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(54) Title: COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

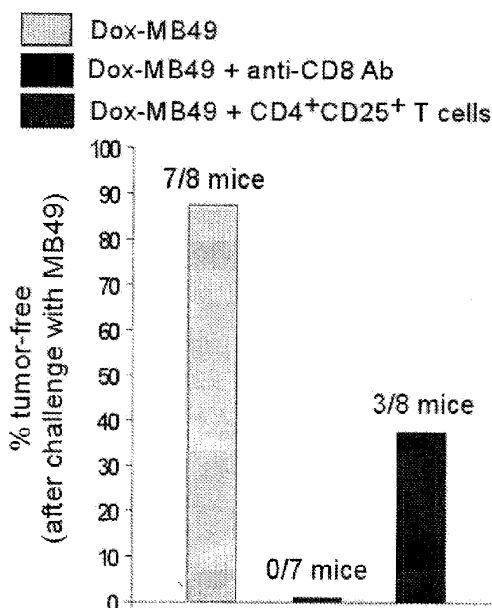


Fig. 53G

(57) Abstract: The present invention is based on the seminal discovery that targeted immunomodulatory antibodies and fusion proteins can counter act or reverse immune tolerance of cancer cells. Cancer cells are able to escape elimination by chemotherapeutic agents or tumor-targeted antibodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. Such immunosuppressive mechanisms include immunosuppressive cytokines (for example, Transforming growth factor beta (TGF- $\beta$ )) and regulatory T cells and/or immunosuppressive myeloid dendritic cells (DCs). By counteracting tumor-induced immune tolerance, the present invention provides effective compositions and methods for cancer treatment, optional in combination with another existing cancer treatment. The present invention provides strategies to counteract tumor-induced immune tolerance and enhance the antitumor efficacy of chemotherapy by activating and leveraging T cell-mediated adaptive antitumor immunity against resistant or disseminated cancer cells.



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## **COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS**

### **BACKGROUND OF THE INVENTION**

#### **FIELD OF THE INVENTION**

[0001] The present invention relates generally to the field of targeted immunomodulatory antibodies and fusion proteins for cancer therapy and more specifically to composition and methods for targeted immunostimulatory or immunosuppressive antibodies and fusion proteins to counteract or induce immune tolerance of cancer cells.

#### **BACKGROUND INFORMATION**

[0002] The immune system provides the human body with a means to recognize and defend itself against microorganisms and substances recognized as foreign or potentially harmful. While passive immunotherapy of cancer with monoclonal antibodies and passive transfer of T cells to attack tumor cells have demonstrated clinical efficacy, the goal of active therapeutic vaccination to induce these immune effectors and establish immunological memory against tumor cells has remained challenging. Several tumor-specific and tumor-associated antigens have been identified, yet these antigens are generally weakly immunogenic and tumors employ diverse mechanisms to create a tolerogenic environment that allows them to evade immunologic attack. Strategies to overcome such immune tolerance and activating robust levels of antibody and/or T cell responses hold the key to effective cancer immunotherapy.

#### **SUMMARY OF THE INVENTION**

[0003] The present invention is based on the seminal discovery that targeted immunomodulatory antibodies and fusion proteins can counteract or reverse immune tolerance of cancer cells. Cancer cells are able to escape elimination by chemotherapeutic agents or tumor-targeted antibodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. Such immunosuppressive mechanisms include immunosuppressive cytokines (for example, Transforming growth factor beta (TGF- $\beta$ )) and regulatory T cells and/or immunosuppressive myeloid dendritic cells (DCs). By counteracting tumor-induced immune tolerance, the present

invention provides effective compositions and methods for cancer treatment, optional in combination with another existing cancer treatment. The present invention provides strategies to counteract tumor-induced immune tolerance and enhance the antitumor efficacy of chemotherapy by activating and leveraging T cell-mediated adaptive antitumor immunity against resistant or disseminated cancer cells.

[0004] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule, and the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF- $\beta$ ); (ii) Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2); (iii) Receptor activator of nuclear factor- $\kappa$ B (RANK) ligand (RANKL); (iv) Transforming growth factor-beta receptor (TGF- $\beta$ R); (v) Programmed death-1 (PD-1); and (vi) Receptor activator of nuclear factor- $\kappa$ B (RANK).

[0005] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. In one aspect, the targeting moiety specifically binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), CD20, Vascular endothelial growth factor (VEGF), insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, CD30, CD33, or CD40.

[0006] In one aspect, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: (i) CD4; (ii) CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R); (iii) cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); (iv) Interleukin-10 (IL-10); (v) Transforming growth factor-beta receptor (TGF- $\beta$ R); (vi) Transforming growth factor-beta (TGF- $\beta$ ); (vii) Programmed Death-1 (PD-1); (viii) Programmed death-1 ligand (PD-L1 or PD-L2); (ix) Receptor activator of nuclear factor- $\kappa$ B (RANK); or (x) Receptor activator of nuclear factor- $\kappa$ B (RANK) ligand (RANKL).

[0007] In one aspect, the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF- $\beta$ ); (ii) Programmed death-1 ligand (PD-L1 or PD-L2); or (iii) Receptor activator of nuclear factor- $\kappa$ B (RANK) ligand (RANKL).

[0008] In one aspect, the immunomodulatory moiety includes a molecule that binds TGF- $\beta$ . In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Transforming growth factor-beta receptor TGF- $\beta$ RII, TGF- $\beta$ RIIb, or TGF- $\beta$ RIII. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- $\beta$ RII. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of TGF- $\beta$ .

[0009] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- $\beta$ RII. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10

[0010] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Programmed Death-1 (PD-1), Programmed death-1 ligand 1 (PD-L1), or Programmed death-1 ligand 2 (PD-L2). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- $\beta$ RII. In another aspect, the molecule includes PD-1 ectodomain, immunoglobulin Fc region, and TGF $\beta$ RII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

[0011] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- $\kappa$ B (RANK) or Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- $\beta$ RII. In another aspect, the molecule includes

RANK ectodomain, immunoglobulin Fc region, and TGF $\beta$ RII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

**[0012]** In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Programmed death-1 ligand 1 (PD-L1).

**[0013]** In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.

**[0014]** In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- $\kappa$ B (RANK) or Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Programmed Death-1 (PD-1). In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and PD-1 ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 25 or 26.

**[0015]** In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK). In an additional aspect, the

immunomodulatory moiety inhibits the activity or function of Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL).

