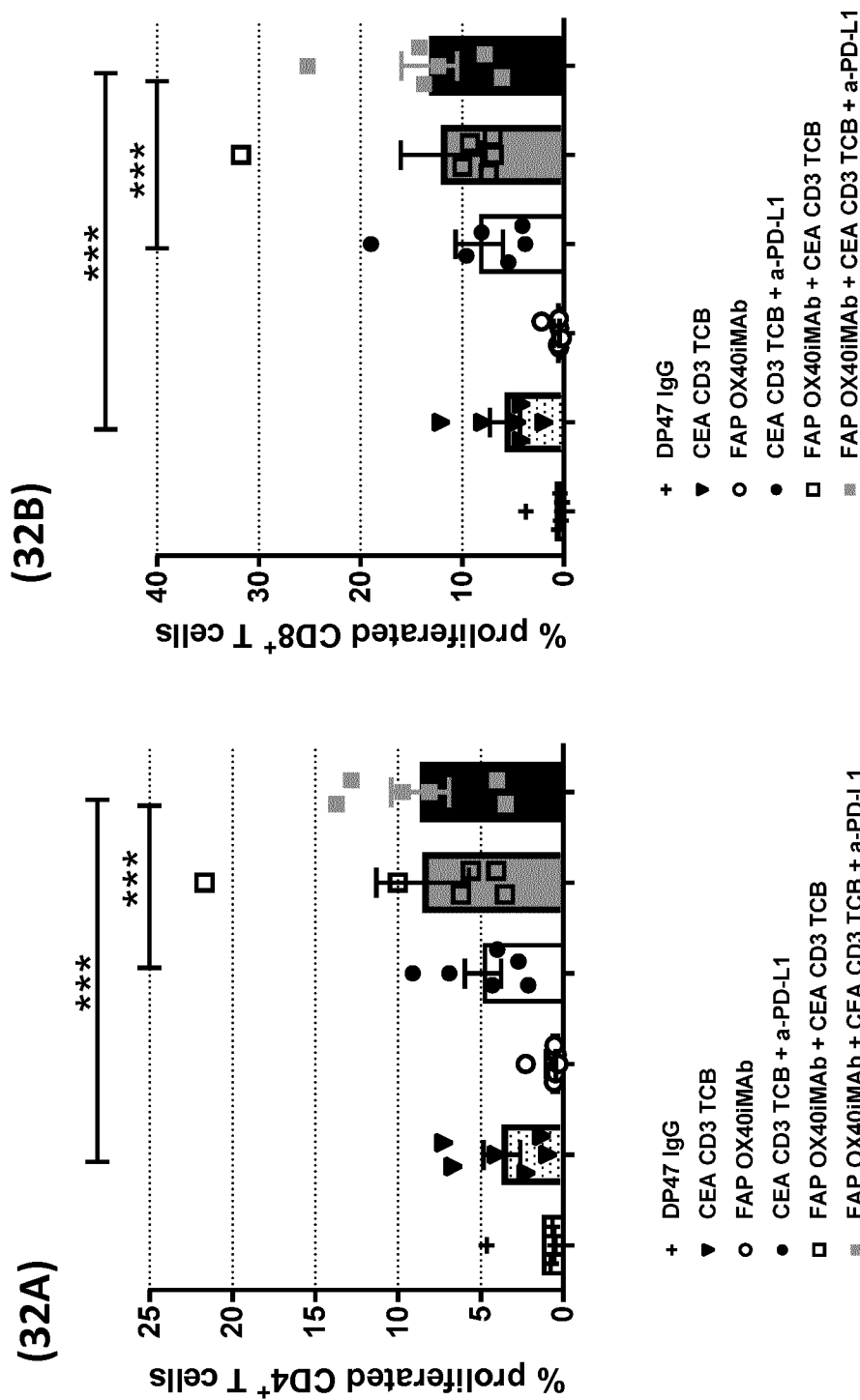


Fig. 33

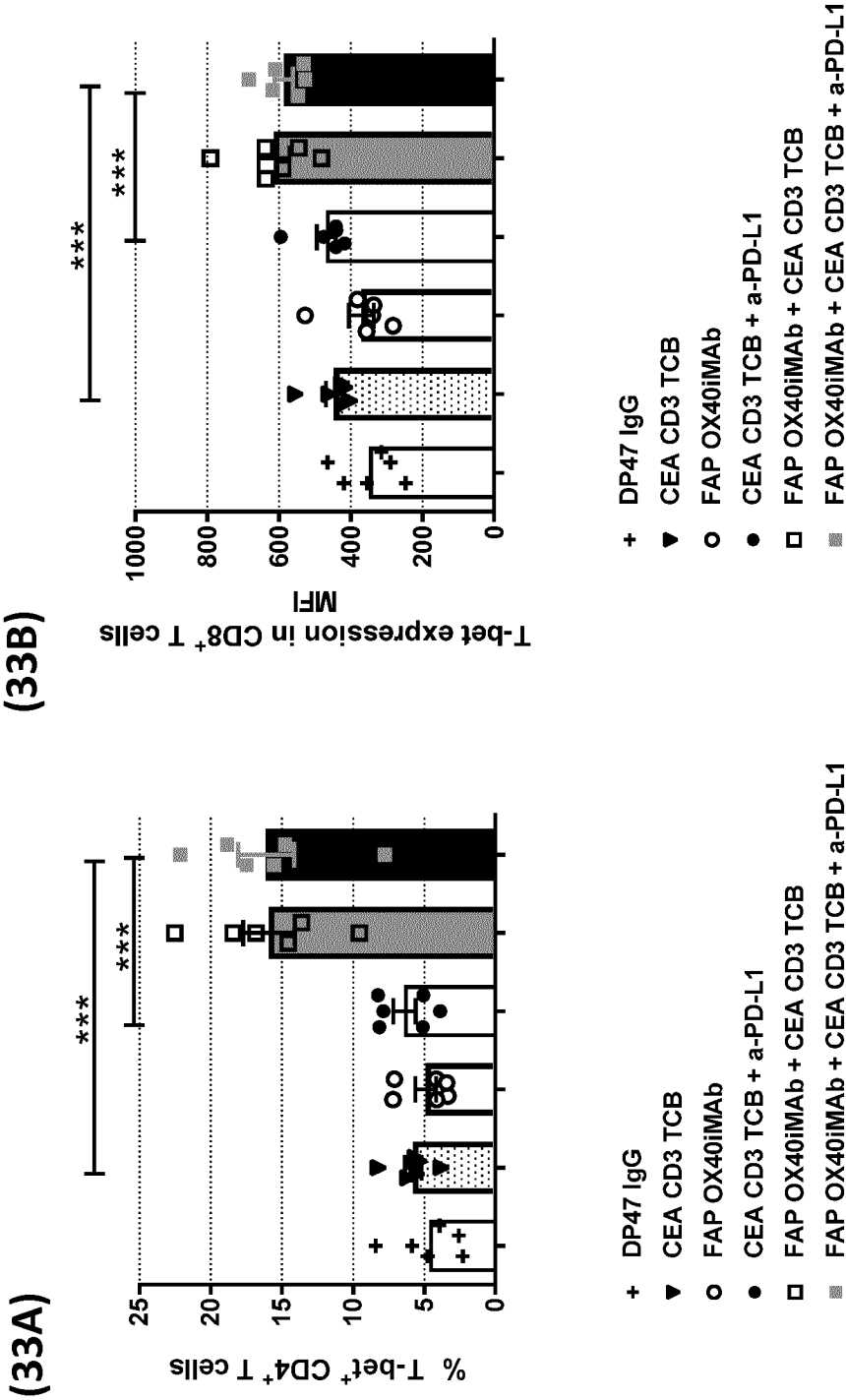


Fig. 33

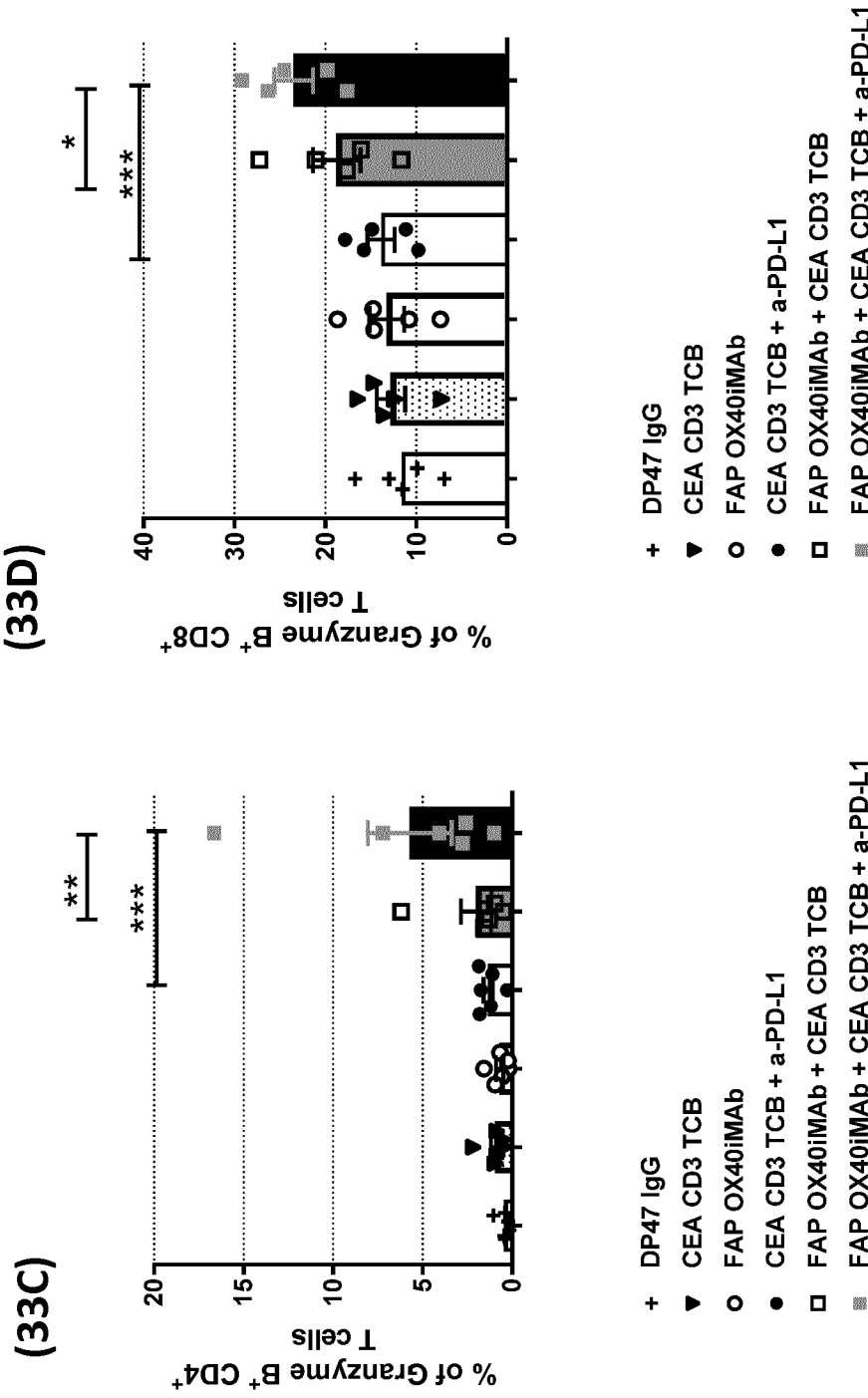


Fig. 34

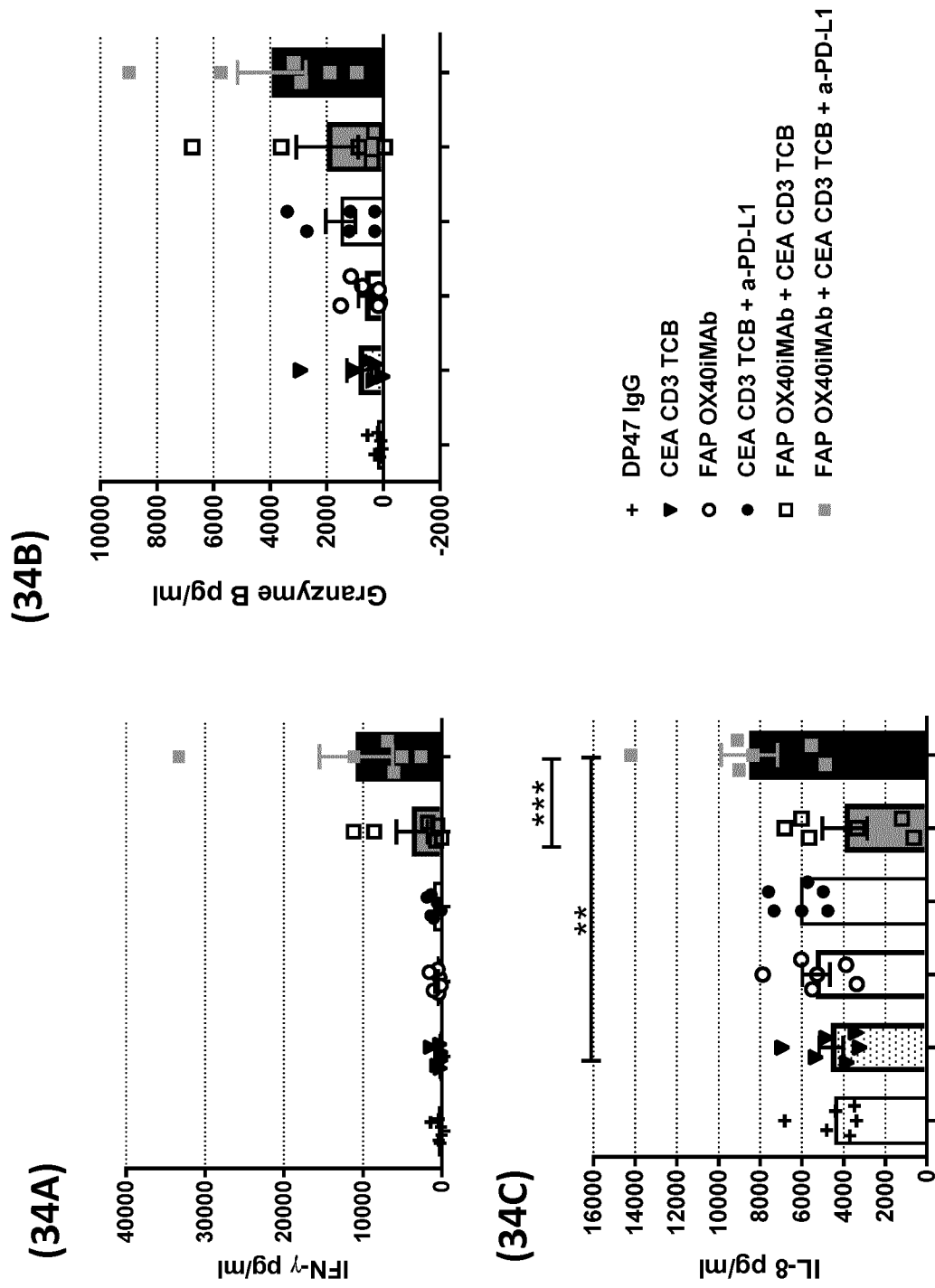
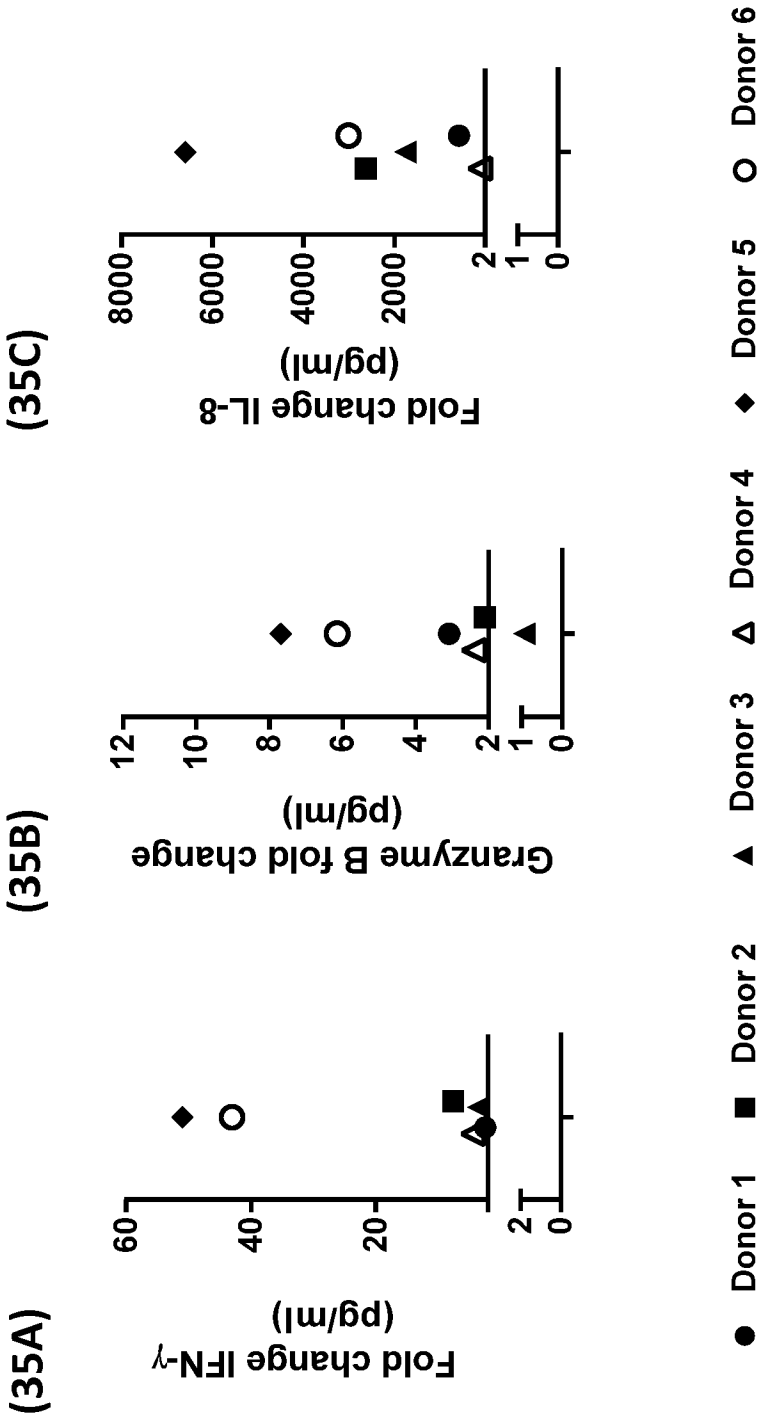


Fig. 35





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35 40 45

Ser Ala Ile Ile Gly Ser Gly Ala Ser Thr Tyr Tyr Ala Asp Ser Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
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Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
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 35 40 45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
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eolf-seql (8).txt

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Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
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Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu

eolf-seql (8).txt

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| Pro | Glu | Asp | Phe | Ala | Val | Tyr |
|     |     |     |     | 85  |     |     |
|     |     |     |     |     | Tyr | Cys |
|     |     |     |     |     | Gln | Gln |
|     |     |     |     |     | Gly | Gln |
|     |     |     |     |     | Val | Ile |
|     |     |     |     |     |     | 95  |
|     |     |     |     |     |     | Pro |

|     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Thr | Phe | Gly | Gln | Gly | Thr | Lys | Val | Glu | Ile | Lys |
|     |     |     | 100 |     |     |     | 105 |     |     |     |     |