**[0016]** In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36.

**[0017]** In one aspect, the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the immunomodulatory moiety increases the function of PD-1.

**[0018]** In one aspect, the targeting moiety specifically binds to Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the targeting moiety includes an antibody that binds TNF- $\alpha$ , and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 37. In another aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2), and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from PD-L1. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 38 or 39.

**[0019]** In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25, or CD4, and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 40, 41, 42, 43, 44, or 45.

**[0020]** In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 46 or 47.

**[0021]** In one aspect, the targeting moiety includes transforming growth factor- $\beta$  (TGF- $\beta$ ) and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 48 or 49.

**[0022]** In one aspect, the immunomodulatory moiety includes a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ). In an additional aspect, the immunomodulatory moiety activates the signaling function of transforming growth factor- $\beta$  (TGF- $\beta$ ) receptor.

**[0023]** In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), and the immunomodulatory moiety includes a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ). In an additional aspect, the targeting moiety includes an antibody that binds TNF- $\alpha$ , and the immunomodulatory moiety includes a sequence from TGF- $\beta$ . In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 50. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 51 or 52.

**[0024]** In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R), or CD4, and the immunomodulatory moiety includes a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 53, 54, 55, 56, 57 or 58.

[0025] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety includes a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 59 or 60.

[0026] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK). In an additional aspect, the targeting moiety includes an antibody that binds TNF- $\alpha$ , and the immunomodulatory moiety includes a sequence from an extracellular RANKL-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 61. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from an extracellular RANK-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 62 or 63.

[0027] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

[0028] In one aspect, the targeting moiety includes includes a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ) and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

[0029] In one aspect, the targeting moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator

of nuclear factor- $\kappa$ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

**[0030]** In various aspects, the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

**[0031]** In another embodiment, the present invention provides a composition including the molecule of the invention and a cell, wherein the cell is a tumor cell, immune cell, or dendritic cell.

**[0032]** In another embodiment, the present invention provides a method of counteracting or overcoming immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

**[0033]** In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof one or more molecule of the invention. In various aspects, the subject is administered one or more molecule of the invention in combination with another anticancer therapy. In one aspect, the anticancer therapy includes a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent or cytotoxic agent. In another aspect, the anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

**[0034]** In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof an antibody that targets and depletes CD4<sup>+</sup> regulatory T cells (Tregs) in combination with another cytotoxic anticancer therapy. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In various aspects, the cytotoxic anticancer therapy includes a chemotherapeutic molecule, tumor-targeted antibody, small molecule kinase inhibitor, hormonal agent or tumor-targeted cytotoxic agent. In another aspect, the cytotoxic anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

[0035] In another embodiment, the subject is administered one or more molecule of the invention in combination with any vaccine. In another aspect, the vaccine includes a tumor antigen, tumor-associated antigen, tumor epitope, tumor antigen-containing fusion protein, tumor cell, or dendritic cell. In another aspect, the vaccine includes a pathogen antigen, pathogen-associated antigen, pathogen epitope, or pathogen antigen-containing fusion protein.

[0036] In another embodiment, the present invention provides a method for treating immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treatment of a neoplastic disease. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

[0037] In another embodiment, the present invention provides a method of inducing or promoting immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

[0038] In another embodiment, the present invention provides a method of preventing or treating an autoimmune or inflammatory disease including administering to a subject in need thereof one or more molecule of the invention. In one aspect, the subject is administered one or more molecule of the invention in combination with another anti-inflammatory or immunosuppressive therapy. In another embodiment, the present invention provides a method of treatment of immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treating an autoimmune or inflammatory disease or preventing rejection of grafted cells or tissue. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0039] Fig. 1 shows exemplary amino acid sequences of transforming growth factor beta receptor type II (TGF- $\beta$ -RII) or TGF- $\beta$ -RIIB or a fragment thereof, including (i) Transforming growth factor beta receptor type II (TGF- $\beta$ -RII) (SEQ ID NO: 79); and (ii) Transforming growth factor beta receptor type IIB (TGF- $\beta$ -RIIB) (SEQ ID NO: 80).

[0040] Also shown in Figure 1 are exemplary truncated mutants of Transforming growth factor beta Receptor II (TGF- $\beta$ -RII) or TGF- $\beta$ -RIIB including the Extracellular domain (ECD) region that binds TGF- $\beta$ , including (i) TGF- $\beta$  R-II ( $\Delta$ C terminus): TGF $\beta$  RII lacking the last 38 amino acids from the C-terminus (SEQ ID NO: 81) and TGF- $\beta$  R-IIB ( $\Delta$ C terminus): TGF $\beta$  RIIB lacking the last 38 aa from the C-terminus (SEQ ID NO: 82); (ii) TGF- $\beta$ R-II ( $\Delta$ cyt): TGF $\beta$ RII lacking the kinase domain & juxtamembrane region (SEQ ID NO: 83) and TGF- $\beta$ R-IIB ( $\Delta$ cyt): TGF $\beta$ RIIB lacking the kinase domain & juxtamembrane region (SEQ ID NO: 84); (iii) TGF- $\beta$  R-II containing the N-terminus region including the extracellular domain (SEQ ID NO: 85) and TGF- $\beta$  R-IIB containing the N-terminus region including the extracellular domain (SEQ ID NO: 86); (iv) TGF- $\beta$  R-II containing the extracellular domain that binds TGF- $\beta$  (SEQ ID NO: 87) and TGF- $\beta$  R-IIB containing the extracellular domain that binds TGF- $\beta$  (SEQ ID NO: 88); and (v) TGF- $\beta$  R-II containing the region of the extracellular domain that binds TGF- $\beta$  (SEQ ID NO: 89).

[0041] In addition, Figure 1 also shows exemplary kinase-deficient mutants, deletion mutants, or point mutants of Transforming growth factor beta Receptor II (TGF $\beta$ -RII) or TGF $\beta$ -RIIB or a fragment thereof which binds TGF- $\beta$ , including (i) Transforming growth factor beta Receptor II containing point mutations - amino acid sequence of TGF- $\beta$  R-II (K277R) contains a point mutation in its ATP-binding site and is inactive as a kinase (SEQ ID NO: 90); and (ii) Transforming growth factor beta Receptor II containing deletions in the amino acid sequence (deletion mutants) - Transforming growth factor beta Receptor II ( $\Delta$ i) - TGF- $\beta$  R-II ( $\Delta$ i2) contains a deletion of amino acids 498 to 508 and is inactive as a kinase (SEQ ID NO: 91).