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eolf-seql (8).txt

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Gly

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<223> anti-OX40 CDR-H2

<400> 20

Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys  
1 5 10 15

Gly

<210> 21  
<211> 7  
<212> PRT  
<213> Artificial sequence

<220>  
<223> anti-OX40 CDR-H3

<400> 21

Glu Tyr Gly Trp Met Asp Tyr  
1 5

<210> 22  
<211> 9  
<212> PRT  
<213> Artificial sequence

<220>  
<223> anti-OX40 CDR-H3

eolf-seql (8).txt

<400> 22

Glu Tyr Tyr Arg Gly Pro Tyr Asp Tyr  
1 5

<210> 23

<211> 7

<212> PRT

<213> Artificial sequence

<220>

<223> anti-OX40 CDR-H3

<400> 23

Glu Tyr Gly Ser Met Asp Tyr  
1 5

<210> 24

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> anti-OX40 CDR-H3

<400> 24

Val Asn Tyr Pro Tyr Ser Tyr Trp Gly Asp Phe Asp Tyr  
1 5 10

<210> 25

<211> 7

<212> PRT

<213> Artificial sequence

<220>

<223> anti-OX40 CDR-H3

<400> 25

Asp Val Gly Ala Phe Asp Tyr  
1 5

eolf-seql (8).txt

<210> 26  
<211> 7  
<212> PRT  
<213> Artificial sequence

<220>  
<223> anti-OX40 CDR-H3

<400> 26

Asp Val Gly Pro Phe Asp Tyr  
1 5

<210> 27  
<211> 11  
<212> PRT  
<213> Artificial sequence

<220>  
<223> anti-OX40 CDR-H3

<400> 27

Val Phe Tyr Arg Gly Gly Val Ser Met Asp Tyr  
1 5 10

<210> 28  
<211> 11  
<212> PRT  
<213> Artificial sequence

<220>  
<223> anti-OX40 CDR-L1

<400> 28

Arg Ala Ser Gln Ser Ile Ser Ser Trp Leu Ala  
1 5 10

<210> 29  
<211> 12  
<212> PRT  
<213> Artificial sequence

eolf-seql (8).txt

<220>

<223> anti-OX40 CDR-L1

<400> 29

Arg Ala Ser Gln Ser Val Ser Ser Ser Tyr Leu Ala  
1 5 10

<210> 30

<211> 11

<212> PRT

<213> Artificial sequence

<220>

<223> anti-OX40 CDR-L1

<400> 30

Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala Ser  
1 5 10

<210> 31

<211> 7

<212> PRT

<213> Artificial sequence

<220>

<223> anti-OX40 CDR-L2

<400> 31

Asp Ala Ser Ser Leu Glu Ser  
1 5

<210> 32

<211> 7

<212> PRT

<213> Artificial sequence

<220>

<223> anti-OX40 CDR-L2

<400> 32

Gly Ala Ser Ser Arg Ala Thr

1 5

<210> 33  
 <211> 7  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> anti-OX40 CDR-L2

<400> 33

Gly Lys Asn Asn Arg Pro Ser  
 1 5

<210> 34  
 <211> 10  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> anti-OX40 CDR-L3

<400> 34

Gln Gln Tyr Leu Thr Tyr Ser Arg Phe Thr  
 1 5 10

<210> 35  
 <211> 9  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> anti-OX40 CDR-L3

<400> 35

Gln Gln Tyr Ser Ser Gln Pro Tyr Thr  
 1 5

<210> 36  
 <211> 10  
 <212> PRT

<213> Artificial sequence

<220>

<223> anti-OX40 CDR-L3

<400> 36

Gln Gln Tyr Ile Ser Tyr Ser Met Leu Thr  
1 5 10

<210> 37

<211> 9

<212> PRT

<213> Artificial sequence

<220>

<223> anti-OX40 CDR-L3

<400> 37

Gln Gln Tyr Gln Ala Phe Ser Leu Thr  
1 5

<210> 38

<211> 9

<212> PRT

<213> Artificial sequence

<220>

<223> anti-OX40 CDR-L3

<400> 38

Gln Gln Tyr Gly Ser Ser Pro Leu Thr  
1 5

<210> 39

<211> 10

<212> PRT

<213> Artificial sequence

<220>

<223> anti-OX40 CDR-L3

<400> 39

eolf-seql (8).txt

Asn Ser Arg Val Met Pro His Asn Arg Val  
1 5 10

<210> 40  
<211> 118  
<212> PRT  
<213> Artificial sequence

<220>  
<223> 49B4 VH

<400> 40

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser  
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr  
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe  
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Glu Tyr Tyr Arg Gly Pro Tyr Asp Tyr Trp Gly Gln Gly Thr  
100 105 110

Thr Val Thr Val Ser Ser  
115

<210> 41

eolf-seql (8).txt

<211> 107  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> 49B4 VL

<400> 41

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp  
 20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
 35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
 65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Ser Gln Pro Tyr  
 85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> 42  
 <211> 116  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> 8H9 VH

<400> 42

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser



eolf-seql (8).txt

|                                                                 |     |     |     |
|-----------------------------------------------------------------|-----|-----|-----|
| 1                                                               | 5   | 10  | 15  |
| Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr | 20  | 25  | 30  |
| Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met | 35  | 40  | 45  |
| Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe | 50  | 55  | 60  |
| Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr | 65  | 70  | 75  |
| Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys | 85  | 90  | 95  |
| Ala Arg Glu Tyr Gly Trp Met Asp Tyr Trp Gly Gln Gly Thr Thr Val | 100 | 105 | 110 |
| Thr Val Ser Ser                                                 | 115 |     |     |

<210> 43  
 <211> 108  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> 8H9 VL

<400> 43

|                                                                 |    |    |    |    |
|-----------------------------------------------------------------|----|----|----|----|
| Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly | 1  | 5  | 10 | 15 |
| Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp | 20 | 25 | 30 |    |

eolf-seql (8).txt

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
 35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
 65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Leu Thr Tyr Ser Arg  
 85 90 95

Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> 44  
 <211> 116  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> 1G4 VH

<400> 44

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser  
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr  
 20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
 35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe  
 50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr

eolf-seql (8).txt

```

65              70              75              80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
      85              90              95

Ala Arg Glu Tyr Gly Ser Met Asp Tyr Trp Gly Gln Gly Thr Thr Val
      100             105             110

Thr Val Ser Ser
      115

<210>  45
<211> 108
<212> PRT
<213> Artificial sequence

<220>
<223> 1G4 VL

<400>  45

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1              5              10             15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
      20             25             30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
      35             40             45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
      50             55             60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65              70              75             80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ile Ser Tyr Ser Met
      85              90             95

```

eolf-seql (8).txt

Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
100 105

<210> 46  
<211> 122  
<212> PRT  
<213> Artificial sequence

<220>  
<223> 20B7 VH

<400> 46

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser  
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr  
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe  
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Val Asn Tyr Pro Tyr Ser Tyr Trp Gly Asp Phe Asp Tyr Trp  
100 105 110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser  
115 120

<210> 47

eolf-seql (8).txt

<211> 107  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> 20B7 VL

<400> 47

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp  
 20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
 35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
 65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Gln Ala Phe Ser Leu  
 85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> 48  
 <211> 116  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> CLC-563 VH

<400> 48

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

eolf-seql (8).txt

|     |     |     |     |
|-----|-----|-----|-----|
| 1   | 5   | 10  | 15  |
| Ser | Leu | Arg | Leu |
|     | 20  | Ser | Cys |
|     |     | Ala | Ala |
|     |     | 25  | Ser |
|     |     | Gly | Phe |
|     |     | Thr | Phe |
|     |     | 30  | Ser |
|     |     | Ser | Tyr |
| Ala | Met | Ser | Trp |
|     | 35  | Val | Arg |
|     |     | Gln | Ala |
|     |     | 40  | Pro |
|     |     | Gly | Lys |
|     |     | Gly | Leu |
|     |     | 45  | Glu |
|     |     | Trp | Val |
| Ser | Ala | Ile | Ser |
|     | 50  | Gly | Ser |
|     |     | Gly | Gly |
|     |     | 55  | Ser |
|     |     | Thr | Tyr |
|     |     | 60  | Tyr |
|     |     | Ala | Asp |
|     |     | Ser | Val |
| Lys | Gly | Arg | Phe |
| 65  |     | Thr | Ile |
|     |     | 70  | Ser |
|     |     | Arg | Asp |
|     |     | 75  | Asn |
|     |     | Ser | Lys |
|     |     | Asn | Thr |
|     |     | Leu | Tyr |
|     |     | 80  |     |
| Leu | Gln | Met | Asn |
|     |     | 85  | Ser |
|     |     | Leu | Arg |
|     |     | Ala | Glu |
|     |     | 90  | Asp |
|     |     | Thr | Ala |
|     |     | Val | Tyr |
|     |     | 95  | Tyr |
|     |     | Cys |     |
| Ala | Leu | Asp | Val |
|     |     | 100 | Gly |
|     |     | Ala | Phe |
|     |     | Asp | Tyr |
|     |     | 105 | Trp |
|     |     | Gly | Gln |
|     |     | Gly | Ala |
|     |     | 110 | Leu |
|     |     | Val |     |
| Thr | Val | Ser | Ser |
|     |     | 115 |     |

<210> 49  
 <211> 108  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> CLC-563 VL

<400> 49

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Ile | Val | Leu | Thr | Gln | Ser | Pro | Gly | Thr | Leu | Ser | Leu | Ser | Pro | Gly |
| 1   |     |     | 5   |     |     |     |     | 10  |     |     |     |     |     | 15  |     |
| Glu | Arg | Ala | Thr | Leu | Ser | Cys | Arg | Ala | Ser | Gln | Ser | Val | Ser | Ser | Ser |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

eolf-seql (8).txt

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro  
85 90 95

Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
100 105

<210> 50  
<211> 116  
<212> PRT  
<213> Artificial sequence

<220>  
<223> CLC-564 VH

<400> 50

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr

eolf-seql (8).txt

|                           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|---------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 65                        |     | 70  |     | 75  |     | 80  |     |     |     |     |     |     |     |     |     |
| Leu                       | Gln | Met | Asn | Ser | Leu | Arg | Ala | Glu | Asp | Thr | Ala | Val | Tyr | Tyr | Cys |
|                           |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |
| Ala                       | Phe | Asp | Val | Gly | Pro | Phe | Asp | Tyr | Trp | Gly | Gln | Gly | Thr | Leu | Val |
|                           |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Thr                       | Val | Ser | Ser |     |     |     |     |     |     |     |     |     |     |     |     |
|                           |     |     | 115 |     |     |     |     |     |     |     |     |     |     |     |     |
| <210> 51                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <211> 108                 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <212> PRT                 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <213> Artificial sequence |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <220>                     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <223> CLC-564 VL          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <400> 51                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu                       | Ile | Val | Leu | Thr | Gln | Ser | Pro | Gly | Thr | Leu | Ser | Leu | Ser | Pro | Gly |
| 1                         |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Glu                       | Arg | Ala | Thr | Leu | Ser | Cys | Arg | Ala | Ser | Gln | Ser | Val | Ser | Ser | Ser |
|                           |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| Tyr                       | Leu | Ala | Trp | Tyr | Gln | Gln | Lys | Pro | Gly | Gln | Ala | Pro | Arg | Leu | Leu |
|                           |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
| Ile                       | Tyr | Gly | Ala | Ser | Ser | Arg | Ala | Thr | Gly | Ile | Pro | Asp | Arg | Phe | Ser |
|                           | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| Gly                       | Ser | Gly | Ser | Gly | Thr | Asp | Phe | Thr | Leu | Thr | Ile | Ser | Arg | Leu | Glu |
| 65                        |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |
| Pro                       | Glu | Asp | Phe | Ala | Val | Tyr | Tyr | Cys | Gln | Gln | Tyr | Gly | Ser | Ser | Pro |
|                           |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |



eolf-seql (8).txt

Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
100 105

<210> 52  
<211> 120  
<212> PRT  
<213> Artificial sequence

<220>  
<223> 17A9 VH

<400> 52

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Val Phe Tyr Arg Gly Gly Val Ser Met Asp Tyr Trp Gly Gln  
100 105 110

Gly Thr Leu Val Thr Val Ser Ser  
115 120

<210> 53

eolf-seql (8).txt

<211> 106  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> 17A9 VL

<400> 53

Ser Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly Gln  
 1 5 10 15

Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala  
 20 25 30

Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr  
 35 40 45

Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser  
 50 55 60

Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu  
 65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Val Met Pro His Asn Arg  
 85 90 95

Val Phe Gly Gly Gly Thr Lys Leu Thr Val  
 100 105

<210> 54  
 <211> 816  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> HC 1 (49B4) VHCH1\_VHCH1 Fc knob VH (4B9)

<400> 54

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser

eolf-seql (8).txt

|                                                                 |     |     |     |
|-----------------------------------------------------------------|-----|-----|-----|
| 1                                                               | 5   | 10  | 15  |
| Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr | 20  | 25  | 30  |
| Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met | 35  | 40  | 45  |
| Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe | 50  | 55  | 60  |
| Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr | 65  | 70  | 75  |
| Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys | 85  | 90  | 95  |
| Ala Arg Glu Tyr Tyr Arg Gly Pro Tyr Asp Tyr Trp Gly Gln Gly Thr | 100 | 105 | 110 |
| Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro | 115 | 120 | 125 |
| Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly | 130 | 135 | 140 |
| Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn | 145 | 150 | 155 |
| Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln | 165 | 170 | 175 |
| Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser | 180 | 185 | 190 |
| Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser |     |     |     |

## eolf-seql (8).txt

195

200

205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Gly Gly  
 210 215 220

Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly  
 225 230 235 240

Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala  
 245 250 255

Ser Gly Gly Thr Phe Ser Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala  
 260 265 270

Pro Gly Gln Gly Leu Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly  
 275 280 285

Thr Ala Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala  
 290 295 300

Asp Lys Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser  
 305 310 315 320

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Glu Tyr Tyr Arg Gly Pro  
 325 330 335

Tyr Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser  
 340 345 350

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr  
 355 360 365

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro  
 370 375 380

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val

eolf-seql (8).txt

385 390 395 400

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser  
405 410 415

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile  
420 425 430

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val  
435 440 445

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala  
450 455 460

Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
465 470 475 480

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val  
485 490 495

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val  
500 505 510

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln  
515 520 525

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln  
530 535 540

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala  
545 550 555 560

Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro  
565 570 575

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr

eolf-seql (8).txt

580

585

590

Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser  
595 600 605

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr  
610 615 620

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr  
625 630 635 640

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe  
645 650 655

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys  
660 665 670

Ser Leu Ser Leu Ser Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly  
675 680 685

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu  
690 695 700

Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser  
705 710 715 720

Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser Trp Val  
725 730 735

Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ala Ile Ile Gly  
740 745 750

Ser Gly Ala Ser Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr  
755 760 765

Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser

eolf-seql (8).txt

770

775

780

Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Gly Trp Phe  
785 790 795 800

Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
805 810 815

<210> 55

<211> 807

<212> PRT

<213> Artificial Sequence

<220>

<223> HC 2 (49B4) VHCH1\_VHCH1 Fc hole VL (4B9)

<400> 55

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser  
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr  
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe  
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Glu Tyr Tyr Arg Gly Pro Tyr Asp Tyr Trp Gly Gln Gly Thr  
100 105 110

eolf-seql (8).txt

Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro  
115 120 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly  
130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn  
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln  
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser  
180 185 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser  
195 200 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Gly Gly  
210 215 220

Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly  
225 230 235 240

Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala  
245 250 255

Ser Gly Gly Thr Phe Ser Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala  
260 265 270

Pro Gly Gln Gly Leu Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly  
275 280 285

Thr Ala Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala  
290 295 300



eolf-seql (8).txt

Asp Lys Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser  
305 310 315 320

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Glu Tyr Tyr Arg Gly Pro  
325 330 335

Tyr Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser  
340 345 350

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr  
355 360 365

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro  
370 375 380

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val  
385 390 395 400

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser  
405 410 415

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile  
420 425 430

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val  
435 440 445

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala  
450 455 460

Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
465 470 475 480

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val  
485 490 495

eolf-seql (8).txt

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val  
500 505 510

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln  
515 520 525

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln  
530 535 540

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala  
545 550 555 560

Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro  
565 570 575

Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr  
580 585 590

Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser  
595 600 605

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr  
610 615 620

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val  
625 630 635 640

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe  
645 650 655

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys  
660 665 670

Ser Leu Ser Leu Ser Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly  
675 680 685

eolf-seql (8).txt

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Leu Thr  
690 695 700

Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu  
705 710 715 720

Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser Tyr Leu Ala Trp Tyr  
725 730 735

Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Asn Val Gly Ser  
740 745 750

Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly  
755 760 765

Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala  
770 775 780

Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro Pro Thr Phe Gly Gln  
785 790 795 800

Gly Thr Lys Val Glu Ile Lys  
805

<210> 56  
<211> 214  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> LC (49B4)

<400> 56

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp

eolf-seql (8).txt

20

25

30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Ser Gln Pro Tyr  
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala  
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly  
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala  
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr  
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser  
195 200 205

Phe Asn Arg Gly Glu Cys

210

&lt;210&gt; 57

&lt;211&gt; 815

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; HC 1 (49B4) VHCH1\_VHCH1 Fc knob VH (28H1)

&lt;400&gt; 57

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser  
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr  
 20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
 35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe  
 50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr  
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Glu Tyr Tyr Arg Gly Pro Tyr Asp Tyr Trp Gly Gln Gly Thr  
 100 105 110

Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro  
 115 120 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly  
 130 135 140

eolf-seql (8).txt

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn  
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln  
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser  
180 185 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser  
195 200 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Gly Gly  
210 215 220

Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly  
225 230 235 240

Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala  
245 250 255

Ser Gly Gly Thr Phe Ser Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala  
260 265 270

Pro Gly Gln Gly Leu Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly  
275 280 285

Thr Ala Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala  
290 295 300

Asp Lys Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser  
305 310 315 320

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Glu Tyr Tyr Arg Gly Pro  
325 330 335

eolf-seql (8).txt

Tyr Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser  
340 345 350

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr  
355 360 365

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro  
370 375 380

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val  
385 390 395 400

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser  
405 410 415

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile  
420 425 430

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val  
435 440 445

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala  
450 455 460

Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
465 470 475 480

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val  
485 490 495

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val  
500 505 510

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln  
515 520 525

eolf-seql (8).txt

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln  
530 535 540

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala  
545 550 555 560

Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro  
565 570 575

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr  
580 585 590

Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser  
595 600 605

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr  
610 615 620

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr  
625 630 635 640

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe  
645 650 655

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys  
660 665 670

Ser Leu Ser Leu Ser Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly  
675 680 685

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu  
690 695 700

Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser  
705 710 715 720



eolf-seql (8).txt

Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His Ala Met Ser Trp Val  
725 730 735

Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ala Ile Trp Ala  
740 745 750

Ser Gly Glu Gln Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile  
755 760 765

Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu  
770 775 780

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Gly Trp Leu Gly  
785 790 795 800

Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
805 810 815

<210> 58  
<211> 807  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> HC 2 (49B4) VHCH1\_VHCH1 Fc hole VL (28H1)

<400> 58

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser  
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr  
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe

eolf-seql (8).txt

50

55

60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Glu Tyr Tyr Arg Gly Pro Tyr Asp Tyr Trp Gly Gln Gly Thr  
100 105 110

Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro  
115 120 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly  
130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn  
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln  
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser  
180 185 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser  
195 200 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Gly Gly  
210 215 220

Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly  
225 230 235 240

Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala

eolf-seql (8).txt

245

250

255

Ser Gly Gly Thr Phe Ser Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala  
260 265 270

Pro Gly Gln Gly Leu Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly  
275 280 285

Thr Ala Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala  
290 295 300

Asp Lys Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser  
305 310 315 320

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Glu Tyr Tyr Arg Gly Pro  
325 330 335

Tyr Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser  
340 345 350

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr  
355 360 365

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro  
370 375 380

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val  
385 390 395 400

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser  
405 410 415

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile  
420 425 430

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val

eolf-seql (8).txt

435

440

445

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala  
450 455 460

Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
465 470 475 480

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val  
485 490 495

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val  
500 505 510

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln  
515 520 525

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln  
530 535 540

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala  
545 550 555 560

Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro  
565 570 575

Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr  
580 585 590

Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser  
595 600 605

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr  
610 615 620

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val

eolf-seql (8).txt

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 625 |     | 630 |     | 635 |     | 640 |     |     |     |     |     |     |     |     |     |
| Ser | Lys | Leu | Thr | Val | Asp | Lys | Ser | Arg | Trp | Gln | Gln | Gly | Asn | Val | Phe |
|     |     |     |     | 645 |     |     |     |     | 650 |     |     |     |     | 655 |     |
| Ser | Cys | Ser | Val | Met | His | Glu | Ala | Leu | His | Asn | His | Tyr | Thr | Gln | Lys |
|     |     |     | 660 |     |     |     |     | 665 |     |     |     |     | 670 |     |     |
| Ser | Leu | Ser | Leu | Ser | Pro | Gly | Gly | Gly | Gly | Gly | Ser | Gly | Gly | Gly | Gly |
|     |     | 675 |     |     |     |     | 680 |     |     |     |     | 685 |     |     |     |
| Ser | Gly | Gly | Gly | Gly | Ser | Gly | Gly | Gly | Gly | Ser | Glu | Ile | Val | Leu | Thr |
|     | 690 |     |     |     |     | 695 |     |     |     |     | 700 |     |     |     |     |
| Gln | Ser | Pro | Gly | Thr | Leu | Ser | Leu | Ser | Pro | Gly | Glu | Arg | Ala | Thr | Leu |
| 705 |     |     |     |     | 710 |     |     |     |     | 715 |     |     |     |     | 720 |
| Ser | Cys | Arg | Ala | Ser | Gln | Ser | Val | Ser | Arg | Ser | Tyr | Leu | Ala | Trp | Tyr |
|     |     |     |     | 725 |     |     |     |     | 730 |     |     |     |     | 735 |     |
| Gln | Gln | Lys | Pro | Gly | Gln | Ala | Pro | Arg | Leu | Leu | Ile | Ile | Gly | Ala | Ser |
|     |     |     | 740 |     |     |     |     | 745 |     |     |     |     | 750 |     |     |
| Thr | Arg | Ala | Thr | Gly | Ile | Pro | Asp | Arg | Phe | Ser | Gly | Ser | Gly | Ser | Gly |
|     |     | 755 |     |     |     |     | 760 |     |     |     |     | 765 |     |     |     |
| Thr | Asp | Phe | Thr | Leu | Thr | Ile | Ser | Arg | Leu | Glu | Pro | Glu | Asp | Phe | Ala |
|     | 770 |     |     |     |     | 775 |     |     |     |     | 780 |     |     |     |     |
| Val | Tyr | Tyr | Cys | Gln | Gln | Gly | Gln | Val | Ile | Pro | Pro | Thr | Phe | Gly | Gln |
| 785 |     |     |     |     | 790 |     |     |     |     | 795 |     |     |     |     | 800 |
| Gly | Thr | Lys | Val | Glu | Ile | Lys |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 805 |     |     |     |     |     |     |     |     |     |     |     |

<210> 59

eolf-seql (8).txt

<211> 807

<212> PRT

<213> Artificial Sequence

<220>

<223> HC 1 (49B4) VHCH1\_VHCH1 Fc knob VL (4B9)

<400> 59

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser  
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr  
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe  
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Glu Tyr Tyr Arg Gly Pro Tyr Asp Tyr Trp Gly Gln Gly Thr  
100 105 110

Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro  
115 120 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly  
130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn  
145 150 155 160

eolf-seql (8).txt

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln  
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser  
180 185 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser  
195 200 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Gly Gly  
210 215 220

Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly  
225 230 235 240

Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala  
245 250 255

Ser Gly Gly Thr Phe Ser Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala  
260 265 270

Pro Gly Gln Gly Leu Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly  
275 280 285

Thr Ala Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala  
290 295 300

Asp Lys Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser  
305 310 315 320

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Glu Tyr Tyr Arg Gly Pro  
325 330 335

Tyr Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser  
340 345 350

eolf-seql (8).txt

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr  
355 360 365

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro  
370 375 380

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val  
385 390 395 400

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser  
405 410 415

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile  
420 425 430

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val  
435 440 445

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala  
450 455 460

Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
465 470 475 480

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val  
485 490 495

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val  
500 505 510

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln  
515 520 525

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln  
530 535 540



eolf-seql (8).txt

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala  
545 550 555 560

Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro  
565 570 575

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr  
580 585 590

Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser  
595 600 605

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr  
610 615 620

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr  
625 630 635 640

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe  
645 650 655

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys  
660 665 670

Ser Leu Ser Leu Ser Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly  
675 680 685

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Leu Thr  
690 695 700

Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu  
705 710 715 720

Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser Tyr Leu Ala Trp Tyr  
725 730 735

eolf-seql (8).txt

Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Asn Val Gly Ser  
740 745 750

Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly  
755 760 765

Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala  
770 775 780

Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro Pro Thr Phe Gly Gln  
785 790 795 800

Gly Thr Lys Val Glu Ile Lys  
805

<210> 60

<211> 816

<212> PRT

<213> Artificial Sequence

<220>

<223> HC 2 (49B4) VHCH1\_VHCH1 Fc hole VH (4B9)

<400> 60

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser  
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr  
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe  
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr

eolf-seql (8).txt

|     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|
| 65  |     | 70  |     | 75  |     | 80  |
| Met | Glu | Leu | Ser | Ser | Leu | Arg |
|     |     |     | 85  |     |     |     |
|     |     |     |     | Glu | Asp | Thr |
|     |     |     |     | 90  |     |     |
|     |     |     |     |     | Ala | Val |
|     |     |     |     |     |     | Tyr |
|     |     |     |     |     |     | Tyr |
|     |     |     |     |     |     | Cys |
|     |     |     |     |     |     | 95  |
| Ala | Arg | Glu | Tyr | Tyr | Arg | Gly |
|     |     |     | 100 |     |     |     |
|     |     |     |     |     | Pro | Tyr |
|     |     |     |     |     | 105 |     |
|     |     |     |     |     |     | Asp |
|     |     |     |     |     |     | Tyr |
|     |     |     |     |     |     | Trp |
|     |     |     |     |     |     | Gly |
|     |     |     |     |     |     | Gln |
|     |     |     |     |     |     | Gly |
|     |     |     |     |     |     | Thr |
|     |     |     |     |     |     | 110 |
| Thr | Val | Thr | Val | Ser | Ser | Ala |
|     |     |     |     |     |     |     |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | Thr |
|     |     |     |     |     |     | Lys |
|     |     |     |     |     |     | Gly |
|     |     |     |     |     |     | Pro |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | Val |
|     |     |     |     |     |     | Phe |
|     |     |     |     |     |     | Pro |
|     |     |     |     |     |     | 115 |
|     |     |     |     |     |     | 120 |
|     |     |     |     |     |     | 125 |
| Leu | Ala | Pro | Ser | Ser | Lys | Ser |
|     |     |     |     |     |     |     |
|     |     |     |     |     |     | Thr |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | Gly |
|     |     |     |     |     |     | Gly |
|     |     |     |     |     |     | Thr |
|     |     |     |     |     |     | Ala |
|     |     |     |     |     |     | Ala |
|     |     |     |     |     |     | Leu |
|     |     |     |     |     |     | Gly |
|     |     |     |     |     |     | 130 |
|     |     |     |     |     |     | 135 |
|     |     |     |     |     |     | 140 |
| Cys | Leu | Val | Lys | Asp | Tyr | Phe |
|     |     |     |     |     |     |     |
|     |     |     |     |     |     | Pro |
|     |     |     |     |     |     | Glu |
|     |     |     |     |     |     | Pro |
|     |     |     |     |     |     | Val |
|     |     |     |     |     |     | Thr |
|     |     |     |     |     |     | Val |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | Trp |
|     |     |     |     |     |     | Asn |
|     |     |     |     |     |     | 145 |
|     |     |     |     |     |     | 150 |
|     |     |     |     |     |     | 155 |
|     |     |     |     |     |     | 160 |
| Ser | Gly | Ala | Leu | Thr | Ser | Gly |
|     |     |     |     |     |     |     |
|     |     |     |     |     |     | Val |
|     |     |     |     |     |     | His |
|     |     |     |     |     |     | Thr |
|     |     |     |     |     |     | Phe |
|     |     |     |     |     |     | Pro |
|     |     |     |     |     |     | Ala |
|     |     |     |     |     |     | Val |
|     |     |     |     |     |     | Leu |
|     |     |     |     |     |     | Gln |
|     |     |     |     |     |     | 165 |
|     |     |     |     |     |     | 170 |
|     |     |     |     |     |     | 175 |
| Ser | Ser | Gly | Leu | Tyr | Ser | Leu |
|     |     |     |     |     |     |     |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | Val |
|     |     |     |     |     |     | Val |
|     |     |     |     |     |     | Thr |
|     |     |     |     |     |     | Val |
|     |     |     |     |     |     | Pro |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | 180 |
|     |     |     |     |     |     | 185 |
|     |     |     |     |     |     | 190 |
| Ser | Leu | Gly | Thr | Gln | Thr | Tyr |
|     |     |     |     |     |     |     |
|     |     |     |     |     |     | Ile |
|     |     |     |     |     |     | Cys |
|     |     |     |     |     |     | Asn |
|     |     |     |     |     |     | Val |
|     |     |     |     |     |     | Asn |
|     |     |     |     |     |     | His |
|     |     |     |     |     |     | Lys |
|     |     |     |     |     |     | Pro |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | 195 |
|     |     |     |     |     |     | 200 |
|     |     |     |     |     |     | 205 |
| Asn | Thr | Lys | Val | Asp | Lys | Lys |
|     |     |     |     |     |     |     |
|     |     |     |     |     |     | Val |
|     |     |     |     |     |     | Glu |
|     |     |     |     |     |     | Pro |
|     |     |     |     |     |     | Lys |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | Cys |
|     |     |     |     |     |     | Asp |
|     |     |     |     |     |     | Gly |
|     |     |     |     |     |     | Gly |
|     |     |     |     |     |     | 210 |
|     |     |     |     |     |     | 215 |
|     |     |     |     |     |     | 220 |
| Gly | Gly | Ser | Gly | Gly | Gly | Gly |
|     |     |     |     |     |     |     |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | Gln |
|     |     |     |     |     |     | Val |
|     |     |     |     |     |     | Gln |
|     |     |     |     |     |     | Leu |
|     |     |     |     |     |     | Val |
|     |     |     |     |     |     | Gln |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | Gly |
|     |     |     |     |     |     | 225 |
|     |     |     |     |     |     | 230 |
|     |     |     |     |     |     | 235 |
|     |     |     |     |     |     | 240 |
| Ala | Glu | Val | Lys | Lys | Pro | Gly |
|     |     |     |     |     |     |     |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | Val |
|     |     |     |     |     |     | Lys |
|     |     |     |     |     |     | Val |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | Cys |
|     |     |     |     |     |     | Lys |
|     |     |     |     |     |     | Ala |
|     |     |     |     |     |     | 245 |
|     |     |     |     |     |     | 250 |
|     |     |     |     |     |     | 255 |
| Ser | Gly | Gly | Thr | Phe | Ser | Ser |
|     |     |     |     |     |     |     |
|     |     |     |     |     |     | Tyr |
|     |     |     |     |     |     | Ala |
|     |     |     |     |     |     | Ile |
|     |     |     |     |     |     | Ser |
|     |     |     |     |     |     | Trp |
|     |     |     |     |     |     | Val |
|     |     |     |     |     |     | Arg |
|     |     |     |     |     |     | Gln |
|     |     |     |     |     |     | Ala |

eolf-seql (8).txt

260

265

270

Pro Gly Gln Gly Leu Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly  
275 280 285

Thr Ala Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala  
290 295 300

Asp Lys Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser  
305 310 315 320

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Glu Tyr Tyr Arg Gly Pro  
325 330 335

Tyr Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser  
340 345 350

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr  
355 360 365

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro  
370 375 380

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val  
385 390 395 400

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser  
405 410 415

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile  
420 425 430

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val  
435 440 445

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala

eolf-seql (8).txt

450

455

460

Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
465 470 475 480

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val  
485 490 495

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val  
500 505 510

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln  
515 520 525

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln  
530 535 540

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala  
545 550 555 560

Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro  
565 570 575

Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr  
580 585 590

Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser  
595 600 605

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr  
610 615 620

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val  
625 630 635 640

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe

eolf-seql (8).txt

645

650

655

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys  
660 665 670

Ser Leu Ser Leu Ser Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly  
675 680 685

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser Glu Val Gln Leu Leu  
690 695 700

Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser  
705 710 715 720

Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser Trp Val  
725 730 735

Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ala Ile Ile Gly  
740 745 750

Ser Gly Ala Ser Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr  
755 760 765

Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser  
770 775 780

Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Gly Trp Phe  
785 790 795 800

Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
805 810 815

<210> 61

<211> 816

<212> PRT

<213> Artificial Sequence

eolf-seql (8).txt

<220>

<223> HC 1 (49B4) VHCH1\_VHCH1 Fc wt knob VH (4B9)

<400> 61

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser  
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr  
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe  
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Glu Tyr Tyr Arg Gly Pro Tyr Asp Tyr Trp Gly Gln Gly Thr  
100 105 110

Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro  
115 120 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly  
130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn  
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln  
165 170 175

eolf-seql (8).txt

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser  
180 185 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser  
195 200 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Gly Gly  
210 215 220

Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly  
225 230 235 240

Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala  
245 250 255

Ser Gly Gly Thr Phe Ser Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala  
260 265 270

Pro Gly Gln Gly Leu Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly  
275 280 285

Thr Ala Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala  
290 295 300

Asp Lys Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser  
305 310 315 320

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Glu Tyr Tyr Arg Gly Pro  
325 330 335

Tyr Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser  
340 345 350

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr  
355 360 365



eolf-seql (8).txt

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro  
370 375 380

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val  
385 390 395 400

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser  
405 410 415

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile  
420 425 430

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val  
435 440 445

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala  
450 455 460

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
465 470 475 480

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val  
485 490 495

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val  
500 505 510

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln  
515 520 525

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln  
530 535 540

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala  
545 550 555 560

eolf-seql (8).txt

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro  
565 570 575

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr  
580 585 590

Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser  
595 600 605

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr  
610 615 620

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr  
625 630 635 640

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe  
645 650 655

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys  
660 665 670

Ser Leu Ser Leu Ser Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly  
675 680 685

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu  
690 695 700

Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser  
705 710 715 720

Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser Trp Val  
725 730 735

Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ala Ile Ile Gly  
740 745 750

eolf-seql (8).txt

Ser Gly Ala Ser Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr  
755 760 765

Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser  
770 775 780

Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Gly Trp Phe  
785 790 795 800

Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
805 810 815

<210> 62

<211> 807

<212> PRT

<213> Artificial Sequence

<220>

<223> HC 2 (49B4) VHCH1\_VHCH1 Fc wt hole VL (4B9)

<400> 62

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser  
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr  
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe  
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys

## eolf-seql (8).txt

85

90

95

Ala Arg Glu Tyr Tyr Arg Gly Pro Tyr Asp Tyr Trp Gly Gln Gly Thr  
 100 105 110

Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro  
 115 120 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly  
 130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn  
 145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln  
 165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser  
 180 185 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser  
 195 200 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Gly Gly  
 210 215 220

Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly  
 225 230 235 240

Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala  
 245 250 255

Ser Gly Gly Thr Phe Ser Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala  
 260 265 270

Pro Gly Gln Gly Leu Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly

## eolf-seql (8).txt

275

280

285

Thr Ala Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala  
 290 295 300

Asp Lys Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser  
 305 310 315 320

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Glu Tyr Tyr Arg Gly Pro  
 325 330 335

Tyr Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser  
 340 345 350

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr  
 355 360 365

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro  
 370 375 380

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val  
 385 390 395 400

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser  
 405 410 415

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile  
 420 425 430

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val  
 435 440 445

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala  
 450 455 460

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro

eolf-seql (8).txt

|     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|
| 465 |     | 470 |     | 475 |     | 480 |
| Lys | Asp | Thr | Leu | Met | Ile | Val |
|     |     |     | 485 |     |     | 490 |
| Val | Asp | Val | Ser | His | Glu | Asp |
|     |     | 500 |     |     | 505 |     |
| Asp | Gly | Val | Glu | Val | His | Asn |
|     |     | 515 |     |     | 520 |     |
| Tyr | Asn | Ser | Thr | Tyr | Arg | Val |
|     |     | 530 |     |     | 535 |     |
| Asp | Trp | Leu | Asn | Gly | Lys | Glu |
| 545 |     |     |     | 550 |     |     |
| Leu | Pro | Ala | Pro | Ile | Glu | Lys |
|     |     |     |     | 565 |     |     |
| Arg | Glu | Pro | Gln | Val | Cys | Thr |
|     |     | 580 |     |     | 585 |     |
| Lys | Asn | Gln | Val | Ser | Leu | Ser |
|     |     | 595 |     |     | 600 |     |
| Asp | Ile | Ala | Val | Glu | Trp | Glu |
|     |     | 610 |     |     | 615 |     |
| Lys | Thr | Thr | Pro | Pro | Val | Leu |
| 625 |     |     |     |     | 630 |     |
| Ser | Lys | Leu | Thr | Val | Asp | Lys |
|     |     |     |     | 645 |     |     |
| Ser | Cys | Ser | Val | Met | His | Glu |
|     |     |     |     |     |     |     |

eolf-seql (8).txt

660

665

670

Ser Leu Ser Leu Ser Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly  
675 680 685

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Leu Thr  
690 695 700

Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu  
705 710 715 720

Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser Tyr Leu Ala Trp Tyr  
725 730 735

Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Asn Val Gly Ser  
740 745 750

Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly  
755 760 765

Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala  
770 775 780

Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro Pro Thr Phe Gly Gln  
785 790 795 800

Gly Thr Lys Val Glu Ile Lys  
805

<210> 63

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> CD3-HCDR1

<400> 63

eolf-seql (8).txt

Thr Tyr Ala Met Asn  
1 5

<210> 64  
<211> 19  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CD3-HCDR2

<400> 64

Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser  
1 5 10 15

Val Lys Gly

<210> 65  
<211> 14  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CD3-HCDR3

<400> 65

His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe Ala Tyr  
1 5 10

<210> 66  
<211> 14  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CD3-LCDR1

<400> 66

Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn



1

5

10

&lt;210&gt; 67

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; CD3-LCDR2

&lt;400&gt; 67

Gly Thr Asn Lys Arg Ala Pro

1

5

&lt;210&gt; 68

&lt;211&gt; 9

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; CD3-LCDR3

&lt;400&gt; 68

Ala Leu Trp Tyr Ser Asn Leu Trp Val

1

5

&lt;210&gt; 69

&lt;211&gt; 125

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; CD3 VH

&lt;400&gt; 69

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

1

5

10

15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr

20

25

30

eolf-seql (8).txt

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

Ser Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp  
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr  
85 90 95

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe  
100 105 110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
115 120 125

<210> 70  
<211> 109  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CD3 VL

<400> 70

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly  
1 5 10 15

Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser  
20 25 30

Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly  
35 40 45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Thr Pro Ala Arg Phe

eolf-seql (8).txt

50

55

60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala  
65 70 75 80

Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn  
85 90 95

Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu  
100 105

<210> 71  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CEA-HCDR1

<400> 71

Glu Phe Gly Met Asn  
1 5

<210> 72  
<211> 17  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CEA-HCDR2

<400> 72

Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe Lys  
1 5 10 15

Gly

<210> 73

eolf-seql (8).txt

<211> 12  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CEA-HCDR3

<400> 73

|     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Trp | Asp | Phe | Ala | Tyr | Tyr | Val | Glu | Ala | Met | Asp | Tyr |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |

<210> 74  
 <211> 11  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CEA-LCDR1

<400> 74

|     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Ala | Ser | Ala | Ala | Val | Gly | Thr | Tyr | Val | Ala |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |

<210> 75  
 <211> 7  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CEA-LCDR2

<400> 75

|     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|
| Ser | Ala | Ser | Tyr | Arg | Lys | Arg |
| 1   |     |     |     | 5   |     |     |

<210> 76  
 <211> 10  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CEA-LCDR3

eolf-seql (8).txt

<400> 76

His Gln Tyr Tyr Thr Tyr Pro Leu Phe Thr  
1 5 10

<210> 77

<211> 121

<212> PRT

<213> Artificial Sequence

<220>

<223> CEA VH

<400> 77

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe  
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45

Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe  
50 55 60

Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly  
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser  
115 120

eolf-seql (8).txt

<210> 78  
 <211> 108  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CEA VL

<400> 78

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Ala Ala Val Gly Thr Tyr  
 20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
 35 40 45

Tyr Ser Ala Ser Tyr Arg Lys Arg Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
 65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr Tyr Thr Tyr Pro Leu  
 85 90 95

Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 79  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CEA-HCDR1 (CEACAM5)

<400> 79

eolf-seql (8).txt

Asp Thr Tyr Met His  
1 5

<210> 80  
<211> 17  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CEA-HCDR2 (CEACAM5)

<400> 80

Arg Ile Asp Pro Ala Asn Gly Asn Ser Lys Tyr Val Pro Lys Phe Gln  
1 5 10 15

Gly

<210> 81  
<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CEA-HCDR3 (CEACAM5)

<400> 81

Phe Gly Tyr Tyr Val Ser Asp Tyr Ala Met Ala Tyr  
1 5 10

<210> 82  
<211> 15  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CEA-LCDR1 (CEACAM5)

<400> 82

Arg Ala Gly Glu Ser Val Asp Ile Phe Gly Val Gly Phe Leu His

eolf-seql (8).txt

1 5 10 15

<210> 83  
 <211> 7  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CEA-LCDR2 (CEACAM5)