[0042] Figure 2 shows exemplary fusion proteins including anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD), including anti-HER2/neu heavy chain + TGF $\beta$ -RII ECD fusion amino acid sequence (SEQ ID NO: 1) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

[0043] Figure 3 shows exemplary fusion proteins including anti-EGFR1 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD), including anti-EGFR1 heavy chain + TGF $\beta$ -RII ECD fusion amino acid sequence (SEQ ID NO: 2) and anti-EGFR1 light chain amino acid sequence (SEQ ID NO: 71).

[0044] Figure 4 shows exemplary fusion proteins including anti-CD20 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD), including anti-CD20 heavy chain+ TGF $\beta$ -RII ECD fusion amino acid sequence (SEQ ID NO: 3) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0045] Figure 5 shows exemplary fusion proteins including anti-VEGF antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD), including anti-VEGF heavy chain+ TGF $\beta$ -RII ECD fusion amino acid sequence (SEQ ID NO: 4) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

[0046] Figure 6 shows exemplary fusion proteins including anti-human CTLA-4 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD), including Anti-CTLA-4 heavy chain + TGF $\beta$ -RII Extracellular domain fusion amino acid sequence (SEQ ID NO: 5) and Anti-CTLA-4 light chain (SEQ ID NO: 74).

[0047] Figure 7 shows exemplary fusion proteins including IL-2, Fc, and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD), including IL-2 + Fc + TGF $\beta$ -RII Extracellular domain (SEQ ID NO: 6) and TGF $\beta$ -RII Extracellular domain + Fc + IL-2 (SEQ ID NO: 7). The linker GGGGSGGGSGGGGS (SEQ ID NO: 104) is optional and can be replaced with EPKSCDK (SEQ ID NO: 105) or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0048] Figure 8 shows exemplary fusion proteins including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD), including anti-CD25 (Daclizumab) heavy chain and TGF $\beta$ -RII Extracellular domain (SEQ ID NO: 8) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (Figure 8A); and anti-CD25 (Basiliximab) heavy chain and TGF $\beta$ -RII Extracellular domain (SEQ ID NO: 9) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76).

[0049] Figure 9 shows exemplary fusion proteins including anti-CD4 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD), including anti-CD4 heavy chain and TGF $\beta$ -RII Extracellular domain (SEQ ID NO: 10) and anti-CD4 light chain (SEQ ID NO: 77)

[0050] Figure 10 shows exemplary fusion proteins including Programmed Death-1 (PD-1) Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ectodomain), including PD-1 ectodomain + Fc + TGF $\beta$ RII ectodomain (SEQ ID NO: 11) and TGF $\beta$ RII ectodomain + Fc + PD-1 ectodomain (SEQ ID NO: 12). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

[0051] Figure 11 shows exemplary fusion proteins including Receptor activator of nuclear factor-kB (RANK) Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ectodomain), including RANK ectodomain + Fc + TGF $\beta$ RII ectodomain (SEQ ID NO: 13) and TGF $\beta$ RII ectodomain + Fc + RANK ectodomain (SEQ ID NO: 14). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

[0052] Figure 12 shows exemplary immunomodulatory moiety that binds Programmed Death-1 ligand 1 (PD-L1 or B7-H1) or Programmed Death-1 ligand 2 (PD-L2 or B7-DC), including full-length PD-1 or fragment thereof (SEQ ID NO: 92), PD-1 extracellular domain (ectodomain) or fragment thereof (SEQ ID NO: 93), and PD-1 extracellular domain (ectodomain) ligand-binding region (SEQ ID NO: 94).

[0053] Figure 13 shows exemplary fusion proteins including anti-HER2/neu antibody and PD-1 Ectodomain, including anti-HER2/neu heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 15) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

[0054] Figure 14 shows exemplary fusion proteins including anti-EGFR1 antibody and PD-1 Ectodomain, including anti-EGFR heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 16) and anti-EGFR light chain amino acid sequence (SEQ ID NO: 71).

[0055] Figure 15 shows exemplary fusion proteins including anti-CD20 antibody and PD-1 Ectodomain, including anti-CD20 heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 17) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0056] Figure 16 shows exemplary fusion proteins including anti-VEGF antibody and PD-1 Ectodomain, including anti-VEGF heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 18) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

[0057] Figure 17 shows exemplary fusion proteins including anti-human CTLA-4 antibody and PD-1 Ectodomain, including anti-CTLA-4 heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 19) and anti-CTLA-4 light chain (SEQ ID NO: 74).

[0058] Figure 18 shows exemplary fusion proteins including anti-CD25 antibody and PD-1 Ectodomain, including anti-CD25 (Daclizumab) heavy chain and PD-1 ectodomain (SEQ ID NO: 20) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (Figure 18A), and anti-CD25 (Basiliximab) heavy chain and PD-1 ectodomain (SEQ ID NO: 21) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (Figure 18B).

[0059] Figure 19 shows exemplary fusion proteins including IL-2, Fc, and PD-1 ectodomain, including IL-2 + Fc + PD-1 ectodomain (SEQ ID NO: 22) and PD-1 ectodomain + Fc + IL-2 (SEQ ID NO: 23). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0060] Figure 20 shows exemplary fusion proteins including anti-CD4 antibody and PD-1 ectodomain, including anti-CD4 heavy chain and PD-1 ectodomain (SEQ ID NO: 24) and anti-CD4 light chain (SEQ ID NO: 77)

[0061] Figure 21 shows exemplary fusion proteins including Receptor activator of nuclear factor-kB (RANK) Ectodomain, Fc, and PD-1 ectodomain, including RANK ectodomain + Fc + PD-1 ectodomain (SEQ ID NO: 25) and PD-1 ectodomain + Fc + RANK ectodomain (SEQ ID NO: 26). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

[0062] Figure 22 shows exemplary immunomodulatory moiety that binds Receptor activator of nuclear factor- $\kappa$ B (RANK) ligand (RANKL) including full-length RANK or fragment thereof (SEQ ID NO: 95), extracellular ligand-binding domain or ectodomain of RANK (SEQ ID NO: 96), RANKL-binding sequences or residues of RANK (SEQ ID NO: 93), or RANKL-binding sequences of Osteoprotegerin (OPG) (SEQ ID NO: 98).

[0063] Figure 23 shows exemplary fusion proteins including anti-HER2/neu antibody and RANK Ectodomain, including anti-HER2/neu heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 27) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

[0064] Figure 24 shows exemplary fusion proteins including anti-EGFR1 antibody and RANK Ectodomain, including anti-EGFR heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 28) and anti-EGFR light chain amino acid sequence (SEQ ID NO: 71).

[0065] Figure 25 shows exemplary fusion proteins including anti-CD20 antibody and RANK Ectodomain, including anti-CD20 heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 29) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0066] Figure 26 shows exemplary fusion proteins including anti-VEGF antibody and RANK Ectodomain, including anti-VEGF heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 30) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

[0067] Figure 27 shows exemplary fusion proteins including anti-human CTLA-4 antibody and RANK Ectodomain, including anti-CTLA-4 heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 31) and anti-CTLA-4 light chain (SEQ ID NO: 74).

[0068] Figure 28 shows exemplary fusion proteins including anti-CD25 antibody and RANK Ectodomain, including anti-CD25 (Daclizumab) heavy chain and RANK ectodomain (SEQ ID NO: 32) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (Figure 28A), and anti-CD25 (Basiliximab) heavy chain and RANK ectodomain (SEQ ID NO: 33) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (Figure 28B).

[0069] Figure 29 shows exemplary fusion proteins including IL-2, Fc, and RANK ectodomain, including IL-2 + Fc + RANK ectodomain (SEQ ID NO: 34) and RANK ectodomain + Fc + IL-2 (SEQ ID NO: 35). The linker GGGSGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0070] Figure 30 shows exemplary fusion proteins including anti-CD4 antibody and RANK ectodomain, including anti-CD4 heavy chain and RANK ectodomain (SEQ ID NO: 36) and anti-CD4 light chain (SEQ ID NO: 77)

[0071] Figure 31 shows exemplary immunomodulatory moiety that binds Programmed Death-1 (PD-1) including a PD-1 ligand 1 (PD-L1 or B7-H1) or PD-1 ligand 2 (PD-L2 or B7-DC) or a fragment thereof (for example, SEQ ID NO: 101), full-length human PD-1 ligand 1 (B7-H1; PDCD1L1; PD-L1; or CD274) protein or a fragment thereof (SEQ ID NO: 99), and PD-L1 extracellular binding domain (ectodomain) or fragment thereof (SEQ ID NO: 100).

[0072] Figure 32 shows exemplary fusion proteins including anti-tumor necrosis factor (TNF $\alpha$ ) antibody and PD-1 ligand, including anti-TNF $\alpha$  heavy chain + PD-1L (SEQ ID NO: 37) and anti-TNF $\alpha$  light chain (SEQ ID NO: 78). The sequence KKAE (SEQ ID NO: 107) can be replaced with KRVE (SEQ ID NO: 108) or KKVE (SEQ ID NO: 109).

[0073] Figure 33 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand, including TNFR2 ECD + IgG C $\gamma$ 1 + PD-L1 (SEQ ID NO: 38) and PD-L1 + IgG C $\gamma$ 1 -TNFR2 ECD (SEQ ID NO: 39).

[0074] Figure 34 shows exemplary fusion proteins including anti-CD20 antibody and PD-1 ligand 1 (PD-L1), including anti-CD20 heavy chain + PD-L1 sequence (SEQ ID NO: 40) and anti-CD20 light chain sequence (SEQ ID NO: 72).

[0075] Figure 35 shows exemplary fusion proteins including anti-CD25 antibody and PD-1 ligand 1 (PD-L1), including anti-CD25 (Daclizumab) heavy chain and PD-L1 (SEQ ID NO: 41) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (Figure 35A), and anti-CD25

(Basiliximab) heavy chain and PD-1 ectodomain (SEQ ID NO: 42) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (Figure 35B).

[0076] Figure 36 shows exemplary fusion proteins including IL-2, Fc, and PD-1 ligand 1 (PD-L1), including fusion protein hPD-1 ligand 1 + Fc + IL-2 (SEQ ID NO: 43) and fusion protein IL-2 + Fc + PD-1 ligand 1 (SEQ ID NO: 44). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0077] Figure 37 shows exemplary fusion proteins including anti-CD4 antibody and PD-1 ligand 1 (PD-L1), including anti-CD4 heavy chain and PD-1 ligand 1 (PD-L1) (SEQ ID NO: 45) and anti-CD4 light chain (SEQ ID NO: 77)

[0078] Figure 38 shows exemplary fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from PD-1 ligand (PD-L1) including Oncostatin M signal peptide + CTLA-4 ECD + IgG C $\gamma$ 1 + PD-L1 (SEQ ID NO: 46) and PD-1L1 + IgG C $\gamma$ 1 + CTLA-4 ECD (SEQ ID NO: 47). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

[0079] Figure 39 shows exemplary fusion proteins including a sequence of transforming growth factor- $\beta$  (TGF- $\beta$ ), Immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence of PD-1 ligand (PD-L1) including TGF- $\beta$ -1 + Fc + PD-L1 (SEQ ID NO: 48), and PD-1L1 + Fc + TGF- $\beta$ -1 (SEQ ID NO: 49). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0080] Figure 40 shows exemplary immunomodulatory moiety that binds Transforming growth factor-beta receptor (TGF- $\beta$ R) including Transforming growth factor-beta (TGF- $\beta$ 1, TGF- $\beta$ 2, or TGF- $\beta$ 3 or a fragment thereof, TGF- $\beta$ 1 full sequence (SEQ ID NO: 102), and mature (active) TGF- $\beta$ 1 sequence (Ala 279 – Ser 390; 112 amino acids) (SEQ ID NO: 103).

[0081] Figure 41 shows exemplary fusion proteins including an antibody that binds TNF- $\alpha$ , and a sequence of transforming growth factor- $\beta$  (TGF- $\beta$ ), including anti-TNF $\alpha$  heavy chain + TGF- $\beta$ 1 (SEQ ID NO: 50) and anti-TNF $\alpha$  light chain (SEQ ID NO: 78). The sequence KKAE (SEQ ID NO: 107) can be replaced with KRVE (SEQ ID NO: 108) or KKVE (SEQ ID NO: 109).

[0082] Figure 42 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain (TNFR2 ECD), immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ) including TNFR2 ECD + IgG C $\gamma$ 1 + TGF- $\beta$ 1 (SEQ ID NO: 51), and TGF- $\beta$ 1 + IgG C $\gamma$ 1 + TNFR2 ECD (SEQ ID NO: 52).