<400> 83

Arg Ala Ser Asn Arg Ala Thr  
 1 5

<210> 84  
 <211> 9  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CEA-LCDR3 (CEACAM5)

<400> 84

Gln Gln Thr Asn Glu Asp Pro Tyr Thr  
 1 5

<210> 85  
 <211> 121  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CEA VH (CEACAM5)

<400> 85

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser  
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Thr  
 20 25 30



eolf-seql (8).txt

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45

Gly Arg Ile Asp Pro Ala Asn Gly Asn Ser Lys Tyr Val Pro Lys Phe  
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Pro Phe Gly Tyr Tyr Val Ser Asp Tyr Ala Met Ala Tyr Trp Gly  
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser  
115 120

<210> 86  
<211> 111  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CEA VL (CEACAM5)

<400> 86

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly  
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Gly Glu Ser Val Asp Ile Phe  
20 25 30

Gly Val Gly Phe Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro  
35 40 45

Arg Leu Leu Ile Tyr Arg Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala

eolf-seql (8).txt

50

55

60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
65 70 75 80

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Thr Asn  
85 90 95

Glu Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys  
100 105 110

<210> 87

<211> 215

<212> PRT

<213> Artificial Sequence

<220>

<223> Light chain CEA (CEA TCB)

<400> 87

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Ala Ala Val Gly Thr Tyr  
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
35 40 45

Tyr Ser Ala Ser Tyr Arg Lys Arg Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr Tyr Thr Tyr Pro Leu  
85 90 95

eolf-seql (8).txt

Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala  
 100 105 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser  
 115 120 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu  
 130 135 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser  
 145 150 155 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu  
 165 170 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val  
 180 185 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys  
 195 200 205

Ser Phe Asn Arg Gly Glu Cys  
 210 215

<210> 88  
 <211> 214  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Light chain CD3 (CEA TCB)

<400> 88

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly  
 1 5 10 15

Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser

eolf-seql (8).txt

20

25

30

Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly  
35 40 45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Thr Pro Ala Arg Phe  
50 55 60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala  
65 70 75 80

Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn  
85 90 95

Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser Ser Ala  
100 105 110

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser  
115 120 125

Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe  
130 135 140

Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly  
145 150 155 160

Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu  
165 170 175

Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr  
180 185 190

Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys  
195 200 205

Val Glu Pro Lys Ser Cys

eolf-seql (8).txt

210

<210> 89  
 <211> 694  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CEA CD3 crossfab VHck fc knob P329GLALA (CEA TCB)

<400> 89

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe  
 20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
 35 40 45

Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe  
 50 55 60

Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr  
 65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly  
 100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
 115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala  
 130 135 140

eolf-seql (8).txt

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His  
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
210 215 220

Asp Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu  
225 230 235 240

Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser  
245 250 255

Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr Ala Met Asn Trp Val  
260 265 270

Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Arg Ile Arg Ser  
275 280 285

Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg  
290 295 300

Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Leu Tyr Leu Gln Met  
305 310 315 320

Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Val Arg His  
325 330 335

eolf-seql (8).txt

Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe Ala Tyr Trp Gly Gln  
 340 345 350

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Val Ala Ala Pro Ser Val  
 355 360 365

Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser  
 370 375 380

Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln  
 385 390 395 400

Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val  
 405 410 415

Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu  
 420 425 430

Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu  
 435 440 445

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg  
 450 455 460

Gly Glu Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
 465 470 475 480

Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
 485 490 495

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
 500 505 510

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
 515 520 525

eolf-seql (8).txt

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
530 535 540

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
545 550 555 560

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly  
565 570 575

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
580 585 590

Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn  
595 600 605

Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
610 615 620

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
625 630 635 640

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
645 650 655

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
660 665 670

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
675 680 685

Ser Leu Ser Pro Gly Lys  
690

<210> 90  
<211> 451  
<212> PRT



eolf-seql (8).txt

<213> Artificial Sequence

<220>

<223> CEA VHCH1 Fc hole P329GLALA (CEA TCB)

<400> 90

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe  
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45

Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe  
50 55 60

Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly  
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala  
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala

## eolf-seql (8).txt

165

170

175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
 180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His  
 195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
 210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly  
 225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met  
 245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His  
 260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val  
 275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr  
 290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly  
 305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile  
 325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val  
 340 345 350

Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser

eolf-seql (8).txt

355

360

365

Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val  
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met  
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
435 440 445

Pro Gly Lys  
450

<210> 91

<211> 232

<212> PRT

<213> Artificial Sequence

<220>

<223> CD3 VH-CL (CEACAM5 TCB)

<400> 91

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr  
20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

eolf-seql (8).txt

Ser Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp  
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr  
85 90 95

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe  
100 105 110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Val  
115 120 125

Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys  
130 135 140

Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg  
145 150 155 160

Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn  
165 170 175

Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser  
180 185 190

Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys  
195 200 205

Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr  
210 215 220

Lys Ser Phe Asn Arg Gly Glu Cys  
225 230

eolf-seql (8).txt

<210> 92  
 <211> 449  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CEACAM5 VH-CH1(EЕ)-Fc (hole, P329G LALA)

<400> 92

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser  
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Thr  
 20 25 30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
 35 40 45

Gly Arg Ile Asp Pro Ala Asn Gly Asn Ser Lys Tyr Val Pro Lys Phe  
 50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr  
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Pro Phe Gly Tyr Tyr Val Ser Asp Tyr Ala Met Ala Tyr Trp Gly  
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
 115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala  
 130 135 140

Ala Leu Gly Cys Leu Val Glu Asp Tyr Phe Pro Glu Pro Val Thr Val

## eolf-seql (8).txt

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 145 |     | 150 |     | 155 |     | 160 |     |     |     |     |     |     |     |     |     |
| Ser | Trp | Asn | Ser | Gly | Ala | Leu | Thr | Ser | Gly | Val | His | Thr | Phe | Pro | Ala |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |
| Val | Leu | Gln | Ser | Ser | Gly | Leu | Tyr | Ser | Leu | Ser | Ser | Val | Val | Thr | Val |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| Pro | Ser | Ser | Ser | Leu | Gly | Thr | Gln | Thr | Tyr | Ile | Cys | Asn | Val | Asn | His |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Lys | Pro | Ser | Asn | Thr | Lys | Val | Asp | Glu | Lys | Val | Glu | Pro | Lys | Ser | Cys |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| Asp | Lys | Thr | His | Thr | Cys | Pro | Pro | Cys | Pro | Ala | Pro | Glu | Ala | Ala | Gly |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
| Gly | Pro | Ser | Val | Phe | Leu | Phe | Pro | Pro | Lys | Pro | Lys | Asp | Thr | Leu | Met |
|     |     |     | 245 |     |     |     |     |     | 250 |     |     |     |     | 255 |     |
| Ile | Ser | Arg | Thr | Pro | Glu | Val | Thr | Cys | Val | Val | Val | Asp | Val | Ser | His |
|     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
| Glu | Asp | Pro | Glu | Val | Lys | Phe | Asn | Trp | Tyr | Val | Asp | Gly | Val | Glu | Val |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
| His | Asn | Ala | Lys | Thr | Lys | Pro | Arg | Glu | Glu | Gln | Tyr | Asn | Ser | Thr | Tyr |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
| Arg | Val | Val | Ser | Val | Leu | Thr | Val | Leu | His | Gln | Asp | Trp | Leu | Asn | Gly |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
| Lys | Glu | Tyr | Lys | Cys | Lys | Val | Ser | Asn | Lys | Ala | Leu | Gly | Ala | Pro | Ile |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |
| Glu | Lys | Thr | Ile | Ser | Lys | Ala | Lys | Gly | Gln | Pro | Arg | Glu | Pro | Gln | Val |

eolf-seql (8).txt

340

345

350

Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser  
355 360 365

Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val  
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met  
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
435 440 445

Pro

<210> 93

<211> 674

<212> PRT

<213> Artificial Sequence

<220>

<223> CEACAM5 VH-CH1(EF)-CD3 VL-CH1-Fc (knob, P329G LALA)

<400> 93

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser  
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Thr  
20 25 30

eolf-seql (8).txt

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45

Gly Arg Ile Asp Pro Ala Asn Gly Asn Ser Lys Tyr Val Pro Lys Phe  
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Pro Phe Gly Tyr Tyr Val Ser Asp Tyr Ala Met Ala Tyr Trp Gly  
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala  
130 135 140

Ala Leu Gly Cys Leu Val Glu Asp Tyr Phe Pro Glu Pro Val Thr Val  
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His  
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Glu Lys Val Glu Pro Lys Ser Cys  
210 215 220



eolf-seql (8).txt

Asp Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val Thr  
225 230 235 240

Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu Thr  
245 250 255

Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp  
260 265 270

Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly Leu Ile Gly Gly Thr  
275 280 285

Asn Lys Arg Ala Pro Gly Thr Pro Ala Arg Phe Ser Gly Ser Leu Leu  
290 295 300

Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala Gln Pro Glu Asp Glu  
305 310 315 320

Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val Phe Gly  
325 330 335

Gly Gly Thr Lys Leu Thr Val Leu Ser Ser Ala Ser Thr Lys Gly Pro  
340 345 350

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr  
355 360 365

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr  
370 375 380

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro  
385 390 395 400

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr  
405 410 415

eolf-seql (8).txt

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn  
420 425 430

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser  
435 440 445

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala  
450 455 460

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu  
465 470 475 480

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser  
485 490 495

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu  
500 505 510

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr  
515 520 525

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn  
530 535 540

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro  
545 550 555 560

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln  
565 570 575

Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val  
580 585 590

Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val  
595 600 605

eolf-seql (8).txt

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
610 615 620

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr  
625 630 635 640

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val  
645 650 655

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu  
660 665 670

Ser Pro

<210> 94  
<211> 218  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CEACAM5 VL-CL(RK)

<400> 94

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly  
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Gly Glu Ser Val Asp Ile Phe  
20 25 30

Gly Val Gly Phe Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro  
35 40 45

Arg Leu Leu Ile Tyr Arg Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala  
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser

eolf-seql (8).txt

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 65  |     | 70  |     | 75  |     | 80  |     |     |     |     |     |     |     |     |     |
| Ser | Leu | Glu | Pro | Glu | Asp | Phe | Ala | Val | Tyr | Tyr | Cys | Gln | Gln | Thr | Asn |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |
| Glu | Asp | Pro | Tyr | Thr | Phe | Gly | Gln | Gly | Thr | Lys | Leu | Glu | Ile | Lys | Arg |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Thr | Val | Ala | Ala | Pro | Ser | Val | Phe | Ile | Phe | Pro | Pro | Ser | Asp | Arg | Lys |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| Leu | Lys | Ser | Gly | Thr | Ala | Ser | Val | Val | Cys | Leu | Leu | Asn | Asn | Phe | Tyr |
|     | 130 |     |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |
| Pro | Arg | Glu | Ala | Lys | Val | Gln | Trp | Lys | Val | Asp | Asn | Ala | Leu | Gln | Ser |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |
| Gly | Asn | Ser | Gln | Glu | Ser | Val | Thr | Glu | Gln | Asp | Ser | Lys | Asp | Ser | Thr |
|     |     |     | 165 |     |     |     |     |     | 170 |     |     |     |     | 175 |     |
| Tyr | Ser | Leu | Ser | Ser | Thr | Leu | Thr | Leu | Ser | Lys | Ala | Asp | Tyr | Glu | Lys |
|     |     | 180 |     |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| His | Lys | Val | Tyr | Ala | Cys | Glu | Val | Thr | His | Gln | Gly | Leu | Ser | Ser | Pro |
|     | 195 |     |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Val | Thr | Lys | Ser | Phe | Asn | Arg | Gly | Glu | Cys |     |     |     |     |     |     |
|     | 210 |     |     |     |     | 215 |     |     |     |     |     |     |     |     |     |

<210> 95  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> (CH2527) CD3-HCDR1

<400> 95

eolf-seql (8).txt

Thr Tyr Ala Met Asn  
1 5

<210> 96  
<211> 19  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> (CH2527) CD3-HCDR2

<400> 96

Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser  
1 5 10 15

Val Lys Gly

<210> 97  
<211> 14  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> (CH2527) CD3-HCDR3

<400> 97

His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe Ala Tyr  
1 5 10

<210> 98  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> (16D5) Fo1R1-HCDR1

<400> 98

Asn Ala Trp Met Ser

1 5

<210> 99  
 <211> 19  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> (16D5) Fo1R1-HCDR2

<400> 99

Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala Pro  
 1 5 10 15

Val Lys Gly

<210> 100  
 <211> 9  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> (16D5) Fo1R1-HCDR3

<400> 100

Pro Trp Glu Trp Ser Trp Tyr Asp Tyr  
 1 5

<210> 101  
 <211> 14  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> (CH2527-VL7-46-13)-LCDR1

<400> 101

Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn  
 1 5 10

eolf-seql (8).txt

<210> 102  
 <211> 7  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> (CH2527-VL7-46-13)-LCDR2

<400> 102

Gly Thr Asn Lys Arg Ala Pro  
 1 5

<210> 103  
 <211> 9  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> (CH2527-VL7-46-13)-LCDR3

<400> 103

Ala Leu Trp Tyr Ser Asn Leu Trp Val  
 1 5

<210> 104  
 <211> 125  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> (CH2527) CD3 VH

<400> 104

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr  
 20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

eolf-seql (8).txt

35

40

45

Ser Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp  
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr  
85 90 95

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe  
100 105 110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
115 120 125

<210> 105

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> (16D5) FolR1 VH

<400> 105

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala  
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala  
50 55 60



eolf-seql (8).txt

Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr  
85 90 95

Tyr Cys Thr Thr Pro Trp Glu Trp Ser Trp Tyr Asp Tyr Trp Gly Gln  
100 105 110

Gly Thr Leu Val Thr Val Ser Ser  
115 120

<210> 106  
<211> 109  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> (CH2527-VL7-46-13)VL

<400> 106

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly  
1 5 10 15

Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser  
20 25 30

Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly  
35 40 45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Thr Pro Ala Arg Phe  
50 55 60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala  
65 70 75 80

Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn

Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu  
 100 105

<210> 107

<211> 689

<212> PRT

<213> Artificial Sequence

<220>

<223> (16D5)VH-CH1-(CH2527)VH-CH1 Fc knob PGLALA

<400> 107

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala  
 20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45

Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala  
 50 55 60

Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
 65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr  
 85 90 95

Tyr Cys Thr Thr Pro Trp Glu Trp Ser Trp Tyr Asp Tyr Trp Gly Gln  
 100 105 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125

eolf-seql (8).txt

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
180 185 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp  
210 215 220

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu  
225 230 235 240

Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys  
245 250 255

Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr Ala Met Asn Trp Val Arg  
260 265 270

Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Arg Ile Arg Ser Lys  
275 280 285

Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe  
290 295 300

Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn  
305 310 315 320

eolf-seql (8).txt

Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Val Arg His Gly  
325 330 335

Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe Ala Tyr Trp Gly Gln Gly  
340 345 350

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe  
355 360 365

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu  
370 375 380

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp  
385 390 395 400

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu  
405 410 415

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser  
420 425 430

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro  
435 440 445

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys  
450 455 460

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro  
465 470 475 480

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser  
485 490 495

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp  
500 505 510

eolf-seql (8).txt

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn  
515 520 525

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val  
530 535 540

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu  
545 550 555 560

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys  
565 570 575

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr  
580 585 590

Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp  
595 600 605

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu  
610 615 620

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu  
625 630 635 640

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys  
645 650 655

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu  
660 665 670

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly  
675 680 685

Lys

eolf-seql (8).txt

<210> 108  
 <211> 450  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> (16D5)VH-CH1-Fc hole PGLALA H435R-Y436F

<400> 108

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala  
 20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45

Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala  
 50 55 60

Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr  
 65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr  
 85 90 95

Tyr Cys Thr Thr Pro Trp Glu Trp Ser Trp Tyr Asp Tyr Trp Gly Gln  
 100 105 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser

145 150 155 160 eolf-seql (8).txt

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
180 185 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp  
210 215 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly  
225 230 235 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
260 265 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu  
325 330 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys

eolf-seql (8).txt

340

345

350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu  
355 360 365

Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp  
405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
420 425 430

Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro  
435 440 445

Gly Lys  
450

<210> 109

<211> 215

<212> PRT

<213> Artificial Sequence

<220>

<223> (CH2527-VL7-46-13)VL-CL

<400> 109

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly  
1 5 10 15

Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser  
20 25 30



eolf-seql (8).txt

Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly  
35 40 45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Thr Pro Ala Arg Phe  
50 55 60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala  
65 70 75 80

Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn  
85 90 95

Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro  
100 105 110

Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu  
115 120 125

Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro  
130 135 140

Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys Ala  
145 150 155 160

Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala  
165 170 175

Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg  
180 185 190

Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr  
195 200 205

Val Ala Pro Thr Glu Cys Ser  
210 215

eolf-seql (8).txt

<210> 110

<211> 290

<212> PRT

<213> Homo sapiens

<400> 110

Met Arg Ile Phe Ala Val Phe Ile Phe Met Thr Tyr Trp His Leu Leu  
1 5 10 15

Asn Ala Phe Thr Val Thr Val Pro Lys Asp Leu Tyr Val Val Glu Tyr  
20 25 30

Gly Ser Asn Met Thr Ile Glu Cys Lys Phe Pro Val Glu Lys Gln Leu  
35 40 45

Asp Leu Ala Ala Leu Ile Val Tyr Trp Glu Met Glu Asp Lys Asn Ile  
50 55 60

Ile Gln Phe Val His Gly Glu Glu Asp Leu Lys Val Gln His Ser Ser  
65 70 75 80

Tyr Arg Gln Arg Ala Arg Leu Leu Lys Asp Gln Leu Ser Leu Gly Asn  
85 90 95

Ala Ala Leu Gln Ile Thr Asp Val Lys Leu Gln Asp Ala Gly Val Tyr  
100 105 110

Arg Cys Met Ile Ser Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Val  
115 120 125

Lys Val Asn Ala Pro Tyr Asn Lys Ile Asn Gln Arg Ile Leu Val Val  
130 135 140

Asp Pro Val Thr Ser Glu His Glu Leu Thr Cys Gln Ala Glu Gly Tyr  
145 150 155 160

eolf-seql (8).txt

Pro Lys Ala Glu Val Ile Trp Thr Ser Ser Asp His Gln Val Leu Ser  
165 170 175

Gly Lys Thr Thr Thr Thr Asn Ser Lys Arg Glu Glu Lys Leu Phe Asn  
180 185 190

Val Thr Ser Thr Leu Arg Ile Asn Thr Thr Thr Asn Glu Ile Phe Tyr  
195 200 205

Cys Thr Phe Arg Arg Leu Asp Pro Glu Glu Asn His Thr Ala Glu Leu  
210 215 220

Val Ile Pro Glu Leu Pro Leu Ala His Pro Pro Asn Glu Arg Thr His  
225 230 235 240

Leu Val Ile Leu Gly Ala Ile Leu Leu Cys Leu Gly Val Ala Leu Thr  
245 250 255

Phe Ile Phe Arg Leu Arg Lys Gly Arg Met Met Asp Val Lys Lys Cys  
260 265 270

Gly Ile Gln Asp Thr Asn Ser Lys Lys Gln Ser Asp Thr His Leu Glu  
275 280 285

Glu Thr  
290

<210> 111  
<211> 288  
<212> PRT  
<213> Homo sapiens

<400> 111

Met Gln Ile Pro Gln Ala Pro Trp Pro Val Val Trp Ala Val Leu Gln  
1 5 10 15

Leu Gly Trp Arg Pro Gly Trp Phe Leu Asp Ser Pro Asp Arg Pro Trp

eolf-seql (8).txt

20

25

30

Asn Pro Pro Thr Phe Ser Pro Ala Leu Leu Val Val Thr Glu Gly Asp  
35 40 45

Asn Ala Thr Phe Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val  
50 55 60

Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp Lys Leu Ala  
65 70 75 80

Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg  
85 90 95

Val Thr Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg  
100 105 110

Ala Arg Arg Asn Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu  
115 120 125

Ala Pro Lys Ala Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val  
130 135 140

Thr Glu Arg Arg Ala Glu Val Pro Thr Ala His Pro Ser Pro Ser Pro  
145 150 155 160

Arg Pro Ala Gly Gln Phe Gln Thr Leu Val Val Gly Val Val Gly Gly  
165 170 175

Leu Leu Gly Ser Leu Val Leu Leu Val Trp Val Leu Ala Val Ile Cys  
180 185 190

Ser Arg Ala Ala Arg Gly Thr Ile Gly Ala Arg Arg Thr Gly Gln Pro  
195 200 205

Leu Lys Glu Asp Pro Ser Ala Val Pro Val Phe Ser Val Asp Tyr Gly

eolf-seql (8).txt

210

215

220

Glu Leu Asp Phe Gln Trp Arg Glu Lys Thr Pro Glu Pro Pro Val Pro  
225 230 235 240