[0083] Figure 43 shows exemplary fusion proteins including anti-CD20 antibody and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ), including anti-CD20 heavy chain + mature TGF $\beta$ 1 sequence (SEQ ID NO: 53) and anti-CD20 light chain sequence (SEQ ID NO: 72).

[0084] Figure 44 shows exemplary fusion proteins including anti-CD25 antibody and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ), including anti-CD25 (Daclizumab) heavy chain and TGF- $\beta$ 1 (SEQ ID NO: 54) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (Figure 44A), and anti-CD25 (Basiliximab) heavy chain and TGF- $\beta$ 1 (SEQ ID NO: 55) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (Figure 44B).

[0085] Figure 45 shows exemplary fusion proteins including IL-2, Fc, and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ), including TGF- $\beta$ 1 + Fc + IL-2 (SEQ ID NO: 56) and IL-2 + Fc + TGF- $\beta$ 1 (SEQ ID NO: 57). The linker GGGGSGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0086] Figure 46 shows exemplary fusion proteins including anti-CD4 antibody and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ), including anti-CD4 heavy chain and TGF- $\beta$  (SEQ ID NO: 58) and anti-CD4 light chain (SEQ ID NO: 77)

[0087] Figure 47 shows exemplary Fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ) including Oncostatin M signal peptide + CTLA-4 ECD + IgG C $\gamma$ 1 + TGF- $\beta$ 1 (SEQ ID NO: 59), and TGF- $\beta$ 1 + IgG C $\gamma$ 1 + CTLA-4 ECD (SEQ ID NO: 60). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

[0088] Figure 48 shows exemplary fusion proteins including an antibody that binds TNF- $\alpha$ , and a sequence of RANK ectodomain, including anti-TNF $\alpha$  heavy chain + RANK ectodomain (SEQ ID NO: 61) and anti-TNF $\alpha$  light chain (SEQ ID NO: 78). The sequence KKAE (SEQ ID NO: 107) can be replaced with KRVE (SEQ ID NO: 108) or KKVE (SEQ ID NO: 109).

[0089] Figure 49 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain (TNFR2 ECD), immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from RANK ectodomain including TNFR2 ECD + IgG C $\gamma$ 1 + RANK ectodomain (SEQ ID NO: 62), and RANK ectodomain + IgG C $\gamma$ 1 + TNFR2 ECD (SEQ ID NO: 63).

[0090] Figure 50 shows exemplary Fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from RANK ectodomain including Oncostatin M signal peptide + CTLA-4 ECD + IgG C $\gamma$ 1 + RANK ectodomain (SEQ ID NO: 64), and RANK ectodomain + IgG C $\gamma$ 1 + CTLA-4 ECD (SEQ ID NO: 65). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

[0091] Figure 51 shows exemplary fusion proteins including a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ), immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from RANK ectodomain including TGF- $\beta$  + IgG C $\gamma$ 1 + RANK ectodomain (SEQ ID NO: 66), and RANK ectodomain + IgG C $\gamma$ 1 + TGF- $\beta$  (SEQ ID NO: 67).

[0092] Figure 52 shows exemplary fusion proteins including a sequence from PD-1 ligand (PD-L1), immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from RANK ectodomain including PD-L1 + IgG C $\gamma$ 1 + RANK ectodomain (SEQ ID NO: 68), and RANK ectodomain + IgG C $\gamma$ 1 + PD-L1 (SEQ ID NO: 69).

[0093] Figure 53 shows Regulatory T cells (Treg) accumulate in the tumor microenvironment and counteract the ability of chemotherapy to activate CD8<sup>+</sup> T cell-mediated antitumor immunity. (a) Surface exposure of calreticulin (CRT) in response to treatment of human (SW780) and murine (MB49) cancer cells with doxorubicin (10  $\mu$ M) for 4h. The surface exposure of CRT was determined by immunofluorescence cytometry of untreated control or doxorubicin-treated cells stained with Dylight-labeled anti-CRT antibody or an isotype control (IgG1) antibody. (b) Priming of tumor-reactive immune responses by MB49 tumor cells treated with doxorubicin *ex vivo* or *in vivo*. 5x10<sup>6</sup> MB49 cells that were pre-treated *ex vivo* with doxorubicin (10  $\mu$ M) for 4h were injected into one flank of syngeneic immunocompetent C57BL/6 mice. Alternatively, C57BL/6 mice were injected with 5x10<sup>5</sup> live MB49 tumor cells and then administered intratumoral doxorubicin (10  $\mu$ g) at 10d following tumor inoculation. Tumor-reactive immune responses were determined by measuring production of IFN- $\gamma$  by draining lymph node (DLN) cells in response to *in vitro* re-challenge with either MB49 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone. (c) Vaccination with doxorubicin-treated tumor cells induces CD8<sup>+</sup> T cell-mediated antitumor immunity that prevents tumor formation following re-challenge with live tumor cells. MB49 cells (5x10<sup>6</sup>) that were pre-treated *in vitro* with doxorubicin (10  $\mu$ M) for 4h were injected subcutaneously into one flank of syngeneic immunocompetent C57BL/6 mice. Naïve or vaccinated mice were challenged with untreated live MB49 tumor cells injected into the opposite flank with or without pre-treatment with an anti-CD8 antibody (Clone GK2.43)(5  $\mu$ g x 2 doses, iv) to deplete CD8<sup>+</sup> T cells. (d) Delayed administration of chemotherapy in mice with pre-established tumors decreases its immunogenicity and antitumor efficacy. C57BL/6 mice were injected with 5x10<sup>5</sup> live syngeneic MB49 tumor cells and then administered intratumoral doxorubicin (10  $\mu$ g) at d3, d7, or d10 following tumor inoculation. (e) Tumors foster the accumulation of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> cells (Tregs) in their microenvironment. Flow cytometric analyses of the percentage of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> cells (Tregs) among CD4<sup>+</sup> T lymphocytes isolated from the spleen, draining lymph nodes (DLN), and tumors of