Cys Val Pro Glu Gln Thr Glu Tyr Ala Thr Ile Val Phe Pro Ser Gly  
245 250 255

Met Gly Thr Ser Ser Pro Ala Arg Arg Gly Ser Ala Asp Gly Pro Arg  
260 265 270

Ser Ala Gln Pro Leu Arg Pro Glu Asp Gly His Cys Ser Trp Pro Leu  
275 280 285

<210> 112

<211> 118

<212> PRT

<213> Artificial Sequence

<220>

<223> VH (PD-L1)

<400> 112

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser  
20 25 30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr  
65 70 75 80

eolf-seql (8).txt

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr  
100 105 110

Leu Val Thr Val Ser Ser  
115

<210> 113  
<211> 107  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> VL (PD-L1)

<400> 113

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala  
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Leu Tyr His Pro Ala  
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys

100

<210> 114  
<211> 121  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> VH (PD-L1) 2

<400> 114

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr  
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr  
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Glu Gly Gly Trp Phe Gly Glu Leu Ala Phe Asp Tyr Trp Gly  
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser  
115 120

<210> 115  
<211> 108  
<212> PRT

<213> Artificial Sequence

<220>

<223> VL (PD-L1) 2

<400> 115

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Val Ser Ser Ser  
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
35 40 45

Ile Tyr Asp Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Leu Pro  
85 90 95

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
100 105

<210> 116

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> VH (PD-1)

<400> 116

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala  
1 5 10 15



eolf-seql (8).txt

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr  
20 25 30

Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45

Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe  
50 55 60

Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr  
65 70 75 80

Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln  
100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
115 120

<210> 117  
<211> 111  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> VL (PD-1)

<400> 117

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly  
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Lys Gly Val Ser Thr Ser  
20 25 30

Gly Tyr Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro

eolf-seql (8).txt

35

40

45

Arg Leu Leu Ile Tyr Leu Ala Ser Tyr Leu Glu Ser Gly Val Pro Ala  
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
65 70 75 80

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Ser Arg  
85 90 95

Asp Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys  
100 105 110

<210> 118

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> VH (PD-1) 2

<400> 118

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg  
1 5 10 15

Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser  
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe  
65 70 75 80

eolf-seql (8).txt

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser  
100 105 110

Ser

<210> 119  
<211> 107  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> VL (PD-1) 2

<400> 119

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly  
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr  
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile  
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro  
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg  
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys

eolf-seql (8).txt

100

105

<210> 120  
 <211> 760  
 <212> PRT  
 <213> Homo sapiens  
 <400> 120

Met Lys Thr Trp Val Lys Ile Val Phe Gly Val Ala Thr Ser Ala Val  
 1 5 10 15

Leu Ala Leu Leu Val Met Cys Ile Val Leu Arg Pro Ser Arg Val His  
 20 25 30

Asn Ser Glu Glu Asn Thr Met Arg Ala Leu Thr Leu Lys Asp Ile Leu  
 35 40 45

Asn Gly Thr Phe Ser Tyr Lys Thr Phe Phe Pro Asn Trp Ile Ser Gly  
 50 55 60

Gln Glu Tyr Leu His Gln Ser Ala Asp Asn Asn Ile Val Leu Tyr Asn  
 65 70 75 80

Ile Glu Thr Gly Gln Ser Tyr Thr Ile Leu Ser Asn Arg Thr Met Lys  
 85 90 95

Ser Val Asn Ala Ser Asn Tyr Gly Leu Ser Pro Asp Arg Gln Phe Val  
 100 105 110

Tyr Leu Glu Ser Asp Tyr Ser Lys Leu Trp Arg Tyr Ser Tyr Thr Ala  
 115 120 125

Thr Tyr Tyr Ile Tyr Asp Leu Ser Asn Gly Glu Phe Val Arg Gly Asn  
 130 135 140

Glu Leu Pro Arg Pro Ile Gln Tyr Leu Cys Trp Ser Pro Val Gly Ser  
 145 150 155 160

eolf-seql (8).txt

Lys Leu Ala Tyr Val Tyr Gln Asn Asn Ile Tyr Leu Lys Gln Arg Pro  
165 170 175

Gly Asp Pro Pro Phe Gln Ile Thr Phe Asn Gly Arg Glu Asn Lys Ile  
180 185 190

Phe Asn Gly Ile Pro Asp Trp Val Tyr Glu Glu Glu Met Leu Ala Thr  
195 200 205

Lys Tyr Ala Leu Trp Trp Ser Pro Asn Gly Lys Phe Leu Ala Tyr Ala  
210 215 220

Glu Phe Asn Asp Thr Asp Ile Pro Val Ile Ala Tyr Ser Tyr Tyr Gly  
225 230 235 240

Asp Glu Gln Tyr Pro Arg Thr Ile Asn Ile Pro Tyr Pro Lys Ala Gly  
245 250 255

Ala Lys Asn Pro Val Val Arg Ile Phe Ile Ile Asp Thr Thr Tyr Pro  
260 265 270

Ala Tyr Val Gly Pro Gln Glu Val Pro Val Pro Ala Met Ile Ala Ser  
275 280 285

Ser Asp Tyr Tyr Phe Ser Trp Leu Thr Trp Val Thr Asp Glu Arg Val  
290 295 300

Cys Leu Gln Trp Leu Lys Arg Val Gln Asn Val Ser Val Leu Ser Ile  
305 310 315 320

Cys Asp Phe Arg Glu Asp Trp Gln Thr Trp Asp Cys Pro Lys Thr Gln  
325 330 335

Glu His Ile Glu Glu Ser Arg Thr Gly Trp Ala Gly Gly Phe Phe Val  
340 345 350

eolf-seql (8).txt

Ser Thr Pro Val Phe Ser Tyr Asp Ala Ile Ser Tyr Tyr Lys Ile Phe  
355 360 365

Ser Asp Lys Asp Gly Tyr Lys His Ile His Tyr Ile Lys Asp Thr Val  
370 375 380

Glu Asn Ala Ile Gln Ile Thr Ser Gly Lys Trp Glu Ala Ile Asn Ile  
385 390 395 400

Phe Arg Val Thr Gln Asp Ser Leu Phe Tyr Ser Ser Asn Glu Phe Glu  
405 410 415

Glu Tyr Pro Gly Arg Arg Asn Ile Tyr Arg Ile Ser Ile Gly Ser Tyr  
420 425 430

Pro Pro Ser Lys Lys Cys Val Thr Cys His Leu Arg Lys Glu Arg Cys  
435 440 445

Gln Tyr Tyr Thr Ala Ser Phe Ser Asp Tyr Ala Lys Tyr Tyr Ala Leu  
450 455 460

Val Cys Tyr Gly Pro Gly Ile Pro Ile Ser Thr Leu His Asp Gly Arg  
465 470 475 480

Thr Asp Gln Glu Ile Lys Ile Leu Glu Glu Asn Lys Glu Leu Glu Asn  
485 490 495

Ala Leu Lys Asn Ile Gln Leu Pro Lys Glu Glu Ile Lys Lys Leu Glu  
500 505 510

Val Asp Glu Ile Thr Leu Trp Tyr Lys Met Ile Leu Pro Pro Gln Phe  
515 520 525

Asp Arg Ser Lys Lys Tyr Pro Leu Leu Ile Gln Val Tyr Gly Gly Pro  
530 535 540

eolf-seql (8).txt

Cys Ser Gln Ser Val Arg Ser Val Phe Ala Val Asn Trp Ile Ser Tyr  
545 550 555 560

Leu Ala Ser Lys Glu Gly Met Val Ile Ala Leu Val Asp Gly Arg Gly  
565 570 575

Thr Ala Phe Gln Gly Asp Lys Leu Leu Tyr Ala Val Tyr Arg Lys Leu  
580 585 590

Gly Val Tyr Glu Val Glu Asp Gln Ile Thr Ala Val Arg Lys Phe Ile  
595 600 605

Glu Met Gly Phe Ile Asp Glu Lys Arg Ile Ala Ile Trp Gly Trp Ser  
610 615 620

Tyr Gly Gly Tyr Val Ser Ser Leu Ala Leu Ala Ser Gly Thr Gly Leu  
625 630 635 640

Phe Lys Cys Gly Ile Ala Val Ala Pro Val Ser Ser Trp Glu Tyr Tyr  
645 650 655

Ala Ser Val Tyr Thr Glu Arg Phe Met Gly Leu Pro Thr Lys Asp Asp  
660 665 670

Asn Leu Glu His Tyr Lys Asn Ser Thr Val Met Ala Arg Ala Glu Tyr  
675 680 685

Phe Arg Asn Val Asp Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn  
690 695 700

Val His Phe Gln Asn Ser Ala Gln Ile Ala Lys Ala Leu Val Asn Ala  
705 710 715 720

Gln Val Asp Phe Gln Ala Met Trp Tyr Ser Asp Gln Asn His Gly Leu  
725 730 735

eolf-seql (8).txt

Ser Gly Leu Ser Thr Asn His Leu Tyr Thr His Met Thr His Phe Leu  
740 745 750

Lys Gln Cys Phe Ser Leu Ser Asp  
755 760

<210> 121  
<211> 748  
<212> PRT  
<213> Artificial sequence

<220>  
<223> hu FAP ectodomain+poly-lys-tag+his6-tag

<400> 121

Arg Pro Ser Arg Val His Asn Ser Glu Glu Asn Thr Met Arg Ala Leu  
1 5 10 15

Thr Leu Lys Asp Ile Leu Asn Gly Thr Phe Ser Tyr Lys Thr Phe Phe  
20 25 30

Pro Asn Trp Ile Ser Gly Gln Glu Tyr Leu His Gln Ser Ala Asp Asn  
35 40 45

Asn Ile Val Leu Tyr Asn Ile Glu Thr Gly Gln Ser Tyr Thr Ile Leu  
50 55 60

Ser Asn Arg Thr Met Lys Ser Val Asn Ala Ser Asn Tyr Gly Leu Ser  
65 70 75 80

Pro Asp Arg Gln Phe Val Tyr Leu Glu Ser Asp Tyr Ser Lys Leu Trp  
85 90 95

Arg Tyr Ser Tyr Thr Ala Thr Tyr Tyr Ile Tyr Asp Leu Ser Asn Gly  
100 105 110



eolf-seql (8).txt

Glu Phe Val Arg Gly Asn Glu Leu Pro Arg Pro Ile Gln Tyr Leu Cys  
 115 120 125

Trp Ser Pro Val Gly Ser Lys Leu Ala Tyr Val Tyr Gln Asn Asn Ile  
 130 135 140

Tyr Leu Lys Gln Arg Pro Gly Asp Pro Pro Phe Gln Ile Thr Phe Asn  
 145 150 155 160

Gly Arg Glu Asn Lys Ile Phe Asn Gly Ile Pro Asp Trp Val Tyr Glu  
 165 170 175

Glu Glu Met Leu Ala Thr Lys Tyr Ala Leu Trp Trp Ser Pro Asn Gly  
 180 185 190

Lys Phe Leu Ala Tyr Ala Glu Phe Asn Asp Thr Asp Ile Pro Val Ile  
 195 200 205

Ala Tyr Ser Tyr Tyr Gly Asp Glu Gln Tyr Pro Arg Thr Ile Asn Ile  
 210 215 220

Pro Tyr Pro Lys Ala Gly Ala Lys Asn Pro Val Val Arg Ile Phe Ile  
 225 230 235 240

Ile Asp Thr Thr Tyr Pro Ala Tyr Val Gly Pro Gln Glu Val Pro Val  
 245 250 255

Pro Ala Met Ile Ala Ser Ser Asp Tyr Tyr Phe Ser Trp Leu Thr Trp  
 260 265 270

Val Thr Asp Glu Arg Val Cys Leu Gln Trp Leu Lys Arg Val Gln Asn  
 275 280 285

Val Ser Val Leu Ser Ile Cys Asp Phe Arg Glu Asp Trp Gln Thr Trp  
 290 295 300

eolf-seql (8).txt

Asp Cys Pro Lys Thr Gln Glu His Ile Glu Glu Ser Arg Thr Gly Trp  
305 310 315 320

Ala Gly Gly Phe Phe Val Ser Thr Pro Val Phe Ser Tyr Asp Ala Ile  
325 330 335

Ser Tyr Tyr Lys Ile Phe Ser Asp Lys Asp Gly Tyr Lys His Ile His  
340 345 350

Tyr Ile Lys Asp Thr Val Glu Asn Ala Ile Gln Ile Thr Ser Gly Lys  
355 360 365

Trp Glu Ala Ile Asn Ile Phe Arg Val Thr Gln Asp Ser Leu Phe Tyr  
370 375 380

Ser Ser Asn Glu Phe Glu Glu Tyr Pro Gly Arg Arg Asn Ile Tyr Arg  
385 390 395 400

Ile Ser Ile Gly Ser Tyr Pro Pro Ser Lys Lys Cys Val Thr Cys His  
405 410 415

Leu Arg Lys Glu Arg Cys Gln Tyr Tyr Thr Ala Ser Phe Ser Asp Tyr  
420 425 430

Ala Lys Tyr Tyr Ala Leu Val Cys Tyr Gly Pro Gly Ile Pro Ile Ser  
435 440 445

Thr Leu His Asp Gly Arg Thr Asp Gln Glu Ile Lys Ile Leu Glu Glu  
450 455 460

Asn Lys Glu Leu Glu Asn Ala Leu Lys Asn Ile Gln Leu Pro Lys Glu  
465 470 475 480

Glu Ile Lys Lys Leu Glu Val Asp Glu Ile Thr Leu Trp Tyr Lys Met  
485 490 495

eolf-seql (8).txt

Ile Leu Pro Pro Gln Phe Asp Arg Ser Lys Lys Tyr Pro Leu Leu Ile  
500 505 510

Gln Val Tyr Gly Gly Pro Cys Ser Gln Ser Val Arg Ser Val Phe Ala  
515 520 525

Val Asn Trp Ile Ser Tyr Leu Ala Ser Lys Glu Gly Met Val Ile Ala  
530 535 540

Leu Val Asp Gly Arg Gly Thr Ala Phe Gln Gly Asp Lys Leu Leu Tyr  
545 550 555 560

Ala Val Tyr Arg Lys Leu Gly Val Tyr Glu Val Glu Asp Gln Ile Thr  
565 570 575

Ala Val Arg Lys Phe Ile Glu Met Gly Phe Ile Asp Glu Lys Arg Ile  
580 585 590

Ala Ile Trp Gly Trp Ser Tyr Gly Gly Tyr Val Ser Ser Leu Ala Leu  
595 600 605

Ala Ser Gly Thr Gly Leu Phe Lys Cys Gly Ile Ala Val Ala Pro Val  
610 615 620

Ser Ser Trp Glu Tyr Tyr Ala Ser Val Tyr Thr Glu Arg Phe Met Gly  
625 630 635 640

Leu Pro Thr Lys Asp Asp Asn Leu Glu His Tyr Lys Asn Ser Thr Val  
645 650 655

Met Ala Arg Ala Glu Tyr Phe Arg Asn Val Asp Tyr Leu Leu Ile His  
660 665 670

Gly Thr Ala Asp Asp Asn Val His Phe Gln Asn Ser Ala Gln Ile Ala  
675 680 685

eolf-seql (8).txt

Lys Ala Leu Val Asn Ala Gln Val Asp Phe Gln Ala Met Trp Tyr Ser  
690 695 700

Asp Gln Asn His Gly Leu Ser Gly Leu Ser Thr Asn His Leu Tyr Thr  
705 710 715 720

His Met Thr His Phe Leu Lys Gln Cys Phe Ser Leu Ser Asp Gly Lys  
725 730 735

Lys Lys Lys Lys Lys Gly His His His His His His  
740 745

<210> 122  
<211> 761  
<212> PRT  
<213> Mus musculus

<400> 122

Met Lys Thr Trp Leu Lys Thr Val Phe Gly Val Thr Thr Leu Ala Ala  
1 5 10 15

Leu Ala Leu Val Val Ile Cys Ile Val Leu Arg Pro Ser Arg Val Tyr  
20 25 30

Lys Pro Glu Gly Asn Thr Lys Arg Ala Leu Thr Leu Lys Asp Ile Leu  
35 40 45

Asn Gly Thr Phe Ser Tyr Lys Thr Tyr Phe Pro Asn Trp Ile Ser Glu  
50 55 60

Gln Glu Tyr Leu His Gln Ser Glu Asp Asp Asn Ile Val Phe Tyr Asn  
65 70 75 80

Ile Glu Thr Arg Glu Ser Tyr Ile Ile Leu Ser Asn Ser Thr Met Lys  
85 90 95

Ser Val Asn Ala Thr Asp Tyr Gly Leu Ser Pro Asp Arg Gln Phe Val

eolf-seql (8).txt

100

105

110

Tyr Leu Glu Ser Asp Tyr Ser Lys Leu Trp Arg Tyr Ser Tyr Thr Ala  
115 120 125

Thr Tyr Tyr Ile Tyr Asp Leu Gln Asn Gly Glu Phe Val Arg Gly Tyr  
130 135 140

Glu Leu Pro Arg Pro Ile Gln Tyr Leu Cys Trp Ser Pro Val Gly Ser  
145 150 155 160

Lys Leu Ala Tyr Val Tyr Gln Asn Asn Ile Tyr Leu Lys Gln Arg Pro  
165 170 175

Gly Asp Pro Pro Phe Gln Ile Thr Tyr Thr Gly Arg Glu Asn Arg Ile  
180 185 190

Phe Asn Gly Ile Pro Asp Trp Val Tyr Glu Glu Glu Met Leu Ala Thr  
195 200 205

Lys Tyr Ala Leu Trp Trp Ser Pro Asp Gly Lys Phe Leu Ala Tyr Val  
210 215 220

Glu Phe Asn Asp Ser Asp Ile Pro Ile Ile Ala Tyr Ser Tyr Tyr Gly  
225 230 235 240

Asp Gly Gln Tyr Pro Arg Thr Ile Asn Ile Pro Tyr Pro Lys Ala Gly  
245 250 255

Ala Lys Asn Pro Val Val Arg Val Phe Ile Val Asp Thr Thr Tyr Pro  
260 265 270

His His Val Gly Pro Met Glu Val Pro Val Pro Glu Met Ile Ala Ser  
275 280 285

Ser Asp Tyr Tyr Phe Ser Trp Leu Thr Trp Val Ser Ser Glu Arg Val

eolf-seql (8).txt

290

295

300

Cys Leu Gln Trp Leu Lys Arg Val Gln Asn Val Ser Val Leu Ser Ile  
305 310 315 320

Cys Asp Phe Arg Glu Asp Trp His Ala Trp Glu Cys Pro Lys Asn Gln  
325 330 335

Glu His Val Glu Glu Ser Arg Thr Gly Trp Ala Gly Gly Phe Phe Val  
340 345 350

Ser Thr Pro Ala Phe Ser Gln Asp Ala Thr Ser Tyr Tyr Lys Ile Phe  
355 360 365

Ser Asp Lys Asp Gly Tyr Lys His Ile His Tyr Ile Lys Asp Thr Val  
370 375 380

Glu Asn Ala Ile Gln Ile Thr Ser Gly Lys Trp Glu Ala Ile Tyr Ile  
385 390 395 400

Phe Arg Val Thr Gln Asp Ser Leu Phe Tyr Ser Ser Asn Glu Phe Glu  
405 410 415

Gly Tyr Pro Gly Arg Arg Asn Ile Tyr Arg Ile Ser Ile Gly Asn Ser  
420 425 430

Pro Pro Ser Lys Lys Cys Val Thr Cys His Leu Arg Lys Glu Arg Cys  
435 440 445

Gln Tyr Tyr Thr Ala Ser Phe Ser Tyr Lys Ala Lys Tyr Tyr Ala Leu  
450 455 460

Val Cys Tyr Gly Pro Gly Leu Pro Ile Ser Thr Leu His Asp Gly Arg  
465 470 475 480

Thr Asp Gln Glu Ile Gln Val Leu Glu Glu Asn Lys Glu Leu Glu Asn

## eolf-seql (8).txt

485

490

495

Ser Leu Arg Asn Ile Gln Leu Pro Lys Val Glu Ile Lys Lys Leu Lys  
 500 505 510

Asp Gly Gly Leu Thr Phe Trp Tyr Lys Met Ile Leu Pro Pro Gln Phe  
 515 520 525

Asp Arg Ser Lys Lys Tyr Pro Leu Leu Ile Gln Val Tyr Gly Gly Pro  
 530 535 540

Cys Ser Gln Ser Val Lys Ser Val Phe Ala Val Asn Trp Ile Thr Tyr  
 545 550 555 560

Leu Ala Ser Lys Glu Gly Ile Val Ile Ala Leu Val Asp Gly Arg Gly  
 565 570 575

Thr Ala Phe Gln Gly Asp Lys Phe Leu His Ala Val Tyr Arg Lys Leu  
 580 585 590

Gly Val Tyr Glu Val Glu Asp Gln Leu Thr Ala Val Arg Lys Phe Ile  
 595 600 605

Glu Met Gly Phe Ile Asp Glu Glu Arg Ile Ala Ile Trp Gly Trp Ser  
 610 615 620

Tyr Gly Gly Tyr Val Ser Ser Leu Ala Leu Ala Ser Gly Thr Gly Leu  
 625 630 635 640

Phe Lys Cys Gly Ile Ala Val Ala Pro Val Ser Ser Trp Glu Tyr Tyr  
 645 650 655

Ala Ser Ile Tyr Ser Glu Arg Phe Met Gly Leu Pro Thr Lys Asp Asp  
 660 665 670

Asn Leu Glu His Tyr Lys Asn Ser Thr Val Met Ala Arg Ala Glu Tyr

eolf-seql (8).txt

675

680

685

Phe Arg Asn Val Asp Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn  
690 695 700

Val His Phe Gln Asn Ser Ala Gln Ile Ala Lys Ala Leu Val Asn Ala  
705 710 715 720

Gln Val Asp Phe Gln Ala Met Trp Tyr Ser Asp Gln Asn His Gly Ile  
725 730 735

Ser Ser Gly Arg Ser Gln Asn His Leu Tyr Thr His Met Thr His Phe  
740 745 750

Leu Lys Gln Cys Phe Ser Leu Ser Asp  
755 760

<210> 123

<211> 749

<212> PRT

<213> Artificial sequence

<220>

<223> Murine FAP ectodomain+poly-lys-tag+his6-tag

<400> 123

Arg Pro Ser Arg Val Tyr Lys Pro Glu Gly Asn Thr Lys Arg Ala Leu  
1 5 10 15

Thr Leu Lys Asp Ile Leu Asn Gly Thr Phe Ser Tyr Lys Thr Tyr Phe  
20 25 30

Pro Asn Trp Ile Ser Glu Gln Glu Tyr Leu His Gln Ser Glu Asp Asp  
35 40 45

Asn Ile Val Phe Tyr Asn Ile Glu Thr Arg Glu Ser Tyr Ile Ile Leu  
50 55 60



eolf-seql (8).txt

Ser Asn Ser Thr Met Lys Ser Val Asn Ala Thr Asp Tyr Gly Leu Ser  
65 70 75 80

Pro Asp Arg Gln Phe Val Tyr Leu Glu Ser Asp Tyr Ser Lys Leu Trp  
85 90 95

Arg Tyr Ser Tyr Thr Ala Thr Tyr Tyr Ile Tyr Asp Leu Gln Asn Gly  
100 105 110

Glu Phe Val Arg Gly Tyr Glu Leu Pro Arg Pro Ile Gln Tyr Leu Cys  
115 120 125

Trp Ser Pro Val Gly Ser Lys Leu Ala Tyr Val Tyr Gln Asn Asn Ile  
130 135 140

Tyr Leu Lys Gln Arg Pro Gly Asp Pro Pro Phe Gln Ile Thr Tyr Thr  
145 150 155 160

Gly Arg Glu Asn Arg Ile Phe Asn Gly Ile Pro Asp Trp Val Tyr Glu  
165 170 175

Glu Glu Met Leu Ala Thr Lys Tyr Ala Leu Trp Trp Ser Pro Asp Gly  
180 185 190

Lys Phe Leu Ala Tyr Val Glu Phe Asn Asp Ser Asp Ile Pro Ile Ile  
195 200 205

Ala Tyr Ser Tyr Tyr Gly Asp Gly Gln Tyr Pro Arg Thr Ile Asn Ile  
210 215 220

Pro Tyr Pro Lys Ala Gly Ala Lys Asn Pro Val Val Arg Val Phe Ile  
225 230 235 240

Val Asp Thr Thr Tyr Pro His His Val Gly Pro Met Glu Val Pro Val  
245 250 255

eolf-seql (8).txt

Pro Glu Met Ile Ala Ser Ser Asp Tyr Tyr Phe Ser Trp Leu Thr Trp  
260 265 270

Val Ser Ser Glu Arg Val Cys Leu Gln Trp Leu Lys Arg Val Gln Asn  
275 280 285

Val Ser Val Leu Ser Ile Cys Asp Phe Arg Glu Asp Trp His Ala Trp  
290 295 300

Glu Cys Pro Lys Asn Gln Glu His Val Glu Glu Ser Arg Thr Gly Trp  
305 310 315 320

Ala Gly Gly Phe Phe Val Ser Thr Pro Ala Phe Ser Gln Asp Ala Thr  
325 330 335

Ser Tyr Tyr Lys Ile Phe Ser Asp Lys Asp Gly Tyr Lys His Ile His  
340 345 350

Tyr Ile Lys Asp Thr Val Glu Asn Ala Ile Gln Ile Thr Ser Gly Lys  
355 360 365

Trp Glu Ala Ile Tyr Ile Phe Arg Val Thr Gln Asp Ser Leu Phe Tyr  
370 375 380

Ser Ser Asn Glu Phe Glu Gly Tyr Pro Gly Arg Arg Asn Ile Tyr Arg  
385 390 395 400

Ile Ser Ile Gly Asn Ser Pro Pro Ser Lys Lys Cys Val Thr Cys His  
405 410 415

Leu Arg Lys Glu Arg Cys Gln Tyr Tyr Thr Ala Ser Phe Ser Tyr Lys  
420 425 430

Ala Lys Tyr Tyr Ala Leu Val Cys Tyr Gly Pro Gly Leu Pro Ile Ser  
435 440 445

eolf-seql (8).txt

Thr Leu His Asp Gly Arg Thr Asp Gln Glu Ile Gln Val Leu Glu Glu  
450 455 460

Asn Lys Glu Leu Glu Asn Ser Leu Arg Asn Ile Gln Leu Pro Lys Val  
465 470 475 480

Glu Ile Lys Lys Leu Lys Asp Gly Gly Leu Thr Phe Trp Tyr Lys Met  
485 490 495

Ile Leu Pro Pro Gln Phe Asp Arg Ser Lys Lys Tyr Pro Leu Leu Ile  
500 505 510

Gln Val Tyr Gly Gly Pro Cys Ser Gln Ser Val Lys Ser Val Phe Ala  
515 520 525

Val Asn Trp Ile Thr Tyr Leu Ala Ser Lys Glu Gly Ile Val Ile Ala  
530 535 540

Leu Val Asp Gly Arg Gly Thr Ala Phe Gln Gly Asp Lys Phe Leu His  
545 550 555 560

Ala Val Tyr Arg Lys Leu Gly Val Tyr Glu Val Glu Asp Gln Leu Thr  
565 570 575

Ala Val Arg Lys Phe Ile Glu Met Gly Phe Ile Asp Glu Glu Arg Ile  
580 585 590

Ala Ile Trp Gly Trp Ser Tyr Gly Gly Tyr Val Ser Ser Leu Ala Leu  
595 600 605

Ala Ser Gly Thr Gly Leu Phe Lys Cys Gly Ile Ala Val Ala Pro Val  
610 615 620

Ser Ser Trp Glu Tyr Tyr Ala Ser Ile Tyr Ser Glu Arg Phe Met Gly  
625 630 635 640

eolf-seql (8).txt

Leu Pro Thr Lys Asp Asp Asn Leu Glu His Tyr Lys Asn Ser Thr Val  
645 650 655

Met Ala Arg Ala Glu Tyr Phe Arg Asn Val Asp Tyr Leu Leu Ile His  
660 665 670

Gly Thr Ala Asp Asp Asn Val His Phe Gln Asn Ser Ala Gln Ile Ala  
675 680 685

Lys Ala Leu Val Asn Ala Gln Val Asp Phe Gln Ala Met Trp Tyr Ser  
690 695 700

Asp Gln Asn His Gly Ile Leu Ser Gly Arg Ser Gln Asn His Leu Tyr  
705 710 715 720

Thr His Met Thr His Phe Leu Lys Gln Cys Phe Ser Leu Ser Asp Gly  
725 730 735

Lys Lys Lys Lys Lys Lys Gly His His His His His His  
740 745

<210> 124  
<211> 748  
<212> PRT  
<213> Artificial sequence

<220>  
<223> Cynomolgus FAP ectodomain+poly-lys-tag+his6-tag

<400> 124

Arg Pro Pro Arg Val His Asn Ser Glu Glu Asn Thr Met Arg Ala Leu  
1 5 10 15

Thr Leu Lys Asp Ile Leu Asn Gly Thr Phe Ser Tyr Lys Thr Phe Phe  
20 25 30

Pro Asn Trp Ile Ser Gly Gln Glu Tyr Leu His Gln Ser Ala Asp Asn

## eolf-seql (8).txt

35

40

45

Asn Ile Val Leu Tyr Asn Ile Glu Thr Gly Gln Ser Tyr Thr Ile Leu  
 50 55 60

Ser Asn Arg Thr Met Lys Ser Val Asn Ala Ser Asn Tyr Gly Leu Ser  
 65 70 75 80

Pro Asp Arg Gln Phe Val Tyr Leu Glu Ser Asp Tyr Ser Lys Leu Trp  
 85 90 95

Arg Tyr Ser Tyr Thr Ala Thr Tyr Tyr Ile Tyr Asp Leu Ser Asn Gly  
 100 105 110

Glu Phe Val Arg Gly Asn Glu Leu Pro Arg Pro Ile Gln Tyr Leu Cys  
 115 120 125

Trp Ser Pro Val Gly Ser Lys Leu Ala Tyr Val Tyr Gln Asn Asn Ile  
 130 135 140

Tyr Leu Lys Gln Arg Pro Gly Asp Pro Pro Phe Gln Ile Thr Phe Asn  
 145 150 155 160

Gly Arg Glu Asn Lys Ile Phe Asn Gly Ile Pro Asp Trp Val Tyr Glu  
 165 170 175

Glu Glu Met Leu Ala Thr Lys Tyr Ala Leu Trp Trp Ser Pro Asn Gly  
 180 185 190

Lys Phe Leu Ala Tyr Ala Glu Phe Asn Asp Thr Asp Ile Pro Val Ile  
 195 200 205

Ala Tyr Ser Tyr Tyr Gly Asp Glu Gln Tyr Pro Arg Thr Ile Asn Ile  
 210 215 220

Pro Tyr Pro Lys Ala Gly Ala Lys Asn Pro Phe Val Arg Ile Phe Ile

## eolf-seql (8).txt

225                      230                      235                      240  
 Ile Asp Thr Thr Tyr Pro Ala Tyr Val Gly Pro Gln Glu Val Pro Val  
                                  245                      250                      255  
 Pro Ala Met Ile Ala Ser Ser Asp Tyr Tyr Phe Ser Trp Leu Thr Trp  
                                  260                      265                      270  
 Val Thr Asp Glu Arg Val Cys Leu Gln Trp Leu Lys Arg Val Gln Asn  
                                  275                      280                      285  
 Val Ser Val Leu Ser Ile Cys Asp Phe Arg Glu Asp Trp Gln Thr Trp  
                                  290                      295                      300  
 Asp Cys Pro Lys Thr Gln Glu His Ile Glu Glu Ser Arg Thr Gly Trp  
 305                                   310                      315                      320  
 Ala Gly Gly Phe Phe Val Ser Thr Pro Val Phe Ser Tyr Asp Ala Ile  
                                  325                      330                      335  
 Ser Tyr Tyr Lys Ile Phe Ser Asp Lys Asp Gly Tyr Lys His Ile His  
                                  340                      345                      350  
 Tyr Ile Lys Asp Thr Val Glu Asn Ala Ile Gln Ile Thr Ser Gly Lys  
                                  355                      360                      365  
 Trp Glu Ala Ile Asn Ile Phe Arg Val Thr Gln Asp Ser Leu Phe Tyr  
                                  370                      375                      380  
 Ser Ser Asn Glu Phe Glu Asp Tyr Pro Gly Arg Arg Asn Ile Tyr Arg  
 385                                   390                      395                      400  
 Ile Ser Ile Gly Ser Tyr Pro Pro Ser Lys Lys Cys Val Thr Cys His  
                                  405                      410                      415  
 Leu Arg Lys Glu Arg Cys Gln Tyr Tyr Thr Ala Ser Phe Ser Asp Tyr

## eolf-seql (8).txt

420

425

430

Ala Lys Tyr Tyr Ala Leu Val Cys Tyr Gly Pro Gly Ile Pro Ile Ser  
 435 440 445

Thr Leu His Asp Gly Arg Thr Asp Gln Glu Ile Lys Ile Leu Glu Glu  
 450 455 460

Asn Lys Glu Leu Glu Asn Ala Leu Lys Asn Ile Gln Leu Pro Lys Glu  
 465 470 475 480

Glu Ile Lys Lys Leu Glu Val Asp Glu Ile Thr Leu Trp Tyr Lys Met  
 485 490 495

Ile Leu Pro Pro Gln Phe Asp Arg Ser Lys Lys Tyr Pro Leu Leu Ile  
 500 505 510

Gln Val Tyr Gly Gly Pro Cys Ser Gln Ser Val Arg Ser Val Phe Ala  
 515 520 525

Val Asn Trp Ile Ser Tyr Leu Ala Ser Lys Glu Gly Met Val Ile Ala  
 530 535 540

Leu Val Asp Gly Arg Gly Thr Ala Phe Gln Gly Asp Lys Leu Leu Tyr  
 545 550 555 560

Ala Val Tyr Arg Lys Leu Gly Val Tyr Glu Val Glu Asp Gln Ile Thr  
 565 570 575

Ala Val Arg Lys Phe Ile Glu Met Gly Phe Ile Asp Glu Lys Arg Ile  
 580 585 590

Ala Ile Trp Gly Trp Ser Tyr Gly Gly Tyr Val Ser Ser Leu Ala Leu  
 595 600 605

Ala Ser Gly Thr Gly Leu Phe Lys Cys Gly Ile Ala Val Ala Pro Val

eolf-seql (8).txt

610

615

620

Ser Ser Trp Glu Tyr Tyr Ala Ser Val Tyr Thr Glu Arg Phe Met Gly  
625 630 635 640

Leu Pro Thr Lys Asp Asp Asn Leu Glu His Tyr Lys Asn Ser Thr Val  
645 650 655

Met Ala Arg Ala Glu Tyr Phe Arg Asn Val Asp Tyr Leu Leu Ile His  
660 665 670

Gly Thr Ala Asp Asp Asn Val His Phe Gln Asn Ser Ala Gln Ile Ala  
675 680 685

Lys Ala Leu Val Asn Ala Gln Val Asp Phe Gln Ala Met Trp Tyr Ser  
690 695 700

Asp Gln Asn His Gly Leu Ser Gly Leu Ser Thr Asn His Leu Tyr Thr  
705 710 715 720

His Met Thr His Phe Leu Lys Gln Cys Phe Ser Leu Ser Asp Gly Lys  
725 730 735

Lys Lys Lys Lys Lys Gly His His His His His His  
740 745

<210> 125

<211> 702

<212> PRT

<213> Homo sapiens

<400> 125

Met Glu Ser Pro Ser Ala Pro Pro His Arg Trp Cys Ile Pro Trp Gln  
1 5 10 15

Arg Leu Leu Leu Thr Ala Ser Leu Leu Thr Phe Trp Asn Pro Pro Thr  
20 25 30



eolf-seql (8).txt

Thr Ala Lys Leu Thr Ile Glu Ser Thr Pro Phe Asn Val Ala Glu Gly  
35 40 45

Lys Glu Val Leu Leu Leu Val His Asn Leu Pro Gln His Leu Phe Gly  
50 55 60

Tyr Ser Trp Tyr Lys Gly Glu Arg Val Asp Gly Asn Arg Gln Ile Ile  
65 70 75 80

Gly Tyr Val Ile Gly Thr Gln Gln Ala Thr Pro Gly Pro Ala Tyr Ser  
85 90 95

Gly Arg Glu Ile Ile Tyr Pro Asn Ala Ser Leu Leu Ile Gln Asn Ile  
100 105 110

Ile Gln Asn Asp Thr Gly Phe Tyr Thr Leu His Val Ile Lys Ser Asp  
115 120 125

Leu Val Asn Glu Glu Ala Thr Gly Gln Phe Arg Val Tyr Pro Glu Leu  
130 135 140

Pro Lys Pro Ser Ile Ser Ser Asn Asn Ser Lys Pro Val Glu Asp Lys  
145 150 155 160

Asp Ala Val Ala Phe Thr Cys Glu Pro Glu Thr Gln Asp Ala Thr Tyr  
165 170 175

Leu Trp Trp Val Asn Asn Gln Ser Leu Pro Val Ser Pro Arg Leu Gln  
180 185 190

Leu Ser Asn Gly Asn Arg Thr Leu Thr Leu Phe Asn Val Thr Arg Asn  
195 200 205

Asp Thr Ala Ser Tyr Lys Cys Glu Thr Gln Asn Pro Val Ser Ala Arg  
210 215 220

eolf-seql (8).txt

Arg Ser Asp Ser Val Ile Leu Asn Val Leu Tyr Gly Pro Asp Ala Pro  
225 230 235 240

Thr Ile Ser Pro Leu Asn Thr Ser Tyr Arg Ser Gly Glu Asn Leu Asn  
245 250 255

Leu Ser Cys His Ala Ala Ser Asn Pro Pro Ala Gln Tyr Ser Trp Phe  
260 265 270

Val Asn Gly Thr Phe Gln Gln Ser Thr Gln Glu Leu Phe Ile Pro Asn  
275 280 285

Ile Thr Val Asn Asn Ser Gly Ser Tyr Thr Cys Gln Ala His Asn Ser  
290 295 300

Asp Thr Gly Leu Asn Arg Thr Thr Val Thr Thr Ile Thr Val Tyr Ala  
305 310 315 320

Glu Pro Pro Lys Pro Phe Ile Thr Ser Asn Asn Ser Asn Pro Val Glu  
325 330 335

Asp Glu Asp Ala Val Ala Leu Thr Cys Glu Pro Glu Ile Gln Asn Thr  
340 345 350

Thr Tyr Leu Trp Trp Val Asn Asn Gln Ser Leu Pro Val Ser Pro Arg  
355 360 365

Leu Gln Leu Ser Asn Asp Asn Arg Thr Leu Thr Leu Leu Ser Val Thr  
370 375 380

Arg Asn Asp Val Gly Pro Tyr Glu Cys Gly Ile Gln Asn Lys Leu Ser  
385 390 395 400

Val Asp His Ser Asp Pro Val Ile Leu Asn Val Leu Tyr Gly Pro Asp  
405 410 415

eolf-seql (8).txt

Asp Pro Thr Ile Ser Pro Ser Tyr Thr Tyr Tyr Arg Pro Gly Val Asn  
420 425 430

Leu Ser Leu Ser Cys His Ala Ala Ser Asn Pro Pro Ala Gln Tyr Ser  
435 440 445

Trp Leu Ile Asp Gly Asn Ile Gln Gln His Thr Gln Glu Leu Phe Ile  
450 455 460

Ser Asn Ile Thr Glu Lys Asn Ser Gly Leu Tyr Thr Cys Gln Ala Asn  
465 470 475 480

Asn Ser Ala Ser Gly His Ser Arg Thr Thr Val Lys Thr Ile Thr Val  
485 490 495

Ser Ala Glu Leu Pro Lys Pro Ser Ile Ser Ser Asn Asn Ser Lys Pro  
500 505 510

Val Glu Asp Lys Asp Ala Val Ala Phe Thr Cys Glu Pro Glu Ala Gln  
515 520 525

Asn Thr Thr Tyr Leu Trp Trp Val Asn Gly Gln Ser Leu Pro Val Ser  
530 535 540

Pro Arg Leu Gln Leu Ser Asn Gly Asn Arg Thr Leu Thr Leu Phe Asn  
545 550 555 560