immunocompetent C57BL/6 mice at d0 and d14 after subcutaneous inoculation of  $5 \times 10^5$  live MB49 tumor cells. (f) Tregs infiltrating the tumor microenvironment suppress priming of tumor-reactive immune responses by doxorubicin-treated tumor cells. Naïve C57BL/6 mice were vaccinated with  $5 \times 10^6$  doxorubicin-killed MB49 cells with or without intravenous adoptive transfer of  $5 \times 10^6$  CD4<sup>+</sup>CD25<sup>+</sup> cells isolated from tumors and DLN of tumor-bearing mice via immunomagnetic separation. Tumor-reactive immune responses were determined by measuring production of IFN- $\gamma$  by draining lymph node (DLN) cells in response to *in vitro* re-challenge with either MB49 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone. (g) Tregs infiltrating the tumor microenvironment suppress the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death. Naïve C57BL/6 mice were vaccinated with  $5 \times 10^6$  doxorubicin-killed MB49 cells (left flank) with or without pre-treatment with either an anti-CD8 antibody (Clo<sup>ne</sup> GK2.43)(5  $\mu$ g x 2 doses, iv) to deplete CD8<sup>+</sup> T cells or adoptive transfer of  $5 \times 10^6$  CD4<sup>+</sup>CD25<sup>+</sup> cells isolated from tumors and DLN of tumor-bearing mice. Protective antitumor immunity in vaccinated mice was determined by assessment of tumor growth upon challenge with untreated live MB49 tumor cells injected into the opposite flank.

[0094] Figure 54 shows inhibition of TGF- $\beta$  in the tumor microenvironment reduces 'adaptive' FoxP3<sup>+</sup> regulatory T cells and enhances the antitumor efficacy chemotherapy. (a) Tumor growth results in a progressive increase in the level of serum TGF- $\beta$ . Levels of TGF- $\beta$  in serum of mice at d0, d14, and d28 following inoculation of  $5 \times 10^5$  live MB49 tumor cells were evaluated utilizing ELISA. (b) Tumor cell-autonomous expression of TGF- $\beta$  is the dominant source of elevated TGF- $\beta$  in tumor-bearing mice. Tumor cells or draining lymph node cells isolated from either tumor-bearing mice or their tumor-free counterparts were cultured *ex vivo* in serum-free medium for 24h and the amount of TGF- $\beta$ /10<sup>6</sup> cells in supernatants was measured by ELISA. (c) TGF $\beta$ RII:Fc sequesters TGF- $\beta$  in supernatants of MB49 tumor cells in a concentration-dependent manner. MB49 tumor cells were cultured in the presence of graded concentrations of TGF $\beta$ RII:Fc (0-400 ng/ml) for 24h followed by measurement of TGF- $\beta$  (pg/ml/10<sup>6</sup> cells) in supernatants via ELISA. (d) TGF- $\beta$  induces 'adaptive' FoxP3<sup>+</sup> regulatory T cells in the tumor microenvironment. At 5d following inoculation of MB49 tumor cells, mice were either left untreated (control) or treated with TGF $\beta$ RII:Fc (1 $\mu$ g intratumoral; twice weekly) for 3 weeks followed by flow cytometric

analyses of intracellular FoxP3 expression in CD4<sup>+</sup>CD25<sup>+</sup> T cells infiltrating the tumors. (e, f) Sequestration of intratumoral TGF- $\beta$  with TGF $\beta$ RII:Fc reduces CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> Tregs in tumor tissue and improves the antitumor efficacy of doxorubicin. MB49 tumor-bearing mice were administered doxorubicin (5mg/kg i.p. weekly x 3) with or without twice weekly treatment with TGF $\beta$ RII:Fc (1 $\mu$ g intratumoral). The percentage of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> cells (Tregs) among tumor cells was assessed by flow cytometry (e), and tumor volume was monitored to determine the effect of counteracting tumor-induced TGF- $\beta$ -mediated immune tolerance on the *in vivo* antitumor efficacy of doxorubicin (f).

[0095] Figure 55 shows that anti-CD4 antibody-mediated depletion of CD4<sup>+</sup> regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive CD8<sup>+</sup> T cells and enhances the antitumor efficacy of chemotherapy. (a) *In vivo* depletion of tumor-infiltrating CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> T cells by treatment of tumor-bearing mice with anti-CD4 antibody. C57BL/6 mice injected with 5 x 10<sup>5</sup> MB49 tumor cells s.c. were left untreated (control) or administered an anti-CD4 antibody (Clone GK1.5) i.p. at 5d and 9d following tumor challenge. CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> T cells infiltrating tumors isolated from mice at d16 following tumor challenge were detected by flow cytometry. (b) Target-specific depletion of either CD4<sup>+</sup> T cells, CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> T cells, or CD8<sup>+</sup> T cells by treatment of tumor-bearing mice with anti-CD4 antibody or anti-CD8 antibody. C57BL/6 mice injected s.c. with 5 x 10<sup>5</sup> MB49 tumor cells were left untreated or treated with doxorubicin (5mg/kg i.p. weekly x 3) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Flow cytometric analyses of peripheral blood mononuclear cells isolated from mice at d16 following tumor challenge determined the percentage of CD4<sup>+</sup> T cells or CD8<sup>+</sup> T cells among total mononuclear cells, and the percentage of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> T cells among total CD4<sup>+</sup> T cells. (c) Depletion of CD4<sup>+</sup> regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive CD8<sup>+</sup> T cells. C57BL/6 mice injected s.c. with 5 x 10<sup>5</sup> MB49 tumor cells were left untreated or treated with doxorubicin (5mg/kg i.p. weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of IFN- $\gamma$  expression in CD8<sup>+</sup> T cells from the tumor and draining lymph node in response to *in vitro* stimulation with MB49 cell lysates. (d)

Depletion of CD4<sup>+</sup> regulatory T cells augments the *in vivo* antitumor efficacy of chemotherapy via activation of tumor-reactive CD8<sup>+</sup> T cells. C57BL/6 mice injected s.c. with 5 x10<sup>5</sup> MB49 tumor cells were left untreated or treated with doxorubicin (5mg/kg i.p. weekly x 3) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting either CD4<sup>+</sup> T cells or CD8<sup>+</sup> T cells on the *in vivo* antitumor efficacy of doxorubicin.

[0096] Figure 56 shows anti-CD4 antibody-mediated depletion of CD4<sup>+</sup> regulatory T cells augments and sustains the antitumor effect of chemotherapy by enabling activation of adaptive antitumor immunity. (a) Surface exposure of calreticulin (CRT) in response to treatment of MB49 cancer cells with either cisplatin or the combination of cisplatin and gemcitabine for 4h. The surface exposure of CRT was determined by immunofluorescence cytometry of untreated control or chemotherapy-treated cells stained with Dylight-labeled anti-CRT antibody or an isotype control (IgG1) antibody. (b,c) Depletion of CD4<sup>+</sup> regulatory T cells enables cisplatin-induced activation of tumor-reactive IFN- $\gamma$ <sup>+</sup>CD8<sup>+</sup> T cells and effector memory (CD8<sup>+</sup>CD62L<sup>-</sup>) T cells. C57BL/6 mice injected s.c. with 5 x10<sup>5</sup> MB49 tumor cells were left untreated or treated with cisplatin (0.5mg/kg i.p. weekly x 4) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of IFN- $\gamma$  expression in CD8<sup>+</sup> T cells from the tumor and draining lymph node (DLN) in response to *in vitro* stimulation with MB49 cell lysates (b). The percentage of effector memory T<sub>EM</sub> cells was determined by flow cytometric analyses of CD8<sup>+</sup>CD62L<sup>-</sup> cells (c). (d,e,f) Depletion of CD4<sup>+</sup> regulatory T cells augments the *in vivo* antitumor efficacy of chemotherapy via activation of tumor-reactive CD8<sup>+</sup> T cells. C57BL/6 mice injected s.c. with 5 x10<sup>5</sup> MB49 tumor cells were left untreated or treated with either cisplatin (0.5mg/kg) or the combination of cisplatin and gemcitabine (i.p. weekly x 4) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting either CD4<sup>+</sup> T cells or CD8<sup>+</sup> T cells on the *in vivo* antitumor efficacy of chemotherapy and the percentage of mice exhibiting complete tumor-regression by d50 following tumor inoculation. Establishment of adaptive antitumor

immunity following regression of primary tumors was determined by re-challenging mice with live MB49 tumor cells in the opposite flank.

[0097] Figure 57 shows that Chemotherapy-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD4<sup>+</sup> regulatory T cells to stimulate CD8<sup>+</sup> T cell-mediated tumor regression. (a) Genotoxic chemotherapeutic agents induce expression of mouse NKG2D ligands (Rae-1) on cancer cells. Kinetics of the upregulation of Rae-1 transcripts in mouse CT26 colon cancer cells was determined by quantitative real-time PCR following treatment with irinotecan (25 µg/ml) or oxaliplatin (10 µg/ml). Quantitative RT-PCR was carried out using Rae-1 specific primers [sense, 5'-CTAGTGCCACCTGGGAATTCA-3' (SEQ ID NO: 111); anti-sense, 5'-CATCATTAGCTGATCTCCAGCTCA-3' (SEQ ID NO: 112)] and probe [5'-6-FAM-CATCAGTGACAGTTACTTCTTACCTTCTACACAGAGA-Tamra-3' (SEQ ID NO: 113)]. (b) Genotoxic chemotherapeutic agents induce p53-independent cell surface expression of human NKG2D ligands (MHC-I-related A and B molecules – MICA/MICB) on cancer cells. Isogenic p53-proficient (p53<sup>+/+</sup>) or p53-deficient (p53<sup>-/-</sup>) HCT116 cells were treated with irinotecan (25 µg/ml) for 16h or left untreated. Irinotecan-induced upregulation of cell surface expression of MICA/B was determined by flow cytometry analysis of tumor cells labeled with an anti-human MICA/B MAb (R&D Systems). (c) & (d) Induction of NKG2D ligands contributes to the antitumor effect of chemotherapy *in vivo*. Immunocompetent Balb/C mice injected s.c. with 2 x 10<sup>5</sup> syngeneic CT26 tumor cells were treated with irinotecan (50 mg/kg i.p. weekly x 3) beginning at d5 following tumor inoculation, with or without pre-treatment with an NKG2D blocking antibody (CX5, eBioscience) (200 µg i.p.) at 16h before each dose of chemotherapy. Tumor volume was monitored to determine the effect of NKG2D blockade on the *in vivo* antitumor efficacy of irinotecan. (e) *In vivo* depletion of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> T cells by treatment of tumor-bearing mice with anti-CD4 antibody. Balb/C mice injected with 2 x 10<sup>5</sup> CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p. weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> T cells in spleen and draining lymph node isolated from mice at d16 following tumor challenge were detected by flow cytometry. (f) Depletion of CD4<sup>+</sup> regulatory T cells facilitates irinotecan-induced activation of tumor-reactive IFN-γ<sup>+</sup>CD8<sup>+</sup> T cells. Balb/C

mice injected with  $2 \times 10^5$  CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of IFN- $\gamma$  expression in CD8 $^+$  T cells from the tumor and draining lymph node (DLN) in response to *in vitro* stimulation with either CT26 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone. (g) & (h) Chemotherapy-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD4 $^+$  regulatory T cells to stimulate CD8 $^+$  T cell-mediated tumor regression. Balb/C mice injected with  $2 \times 10^5$  CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) and/or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting CD4 $^+$  T cells and/or CD8 $^+$  T cells on the *in vivo* antitumor efficacy of irinotecan.

#### DETAILED DESCRIPTION OF THE INVENTION

[0098] Targeted immunostimulatory antibodies and/or fusion proteins for prevention or treatment of cancer: Chemotherapy is a cornerstone of systemic treatment of patients with most common types of advanced cancers. The vast majority of human cancers harbor genetic alterations and signaling mechanisms that impair the direct death signaling pathways entrained by chemotherapeutic agents. Although chemotherapeutic agents employ diverse mechanisms to directly kill tumor cells, the present invention provides that these agents have immuno-adjutant effects which activate innate and adaptive antitumor immune responses that are crucial for their antitumor efficacy *in vivo*. The present invention also provides that antitumor CD8 $^+$  T cells play an instrumental role in the *in vivo* response of tumors to diverse cytotoxic chemotherapeutic agents. Although chemotherapeutic agents can induce “immunogenic” tumor cell death and facilitate cross-presentation of antigens by dendritic cells, tumors create a tolerogenic environment that allows them to suppress the activation of innate and adaptive immune responses and evade immunologic attack by immune effector cells. The present invention provides that strategies to counteract tumor-induced immune tolerance in the tumor microenvironment can enhance the antitumor efficacy of chemotherapy by activating and leveraging T cell-mediated adaptive antitumor immunity against disseminated cancer cells.

[0099] The present invention is based on the seminal discovery that targeted immunomodulatory antibodies and fusion proteins can counteract or reverse immune tolerance of cancer cells. Cancer cells are able to escape elimination by chemotherapeutic agents or tumor-targeted antibodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. By counteracting tumor-induced immune tolerance, the present invention provides effective compositions and methods for cancer treatment, optional in combination with another existing cancer treatment.