Val Thr Arg Asn Asp Ala Arg Ala Tyr Val Cys Gly Ile Gln Asn Ser  
565 570 575

Val Ser Ala Asn Arg Ser Asp Pro Val Thr Leu Asp Val Leu Tyr Gly  
580 585 590

Pro Asp Thr Pro Ile Ile Ser Pro Pro Asp Ser Ser Tyr Leu Ser Gly  
595 600 605

eolf-seql (8).txt

Ala Asn Leu Asn Leu Ser Cys His Ser Ala Ser Asn Pro Ser Pro Gln  
610 615 620

Tyr Ser Trp Arg Ile Asn Gly Ile Pro Gln Gln His Thr Gln Val Leu  
625 630 635 640

Phe Ile Ala Lys Ile Thr Pro Asn Asn Asn Gly Thr Tyr Ala Cys Phe  
645 650 655

Val Ser Asn Leu Ala Thr Gly Arg Asn Asn Ser Ile Val Lys Ser Ile  
660 665 670

Thr Val Ser Ala Ser Gly Thr Ser Pro Gly Leu Ser Ala Gly Ala Thr  
675 680 685

Val Gly Ile Met Ile Gly Val Leu Val Gly Val Ala Leu Ile  
690 695 700

<210> 126  
<211> 257  
<212> PRT  
<213> Homo sapiens

<400> 126

Met Ala Gln Arg Met Thr Thr Gln Leu Leu Leu Leu Val Trp Val  
1 5 10 15

Ala Val Val Gly Glu Ala Gln Thr Arg Ile Ala Trp Ala Arg Thr Glu  
20 25 30

Leu Leu Asn Val Cys Met Asn Ala Lys His His Lys Glu Lys Pro Gly  
35 40 45

Pro Glu Asp Lys Leu His Glu Gln Cys Arg Pro Trp Arg Lys Asn Ala  
50 55 60

eolf-seql (8).txt

Cys Cys Ser Thr Asn Thr Ser Gln Glu Ala His Lys Asp Val Ser Tyr  
65 70 75 80

Leu Tyr Arg Phe Asn Trp Asn His Cys Gly Glu Met Ala Pro Ala Cys  
85 90 95

Lys Arg His Phe Ile Gln Asp Thr Cys Leu Tyr Glu Cys Ser Pro Asn  
100 105 110

Leu Gly Pro Trp Ile Gln Gln Val Asp Gln Ser Trp Arg Lys Glu Arg  
115 120 125

Val Leu Asn Val Pro Leu Cys Lys Glu Asp Cys Glu Gln Trp Trp Glu  
130 135 140

Asp Cys Arg Thr Ser Tyr Thr Cys Lys Ser Asn Trp His Lys Gly Trp  
145 150 155 160

Asn Trp Thr Ser Gly Phe Asn Lys Cys Ala Val Gly Ala Ala Cys Gln  
165 170 175

Pro Phe His Phe Tyr Phe Pro Thr Pro Thr Val Leu Cys Asn Glu Ile  
180 185 190

Trp Thr His Ser Tyr Lys Val Ser Asn Tyr Ser Arg Gly Ser Gly Arg  
195 200 205

Cys Ile Gln Met Trp Phe Asp Pro Ala Gln Gly Asn Pro Asn Glu Glu  
210 215 220

Val Ala Arg Phe Tyr Ala Ala Ala Met Ser Gly Ala Gly Pro Trp Ala  
225 230 235 240

Ala Trp Pro Phe Leu Leu Ser Leu Ala Leu Met Leu Leu Trp Leu Leu  
245 250 255

eolf-seql (8).txt

Ser

<210> 127  
 <211> 255  
 <212> PRT  
 <213> Mus musculus

<400> 127

Met Ala His Leu Met Thr Val Gln Leu Leu Leu Leu Val Met Trp Met  
 1 5 10 15

Ala Glu Cys Ala Gln Ser Arg Ala Thr Arg Ala Arg Thr Glu Leu Leu  
 20 25 30

Asn Val Cys Met Asp Ala Lys His His Lys Glu Lys Pro Gly Pro Glu  
 35 40 45

Asp Asn Leu His Asp Gln Cys Ser Pro Trp Lys Thr Asn Ser Cys Cys  
 50 55 60

Ser Thr Asn Thr Ser Gln Glu Ala His Lys Asp Ile Ser Tyr Leu Tyr  
 65 70 75 80

Arg Phe Asn Trp Asn His Cys Gly Thr Met Thr Ser Glu Cys Lys Arg  
 85 90 95

His Phe Ile Gln Asp Thr Cys Leu Tyr Glu Cys Ser Pro Asn Leu Gly  
 100 105 110

Pro Trp Ile Gln Gln Val Asp Gln Ser Trp Arg Lys Glu Arg Ile Leu  
 115 120 125

Asp Val Pro Leu Cys Lys Glu Asp Cys Gln Gln Trp Trp Glu Asp Cys  
 130 135 140

eolf-seql (8).txt

Gln Ser Ser Phe Thr Cys Lys Ser Asn Trp His Lys Gly Trp Asn Trp  
145 150 155 160

Ser Ser Gly His Asn Glu Cys Pro Val Gly Ala Ser Cys His Pro Phe  
165 170 175

Thr Phe Tyr Phe Pro Thr Ser Ala Ala Leu Cys Glu Glu Ile Trp Ser  
180 185 190

His Ser Tyr Lys Leu Ser Asn Tyr Ser Arg Gly Ser Gly Arg Cys Ile  
195 200 205

Gln Met Trp Phe Asp Pro Ala Gln Gly Asn Pro Asn Glu Glu Val Ala  
210 215 220

Arg Phe Tyr Ala Glu Ala Met Ser Gly Ala Gly Phe His Gly Thr Trp  
225 230 235 240

Pro Leu Leu Cys Ser Leu Ser Leu Val Leu Leu Trp Val Ile Ser  
245 250 255

<210> 128  
<211> 257  
<212> PRT  
<213> Macaca fascicularis

<400> 128

Met Ala Gln Arg Met Thr Thr Gln Leu Leu Leu Leu Val Trp Val  
1 5 10 15

Ala Val Val Gly Glu Ala Gln Thr Arg Thr Ala Arg Ala Arg Thr Glu  
20 25 30

Leu Leu Asn Val Cys Met Asn Ala Lys His His Lys Glu Lys Pro Gly  
35 40 45

Pro Glu Asp Lys Leu His Glu Gln Cys Arg Pro Trp Lys Lys Asn Ala

eolf-seql (8).txt

50

55

60

Cys Cys Ser Thr Asn Thr Ser Gln Glu Ala His Lys Asp Val Ser Tyr  
65 70 75 80

Leu Tyr Arg Phe Asn Trp Asn His Cys Gly Glu Met Ala Pro Ala Cys  
85 90 95

Lys Arg His Phe Ile Gln Asp Thr Cys Leu Tyr Glu Cys Ser Pro Asn  
100 105 110

Leu Gly Pro Trp Ile Gln Gln Val Asp Gln Ser Trp Arg Lys Glu Arg  
115 120 125

Val Leu Asn Val Pro Leu Cys Lys Glu Asp Cys Glu Arg Trp Trp Glu  
130 135 140

Asp Cys Arg Thr Ser Tyr Thr Cys Lys Ser Asn Trp His Lys Gly Trp  
145 150 155 160

Asn Trp Thr Ser Gly Phe Asn Lys Cys Pro Val Gly Ala Ala Cys Gln  
165 170 175

Pro Phe His Phe Tyr Phe Pro Thr Pro Thr Val Leu Cys Asn Glu Ile  
180 185 190

Trp Thr Tyr Ser Tyr Lys Val Ser Asn Tyr Ser Arg Gly Ser Gly Arg  
195 200 205

Cys Ile Gln Met Trp Phe Asp Pro Ala Gln Gly Asn Pro Asn Glu Glu  
210 215 220

Val Ala Arg Phe Tyr Ala Ala Ala Met Ser Gly Ala Gly Pro Trp Ala  
225 230 235 240

Ala Trp Pro Leu Leu Leu Ser Leu Ala Leu Thr Leu Leu Trp Leu Leu



Ser

<210> 129  
 <211> 2322  
 <212> PRT  
 <213> Homo sapiens

<400> 129

Met Gln Ser Gly Pro Arg Pro Pro Leu Pro Ala Pro Gly Leu Ala Leu  
 1 5 10 15

Ala Leu Thr Leu Thr Met Leu Ala Arg Leu Ala Ser Ala Ala Ser Phe  
 20 25 30

Phe Gly Glu Asn His Leu Glu Val Pro Val Ala Thr Ala Leu Thr Asp  
 35 40 45

Ile Asp Leu Gln Leu Gln Phe Ser Thr Ser Gln Pro Glu Ala Leu Leu  
 50 55 60

Leu Leu Ala Ala Gly Pro Ala Asp His Leu Leu Leu Gln Leu Tyr Ser  
 65 70 75 80

Gly Arg Leu Gln Val Arg Leu Val Leu Gly Gln Glu Glu Leu Arg Leu  
 85 90 95

Gln Thr Pro Ala Glu Thr Leu Leu Ser Asp Ser Ile Pro His Thr Val  
 100 105 110

Val Leu Thr Val Val Glu Gly Trp Ala Thr Leu Ser Val Asp Gly Phe  
 115 120 125

Leu Asn Ala Ser Ser Ala Val Pro Gly Ala Pro Leu Glu Val Pro Tyr  
 130 135 140

eolf-seql (8).txt

Gly Leu Phe Val Gly Gly Thr Gly Thr Leu Gly Leu Pro Tyr Leu Arg  
145 150 155 160

Gly Thr Ser Arg Pro Leu Arg Gly Cys Leu His Ala Ala Thr Leu Asn  
165 170 175

Gly Arg Ser Leu Leu Arg Pro Leu Thr Pro Asp Val His Glu Gly Cys  
180 185 190

Ala Glu Glu Phe Ser Ala Ser Asp Asp Val Ala Leu Gly Phe Ser Gly  
195 200 205

Pro His Ser Leu Ala Ala Phe Pro Ala Trp Gly Thr Gln Asp Glu Gly  
210 215 220

Thr Leu Glu Phe Thr Leu Thr Thr Gln Ser Arg Gln Ala Pro Leu Ala  
225 230 235 240

Phe Gln Ala Gly Gly Arg Arg Gly Asp Phe Ile Tyr Val Asp Ile Phe  
245 250 255

Glu Gly His Leu Arg Ala Val Val Glu Lys Gly Gln Gly Thr Val Leu  
260 265 270

Leu His Asn Ser Val Pro Val Ala Asp Gly Gln Pro His Glu Val Ser  
275 280 285

Val His Ile Asn Ala His Arg Leu Glu Ile Ser Val Asp Gln Tyr Pro  
290 295 300

Thr His Thr Ser Asn Arg Gly Val Leu Ser Tyr Leu Glu Pro Arg Gly  
305 310 315 320

Ser Leu Leu Leu Gly Gly Leu Asp Ala Glu Ala Ser Arg His Leu Gln  
325 330 335

eolf-seql (8).txt

Glu His Arg Leu Gly Leu Thr Pro Glu Ala Thr Asn Ala Ser Leu Leu  
340 345 350

Gly Cys Met Glu Asp Leu Ser Val Asn Gly Gln Arg Arg Gly Leu Arg  
355 360 365

Glu Ala Leu Leu Thr Arg Asn Met Ala Ala Gly Cys Arg Leu Glu Glu  
370 375 380

Glu Glu Tyr Glu Asp Asp Ala Tyr Gly His Tyr Glu Ala Phe Ser Thr  
385 390 395 400

Leu Ala Pro Glu Ala Trp Pro Ala Met Glu Leu Pro Glu Pro Cys Val  
405 410 415

Pro Glu Pro Gly Leu Pro Pro Val Phe Ala Asn Phe Thr Gln Leu Leu  
420 425 430

Thr Ile Ser Pro Leu Val Val Ala Glu Gly Gly Thr Ala Trp Leu Glu  
435 440 445

Trp Arg His Val Gln Pro Thr Leu Asp Leu Met Glu Ala Glu Leu Arg  
450 455 460

Lys Ser Gln Val Leu Phe Ser Val Thr Arg Gly Ala Arg His Gly Glu  
465 470 475 480

Leu Glu Leu Asp Ile Pro Gly Ala Gln Ala Arg Lys Met Phe Thr Leu  
485 490 495

Leu Asp Val Val Asn Arg Lys Ala Arg Phe Ile His Asp Gly Ser Glu  
500 505 510

Asp Thr Ser Asp Gln Leu Val Leu Glu Val Ser Val Thr Ala Arg Val  
515 520 525

eolf-seql (8).txt

Pro Met Pro Ser Cys Leu Arg Arg Gly Gln Thr Tyr Leu Leu Pro Ile  
530 535 540

Gln Val Asn Pro Val Asn Asp Pro Pro His Ile Ile Phe Pro His Gly  
545 550 555 560

Ser Leu Met Val Ile Leu Glu His Thr Gln Lys Pro Leu Gly Pro Glu  
565 570 575

Val Phe Gln Ala Tyr Asp Pro Asp Ser Ala Cys Glu Gly Leu Thr Phe  
580 585 590

Gln Val Leu Gly Thr Ser Ser Gly Leu Pro Val Glu Arg Arg Asp Gln  
595 600 605

Pro Gly Glu Pro Ala Thr Glu Phe Ser Cys Arg Glu Leu Glu Ala Gly  
610 615 620

Ser Leu Val Tyr Val His Arg Gly Gly Pro Ala Gln Asp Leu Thr Phe  
625 630 635 640

Arg Val Ser Asp Gly Leu Gln Ala Ser Pro Pro Ala Thr Leu Lys Val  
645 650 655

Val Ala Ile Arg Pro Ala Ile Gln Ile His Arg Ser Thr Gly Leu Arg  
660 665 670

Leu Ala Gln Gly Ser Ala Met Pro Ile Leu Pro Ala Asn Leu Ser Val  
675 680 685

Glu Thr Asn Ala Val Gly Gln Asp Val Ser Val Leu Phe Arg Val Thr  
690 695 700

Gly Ala Leu Gln Phe Gly Glu Leu Gln Lys Gln Gly Ala Gly Gly Val  
705 710 715 720

eolf-seql (8).txt

Glu Gly Ala Glu Trp Trp Ala Thr Gln Ala Phe His Gln Arg Asp Val  
725 730 735

Glu Gln Gly Arg Val Arg Tyr Leu Ser Thr Asp Pro Gln His His Ala  
740 745 750

Tyr Asp Thr Val Glu Asn Leu Ala Leu Glu Val Gln Val Gly Gln Glu  
755 760 765

Ile Leu Ser Asn Leu Ser Phe Pro Val Thr Ile Gln Arg Ala Thr Val  
770 775 780

Trp Met Leu Arg Leu Glu Pro Leu His Thr Gln Asn Thr Gln Gln Glu  
785 790 795 800

Thr Leu Thr Thr Ala His Leu Glu Ala Thr Leu Glu Glu Ala Gly Pro  
805 810 815

Ser Pro Pro Thr Phe His Tyr Glu Val Val Gln Ala Pro Arg Lys Gly  
820 825 830

Asn Leu Gln Leu Gln Gly Thr Arg Leu Ser Asp Gly Gln Gly Phe Thr  
835 840 845

Gln Asp Asp Ile Gln Ala Gly Arg Val Thr Tyr Gly Ala Thr Ala Arg  
850 855 860

Ala Ser Glu Ala Val Glu Asp Thr Phe Arg Phe Arg Val Thr Ala Pro  
865 870 875 880

Pro Tyr Phe Ser Pro Leu Tyr Thr Phe Pro Ile His Ile Gly Gly Asp  
885 890 895

Pro Asp Ala Pro Val Leu Thr Asn Val Leu Leu Val Val Pro Glu Gly  
900 905 910

eolf-seql (8).txt

Gly Glu Gly Val Leu Ser Ala Asp His Leu Phe Val Lys Ser Leu Asn  
 915 920 925

Ser Ala Ser Tyr Leu Tyr Glu Val Met Glu Arg Pro Arg His Gly Arg  
 930 935 940

Leu Ala Trp Arg Gly Thr Gln Asp Lys Thr Thr Met Val Thr Ser Phe  
 945 950 955 960

Thr Asn Glu Asp Leu Leu Arg Gly Arg Leu Val Tyr Gln His Asp Asp  
 965 970 975

Ser Glu Thr Thr Glu Asp Asp Ile Pro Phe Val Ala Thr Arg Gln Gly  
 980 985 990

Glu Ser Ser Gly Asp Met Ala Trp Glu Glu Val Arg Gly Val Phe Arg  
 995 1000 1005

Val Ala Ile Gln Pro Val Asn Asp His Ala Pro Val Gln Thr Ile  
 1010 1015 1020

Ser Arg Ile Phe His Val Ala Arg Gly Gly Arg Arg Leu Leu Thr  
 1025 1030 1035

Thr Asp Asp Val Ala Phe Ser Asp Ala Asp Ser Gly Phe Ala Asp  
 1040 1045 1050

Ala Gln Leu Val Leu Thr Arg Lys Asp Leu Leu Phe Gly Ser Ile  
 1055 1060 1065

Val Ala Val Asp Glu Pro Thr Arg Pro Ile Tyr Arg Phe Thr Gln  
 1070 1075 1080

Glu Asp Leu Arg Lys Arg Arg Val Leu Phe Val His Ser Gly Ala  
 1085 1090 1095

eolf-seql (8).txt

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Asp  | Arg | Gly | Trp | Ile | Gln | Leu  | Gln | Val | Ser | Asp | Gly  | Gln | His | Gln |
| 1100 |     |     |     |     |     | 1105 |     |     |     |     | 1110 |     |     |     |
| Ala  | Thr | Ala | Leu | Leu | Glu | Val  | Gln | Ala | Ser | Glu | Pro  | Tyr | Leu | Arg |
| 1115 |     |     |     |     |     | 1120 |     |     |     |     | 1125 |     |     |     |
| Val  | Ala | Asn | Gly | Ser | Ser | Leu  | Val | Val | Pro | Gln | Gly  | Gly | Gln | Gly |
| 1130 |     |     |     |     |     | 1135 |     |     |     |     | 1140 |     |     |     |
| Thr  | Ile | Asp | Thr | Ala | Val | Leu  | His | Leu | Asp | Thr | Asn  | Leu | Asp | Ile |
| 1145 |     |     |     |     |     | 1150 |     |     |     |     | 1155 |     |     |     |
| Arg  | Ser | Gly | Asp | Glu | Val | His  | Tyr | His | Val | Thr | Ala  | Gly | Pro | Arg |
| 1160 |     |     |     |     |     | 1165 |     |     |     |     | 1170 |     |     |     |
| Trp  | Gly | Gln | Leu | Val | Arg | Ala  | Gly | Gln | Pro | Ala | Thr  | Ala | Phe | Ser |
| 1175 |     |     |     |     |     | 1180 |     |     |     |     | 1185 |     |     |     |
| Gln  | Gln | Asp | Leu | Leu | Asp | Gly  | Ala | Val | Leu | Tyr | Ser  | His | Asn | Gly |
| 1190 |     |     |     |     |     | 1195 |     |     |     |     | 1200 |     |     |     |
| Ser  | Leu | Ser | Pro | Arg | Asp | Thr  | Met | Ala | Phe | Ser | Val  | Glu | Ala | Gly |
| 1205 |     |     |     |     |     | 1210 |     |     |     |     | 1215 |     |     |     |
| Pro  | Val | His | Thr | Asp | Ala | Thr  | Leu | Gln | Val | Thr | Ile  | Ala | Leu | Glu |
| 1220 |     |     |     |     |     | 1225 |     |     |     |     | 1230 |     |     |     |
| Gly  | Pro | Leu | Ala | Pro | Leu | Lys  | Leu | Val | Arg | His | Lys  | Lys | Ile | Tyr |
| 1235 |     |     |     |     |     | 1240 |     |     |     |     | 1245 |     |     |     |
| Val  | Phe | Gln | Gly | Glu | Ala | Ala  | Glu | Ile | Arg | Arg | Asp  | Gln | Leu | Glu |
| 1250 |     |     |     |     |     | 1255 |     |     |     |     | 1260 |     |     |     |
| Ala  | Ala | Gln | Glu | Ala | Val | Pro  | Pro | Ala | Asp | Ile | Val  | Phe | Ser | Val |
| 1265 |     |     |     |     |     | 1270 |     |     |     |     | 1275 |     |     |     |

eolf-seql (8).txt

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Lys  | Ser | Pro | Pro | Ser | Ala | Gly  | Tyr | Leu | Val | Met | Val  | Ser | Arg | Gly |
| 1280 |     |     |     |     |     | 1285 |     |     |     |     | 1290 |     |     |     |
| Ala  | Leu | Ala | Asp | Glu | Pro | Pro  | Ser | Leu | Asp | Pro | Val  | Gln | Ser | Phe |
| 1295 |     |     |     |     |     | 1300 |     |     |     |     | 1305 |     |     |     |
| Ser  | Gln | Glu | Ala | Val | Asp | Thr  | Gly | Arg | Val | Leu | Tyr  | Leu | His | Ser |
| 1310 |     |     |     |     |     | 1315 |     |     |     |     | 1320 |     |     |     |
| Arg  | Pro | Glu | Ala | Trp | Ser | Asp  | Ala | Phe | Ser | Leu | Asp  | Val | Ala | Ser |
| 1325 |     |     |     |     |     | 1330 |     |     |     |     | 1335 |     |     |     |
| Gly  | Leu | Gly | Ala | Pro | Leu | Glu  | Gly | Val | Leu | Val | Glu  | Leu | Glu | Val |
| 1340 |     |     |     |     |     | 1345 |     |     |     |     | 1350 |     |     |     |
| Leu  | Pro | Ala | Ala | Ile | Pro | Leu  | Glu | Ala | Gln | Asn | Phe  | Ser | Val | Pro |
| 1355 |     |     |     |     |     | 1360 |     |     |     |     | 1365 |     |     |     |
| Glu  | Gly | Gly | Ser | Leu | Thr | Leu  | Ala | Pro | Pro | Leu | Leu  | Arg | Val | Ser |
| 1370 |     |     |     |     |     | 1375 |     |     |     |     | 1380 |     |     |     |
| Gly  | Pro | Tyr | Phe | Pro | Thr | Leu  | Leu | Gly | Leu | Ser | Leu  | Gln | Val | Leu |
| 1385 |     |     |     |     |     | 1390 |     |     |     |     | 1395 |     |     |     |
| Glu  | Pro | Pro | Gln | His | Gly | Ala  | Leu | Gln | Lys | Glu | Asp  | Gly | Pro | Gln |
| 1400 |     |     |     |     |     | 1405 |     |     |     |     | 1410 |     |     |     |
| Ala  | Arg | Thr | Leu | Ser | Ala | Phe  | Ser | Trp | Arg | Met | Val  | Glu | Glu | Gln |
| 1415 |     |     |     |     |     | 1420 |     |     |     |     | 1425 |     |     |     |
| Leu  | Ile | Arg | Tyr | Val | His | Asp  | Gly | Ser | Glu | Thr | Leu  | Thr | Asp | Ser |
| 1430 |     |     |     |     |     | 1435 |     |     |     |     | 1440 |     |     |     |
| Phe  | Val | Leu | Met | Ala | Asn | Ala  | Ser | Glu | Met | Asp | Arg  | Gln | Ser | His |
| 1445 |     |     |     |     |     | 1450 |     |     |     |     | 1455 |     |     |     |



eolf-seql (8).txt

Pro Val Ala Phe Thr Val Thr Val Leu Pro Val Asn Asp Gln Pro  
1460 1465 1470

Pro Ile Leu Thr Thr Asn Thr Gly Leu Gln Met Trp Glu Gly Ala  
1475 1480 1485

Thr Ala Pro Ile Pro Ala Glu Ala Leu Arg Ser Thr Asp Gly Asp  
1490 1495 1500

Ser Gly Ser Glu Asp Leu Val Tyr Thr Ile Glu Gln Pro Ser Asn  
1505 1510 1515

Gly Arg Val Val Leu Arg Gly Ala Pro Gly Thr Glu Val Arg Ser  
1520 1525 1530

Phe Thr Gln Ala Gln Leu Asp Gly Gly Leu Val Leu Phe Ser His  
1535 1540 1545

Arg Gly Thr Leu Asp Gly Gly Phe Arg Phe Arg Leu Ser Asp Gly  
1550 1555 1560

Glu His Thr Ser Pro Gly His Phe Phe Arg Val Thr Ala Gln Lys  
1565 1570 1575

Gln Val Leu Leu Ser Leu Lys Gly Ser Gln Thr Leu Thr Val Cys  
1580 1585 1590

Pro Gly Ser Val Gln Pro Leu Ser Ser Gln Thr Leu Arg Ala Ser  
1595 1600 1605

Ser Ser Ala Gly Thr Asp Pro Gln Leu Leu Leu Tyr Arg Val Val  
1610 1615 1620

Arg Gly Pro Gln Leu Gly Arg Leu Phe His Ala Gln Gln Asp Ser  
1625 1630 1635

eolf-seql (8).txt

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Thr  | Gly | Glu | Ala | Leu | Val | Asn  | Phe | Thr | Gln | Ala | Glu  | Val | Tyr | Ala |
| 1640 |     |     |     |     |     | 1645 |     |     |     |     | 1650 |     |     |     |
| Gly  | Asn | Ile | Leu | Tyr | Glu | His  | Glu | Met | Pro | Pro | Glu  | Pro | Phe | Trp |
| 1655 |     |     |     |     |     | 1660 |     |     |     |     | 1665 |     |     |     |
| Glu  | Ala | His | Asp | Thr | Leu | Glu  | Leu | Gln | Leu | Ser | Ser  | Pro | Pro | Ala |
| 1670 |     |     |     |     |     | 1675 |     |     |     |     | 1680 |     |     |     |
| Arg  | Asp | Val | Ala | Ala | Thr | Leu  | Ala | Val | Ala | Val | Ser  | Phe | Glu | Ala |
| 1685 |     |     |     |     |     | 1690 |     |     |     |     | 1695 |     |     |     |
| Ala  | Cys | Pro | Gln | Arg | Pro | Ser  | His | Leu | Trp | Lys | Asn  | Lys | Gly | Leu |
| 1700 |     |     |     |     |     | 1705 |     |     |     |     | 1710 |     |     |     |
| Trp  | Val | Pro | Glu | Gly | Gln | Arg  | Ala | Arg | Ile | Thr | Val  | Ala | Ala | Leu |
| 1715 |     |     |     |     |     | 1720 |     |     |     |     | 1725 |     |     |     |
| Asp  | Ala | Ser | Asn | Leu | Leu | Ala  | Ser | Val | Pro | Ser | Pro  | Gln | Arg | Ser |
| 1730 |     |     |     |     |     | 1735 |     |     |     |     | 1740 |     |     |     |
| Glu  | His | Asp | Val | Leu | Phe | Gln  | Val | Thr | Gln | Phe | Pro  | Ser | Arg | Gly |
| 1745 |     |     |     |     |     | 1750 |     |     |     |     | 1755 |     |     |     |
| Gln  | Leu | Leu | Val | Ser | Glu | Glu  | Pro | Leu | His | Ala | Gly  | Gln | Pro | His |
| 1760 |     |     |     |     |     | 1765 |     |     |     |     | 1770 |     |     |     |
| Phe  | Leu | Gln | Ser | Gln | Leu | Ala  | Ala | Gly | Gln | Leu | Val  | Tyr | Ala | His |
| 1775 |     |     |     |     |     | 1780 |     |     |     |     | 1785 |     |     |     |
| Gly  | Gly | Gly | Gly | Thr | Gln | Gln  | Asp | Gly | Phe | His | Phe  | Arg | Ala | His |
| 1790 |     |     |     |     |     | 1795 |     |     |     |     | 1800 |     |     |     |
| Leu  | Gln | Gly | Pro | Ala | Gly | Ala  | Ser | Val | Ala | Gly | Pro  | Gln | Thr | Ser |
| 1805 |     |     |     |     |     | 1810 |     |     |     |     | 1815 |     |     |     |

eolf-seql (8).txt

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Glu  | Ala | Phe | Ala | Ile | Thr | Val  | Arg | Asp | Val | Asn | Glu  | Arg | Pro | Pro |
| 1820 |     |     |     |     |     | 1825 |     |     |     |     | 1830 |     |     |     |
| Gln  | Pro | Gln | Ala | Ser | Val | Pro  | Leu | Arg | Leu | Thr | Arg  | Gly | Ser | Arg |
| 1835 |     |     |     |     |     | 1840 |     |     |     |     | 1845 |     |     |     |
| Ala  | Pro | Ile | Ser | Arg | Ala | Gln  | Leu | Ser | Val | Val | Asp  | Pro | Asp | Ser |
| 1850 |     |     |     |     |     | 1855 |     |     |     |     | 1860 |     |     |     |
| Ala  | Pro | Gly | Glu | Ile | Glu | Tyr  | Glu | Val | Gln | Arg | Ala  | Pro | His | Asn |
| 1865 |     |     |     |     |     | 1870 |     |     |     |     | 1875 |     |     |     |
| Gly  | Phe | Leu | Ser | Leu | Val | Gly  | Gly | Gly | Leu | Gly | Pro  | Val | Thr | Arg |
| 1880 |     |     |     |     |     | 1885 |     |     |     |     | 1890 |     |     |     |
| Phe  | Thr | Gln | Ala | Asp | Val | Asp  | Ser | Gly | Arg | Leu | Ala  | Phe | Val | Ala |
| 1895 |     |     |     |     |     | 1900 |     |     |     |     | 1905 |     |     |     |
| Asn  | Gly | Ser | Ser | Val | Ala | Gly  | Ile | Phe | Gln | Leu | Ser  | Met | Ser | Asp |
| 1910 |     |     |     |     |     | 1915 |     |     |     |     | 1920 |     |     |     |
| Gly  | Ala | Ser | Pro | Pro | Leu | Pro  | Met | Ser | Leu | Ala | Val  | Asp | Ile | Leu |
| 1925 |     |     |     |     |     | 1930 |     |     |     |     | 1935 |     |     |     |
| Pro  | Ser | Ala | Ile | Glu | Val | Gln  | Leu | Arg | Ala | Pro | Leu  | Glu | Val | Pro |
| 1940 |     |     |     |     |     | 1945 |     |     |     |     | 1950 |     |     |     |
| Gln  | Ala | Leu | Gly | Arg | Ser | Ser  | Leu | Ser | Gln | Gln | Gln  | Leu | Arg | Val |
| 1955 |     |     |     |     |     | 1960 |     |     |     |     | 1965 |     |     |     |
| Val  | Ser | Asp | Arg | Glu | Glu | Pro  | Glu | Ala | Ala | Tyr | Arg  | Leu | Ile | Gln |
| 1970 |     |     |     |     |     | 1975 |     |     |     |     | 1980 |     |     |     |
| Gly  | Pro | Gln | Tyr | Gly | His | Leu  | Leu | Val | Gly | Gly | Arg  | Pro | Thr | Ser |
| 1985 |     |     |     |     |     | 1990 |     |     |     |     | 1995 |     |     |     |

eolf-seql (8).txt

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ala  | Phe | Ser | Gln | Phe | Gln | Ile  | Asp | Gln | Gly | Glu | Val  | Val | Phe | Ala |
| 2000 |     |     |     |     |     | 2005 |     |     |     |     | 2010 |     |     |     |
| Phe  | Thr | Asn | Phe | Ser | Ser | Ser  | His | Asp | His | Phe | Arg  | Val | Leu | Ala |
| 2015 |     |     |     |     |     | 2020 |     |     |     |     | 2025 |     |     |     |
| Leu  | Ala | Arg | Gly | Val | Asn | Ala  | Ser | Ala | Val | Val | Asn  | Val | Thr | Val |
| 2030 |     |     |     |     |     | 2035 |     |     |     |     | 2040 |     |     |     |
| Arg  | Ala | Leu | Leu | His | Val | Trp  | Ala | Gly | Gly | Pro | Trp  | Pro | Gln | Gly |
| 2045 |     |     |     |     |     | 2050 |     |     |     |     | 2055 |     |     |     |
| Ala  | Thr | Leu | Arg | Leu | Asp | Pro  | Thr | Val | Leu | Asp | Ala  | Gly | Glu | Leu |
| 2060 |     |     |     |     |     | 2065 |     |     |     |     | 2070 |     |     |     |
| Ala  | Asn | Arg | Thr | Gly | Ser | Val  | Pro | Arg | Phe | Arg | Leu  | Leu | Glu | Gly |
| 2075 |     |     |     |     |     | 2080 |     |     |     |     | 2085 |     |     |     |
| Pro  | Arg | His | Gly | Arg | Val | Val  | Arg | Val | Pro | Arg | Ala  | Arg | Thr | Glu |
| 2090 |     |     |     |     |     | 2095 |     |     |     |     | 2100 |     |     |     |
| Pro  | Gly | Gly | Ser | Gln | Leu | Val  | Glu | Gln | Phe | Thr | Gln  | Gln | Asp | Leu |
| 2105 |     |     |     |     |     | 2110 |     |     |     |     | 2115 |     |     |     |
| Glu  | Asp | Gly | Arg | Leu | Gly | Leu  | Glu | Val | Gly | Arg | Pro  | Glu | Gly | Arg |
| 2120 |     |     |     |     |     | 2125 |     |     |     |     | 2130 |     |     |     |
| Ala  | Pro | Gly | Pro | Ala | Gly | Asp  | Ser | Leu | Thr | Leu | Glu  | Leu | Trp | Ala |
| 2135 |     |     |     |     |     | 2140 |     |     |     |     | 2145 |     |     |     |
| Gln  | Gly | Val | Pro | Pro | Ala | Val  | Ala | Ser | Leu | Asp | Phe  | Ala | Thr | Glu |
| 2150 |     |     |     |     |     | 2155 |     |     |     |     | 2160 |     |     |     |
| Pro  | Tyr | Asn | Ala | Ala | Arg | Pro  | Tyr | Ser | Val | Ala | Leu  | Leu | Ser | Val |
| 2165 |     |     |     |     |     | 2170 |     |     |     |     | 2175 |     |     |     |

eolf-seql (8).txt

Pro Glu Ala Ala Arg Thr Glu Ala Gly Lys Pro Glu Ser Ser Thr  
2180 2185 2190

Pro Thr Gly Glu Pro Gly Pro Met Ala Ser Ser Pro Glu Pro Ala  
2195 2200 2205

Val Ala Lys Gly Gly Phe Leu Ser Phe Leu Glu Ala Asn Met Phe  
2210 2215 2220

Ser Val Ile Ile Pro Met Cys Leu Val Leu Leu Leu Leu Ala Leu  
2225 2230 2235

Ile Leu Pro Leu Leu Phe Tyr Leu Arg Lys Arg Asn Lys Thr Gly  
2240 2245 2250

Lys His Asp Val Gln Val Leu Thr Ala Lys Pro Arg Asn Gly Leu  
2255 2260 2265

Ala Gly Asp Thr Glu Thr Phe Arg Lys Val Glu Pro Gly Gln Ala  
2270 2275 2280

Ile Pro Leu Thr Ala Val Pro Gly Gln Gly Pro Pro Pro Gly Gly  
2285 2290 2295

Gln Pro Asp Pro Glu Leu Leu Gln Phe Cys Arg Thr Pro Asn Pro  
2300 2305 2310

Ala Leu Lys Asn Gly Gln Tyr Trp Val  
2315 2320

<210> 130

<211> 207

<212> PRT

<213> Homo sapiens

<400> 130

eolf-seql (8).txt

Met Gln Ser Gly Thr His Trp Arg Val Leu Gly Leu Cys Leu Leu Ser  
1 5 10 15

Val Gly Val Trp Gly Gln Asp Gly Asn Glu Glu Met Gly Gly Ile Thr  
20 25 30

Gln Thr Pro Tyr Lys Val Ser Ile Ser Gly Thr Thr Val Ile Leu Thr  
35 40 45

Cys Pro Gln Tyr Pro Gly Ser Glu Ile Leu Trp Gln His Asn Asp Lys  
50 55 60

Asn Ile Gly Gly Asp Glu Asp Asp Lys Asn Ile Gly Ser Asp Glu Asp  
65 70 75 80

His Leu Ser Leu Lys Glu Phe Ser Glu Leu Glu Gln Ser Gly Tyr Tyr  
85 90 95

Val Cys Tyr Pro Arg Gly Ser Lys Pro Glu Asp Ala Asn Phe Tyr Leu  
100 105 110

Tyr Leu Arg Ala Arg Val Cys Glu Asn Cys Met Glu Met Asp Val Met  
115 120 125

Ser Val Ala Thr Ile Val Ile Val Asp Ile Cys Ile Thr Gly Gly Leu  
130 135 140

Leu Leu Leu Val Tyr Tyr Trp Ser Lys Asn Arg Lys Ala Lys Ala Lys  
145 150 155 160

Pro Val Thr Arg Gly Ala Gly Ala Gly Gly Arg Gln Arg Gly Gln Asn  
165 170 175

Lys Glu Arg Pro Pro Pro Val Pro Asn Pro Asp Tyr Glu Pro Ile Arg  
180 185 190

eolf-seql (8).txt

Lys Gly Gln Arg Asp Leu Tyr Ser Gly Leu Asn Gln Arg Arg Ile  
 195 200 205

<210> 131  
 <211> 198  
 <212> PRT  
 <213> Macacis fascularis

<400> 131

Met Gln Ser Gly Thr Arg Trp Arg Val Leu Gly Leu Cys Leu Leu Ser  
 1 5 10 15

Ile Gly Val Trp Gly Gln Asp Gly Asn Glu Glu Met Gly Ser Ile Thr  
 20 25 30

Gln Thr Pro Tyr Gln Val Ser Ile Ser Gly Thr Thr Val Ile Leu Thr  
 35 40 45

Cys Ser Gln His Leu Gly Ser Glu Ala Gln Trp Gln His Asn Gly Lys  
 50 55 60

Asn Lys Glu Asp Ser Gly Asp Arg Leu Phe Leu Pro Glu Phe Ser Glu  
 65 70 75 80

Met Glu Gln Ser Gly Tyr Tyr Val Cys Tyr Pro Arg Gly Ser Asn Pro  
 85 90 95

Glu Asp Ala Ser His His Leu Tyr Leu Lys Ala Arg Val Cys Glu Asn  
 100 105 110

Cys Met Glu Met Asp Val Met Ala Val Ala Thr Ile Val Ile Val Asp  
 115 120 125

Ile Cys Ile Thr Leu Gly Leu Leu Leu Leu Val Tyr Tyr Trp Ser Lys  
 130 135 140

eolf-seql (8).txt

Asn Arg Lys Ala Lys Ala Lys Pro Val Thr Arg Gly Ala Gly Ala Gly  
145 150 155 160

Gly Arg Gln Arg Gly Gln Asn Lys Glu Arg Pro Pro Pro Val Pro Asn  
165 170 175

Pro Asp Tyr Glu Pro Ile Arg Lys Gly Gln Gln Asp Leu Tyr Ser Gly  
180 185 190

Leu Asn Gln Arg Arg Ile  
195

<210> 132  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> G4S peptide linker

<400> 132

Gly Gly Gly Gly Ser  
1 5

<210> 133  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> (G4S)2

<400> 133

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
1 5 10

<210> 134  
<211> 10  
<212> PRT  
<213> Artificial Sequence



eolf-seql (8).txt

<220>

<223> (SG4)2

<400> 134

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly  
1 5 10

<210> 135

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> peptide linker

<400> 135

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly  
1 5 10

<210> 136

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> peptide linker

<400> 136

Gly Ser Pro Gly Ser Ser Ser Ser Gly Ser  
1 5 10

<210> 137

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> peptide linker 2

<400> 137

eolf-seql (8).txt

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
1 5 10 15

<210> 138  
<211> 20  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> peptide linker 3

<400> 138

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly  
1 5 10 15

Gly Gly Gly Ser  
20

<210> 139  
<211> 8  
<212> PRT  
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<220>  
<223> peptide linker 4

<400> 139

Gly Ser Gly Ser Gly Ser Gly Ser  
1 5

<210> 140  
<211> 8  
<212> PRT  
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<220>  
<223> peptide linker 5

<400> 140

Gly Ser Gly Ser Gly Asn Gly Ser  
1 5

<210> 141  
<211> 8  
<212> PRT  
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<220>  
<223> peptide linker 6

<400> 141

Gly Gly Ser Gly Ser Gly Ser Gly  
1 5

<210> 142  
<211> 6  
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<220>  
<223> peptide linker 7

<400> 142

Gly Gly Ser Gly Ser Gly  
1 5

<210> 143  
<211> 4  
<212> PRT  
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<220>  
<223> peptide linker 8

<400> 143

Gly Gly Ser Gly  
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<210> 144  
<211> 8  
<212> PRT  
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<220>

<223> peptide linker 9

<400> 144

Gly Gly Ser Gly Asn Gly Ser Gly  
1 5

<210> 145

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> peptide linker 10

<400> 145

Gly Gly Asn Gly Ser Gly Ser Gly  
1 5

<210> 146

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> peptide linker 11

<400> 146

Gly Gly Asn Gly Ser Gly  
1 5