[0100] The present invention provides compositions and methods for targeted immunostimulatory antibodies and fusion proteins that counteract immune tolerance in the tumor microenvironment and promote T cell-mediated adaptive antitumor immunity for maintenance of durable long-term protection against recurrent or disseminated cancers. These tumor-targeted immunostimulatory molecules are designed to facilitate effective long term T cell-mediated immune responses against tumor cells by at least one of the following:

- (i) promoting death of tumor cells via enhancement of antibody-dependent cellular cytotoxicity (ADCC);
- (ii) facilitating effective cross-presentation of tumor antigen(s) from dying tumor cells by augmenting maturation of dendritic cells (DCs); and
- (iii) increasing activation and proliferation of antitumor CD8<sup>+</sup> T cells by negating immune suppression mediated by regulatory T cells and myeloid suppressor cells. These antitumor immune responses may be activated in tandem with the sensitization of tumor cells to immune effector-mediated cytotoxicity, thereby establishing a positive feedback loop that augments tumor cyoreduction and reinforces adaptive antitumor immunity. The tumor-targeted immunostimulatory monoclonal antibodies (mAbs) of the present invention provides the ability to generate and boost antitumor immunity to multiple cross-presented tumor antigens obtained from endogenous tumor cells during the course of therapy (as an *in situ* tumor vaccine), while simultaneously leveraging the antitumor immune response to eliminate disseminated cancer cells. Accordingly, the targeted immunostimulatory antibodies and fusion proteins of the invention can integrate the hitherto distinct fields of passive and active immunotherapy and provide a novel platform for simultaneously leveraging the synergistic

benefits of these strategies to entrain effective innate and adaptive immune responses against targeted cancers.

[0101] While passive immunotherapy of cancer with tumor-targeted monoclonal antibodies has demonstrated clinical efficacy, the goal of active therapeutic vaccination to induce T cell-mediated immunity and establish immunological memory against tumor cells has remained challenging. Several tumor-specific and tumor-associated antigens have been identified, yet tumors employ diverse mechanisms to create a tolerogenic environment that allows them to suppress the activation of a T cell-mediated antitumor immune response. The tumor-targeted immunostimulatory antibodies and/or fusion proteins of the invention are designed to overcome such immune tolerance in the tumor microenvironment and activate robust levels of T cell responses for effective cancer immunotherapy or chemo-immunotherapy. Accordingly, the tumor-targeted immunostimulatory antibodies and/or fusion proteins of the invention have broad clinical relevance for advancing the treatment of many types of human cancers.

[0102] The tumor-targeted immunostimulatory mAbs and/or fusion proteins of the invention provide their ability to generate and boost antitumor immunity to multiple cross-presented tumor antigens obtained from endogenous tumor cells during the course of therapy (as an *in situ* tumor vaccine), while simultaneously leveraging the antitumor immune response to eliminate disseminated cancer cells. Accordingly, the tumor-targeted immunostimulatory antibodies and/or fusion proteins of the invention can integrate the hitherto distinct fields of passive and active immunotherapy and provide a novel platform for simultaneously leveraging the synergistic benefits of these strategies to entrain effective innate and adaptive immune responses against targeted cancers. This approach of the present invention is distinguished from and superior to conventional tumor antigen-, allogeneic tumor cell- or DC-based vaccines in at least one of the following aspects: (i) There is no *a priori* requirement to define, clone and purify individual tumor antigens, since the patient's tumor itself is the *in vivo* source of antigens; (ii) Multivalent antitumor immune responses that are naturally tailored against antigens from the patient's own tumor are less likely to allow immune escape than a pre-selected tumor antigen; (iii) The activation of antitumor immune responses by the immuno-adjuvant effects of tumor-targeted immunostimulatory antibodies or fusion proteins occurs in tandem with the sensitization of tumor cells to immune effector-mediated cytotoxicity, thereby establishing a positive feedback loop that augments tumor cytorreduction and reinforces

adaptive antitumor immunity; and (iv) The molecules of the invention have broad clinical relevance for advancing the treatment of many types of human cancers.

**[0103]** In addition, the targeted immunostimulatory antibodies and/or fusion proteins of the invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) To counteract immune tolerance in the tumor microenvironment and promote T cell-mediated adaptive antitumor immunity for maintenance of long-term protection against recurrent or disseminated cancers (for prevention or treatment of diverse cancers); (ii) To produce immune cell compositions for adoptive cellular therapy of diverse cancers; and (iii) To serve as immune adjuvants or vaccines for prophylaxis of diverse cancers or infectious diseases.

**[0104]** The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to disrupt immunosuppressive networks in the tumor microenvironment. Tumors employ a wide array of regulatory mechanisms to avoid or suppress the immune response. Cancer cells actively promote immune tolerance in the tumor microenvironment via the expression of cytokines and molecules that inhibit the differentiation and maturation of antigen-presenting dendritic cells. The immunosuppressive cytokines and ligands produced by tumor cells include the following: (i) Transforming growth factor-beta (TGF- $\beta$ ); (ii) Programmed death-1 ligand 1 (PD-L1; B7-H1); (iii) Vascular endothelial growth factor (VEGF); and (iv) Interleukin-10 (IL-10). In addition to blocking dendritic cell (DC) maturation, these molecules promote the development of specialized subsets of immunosuppressive CD4<sup>+</sup> T cells (regulatory T cells; Treg cells) and myeloid-derived suppressor cells (MDSC). Tregs are a minority sub-population of CD4<sup>+</sup> T cells that constitutively express CD25 [the interleukin-2 (IL-2) receptor  $\alpha$ -chain] and the forkhead box P3 (FOXP3) transcription factor. Tregs (CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> cells) maintain immune tolerance by restraining the activation, proliferation, and effector functions of a wide range of immune cells, including CD4<sup>+</sup> and CD8<sup>+</sup> T cells, natural killer (NK) and NKT cells, B cells and antigen presenting cells (APCs) *in vitro* and *in vivo*. The accumulation of Treg cells in the tumor microenvironment re-inforces tumor immune tolerance and facilitates tumor progression and metastases. The increased expression of immunosuppressive cytokines (TGF- $\beta$ ; PD-L1) and tumor-infiltrating Tregs is correlated with a reduction of survival of patients with diverse types of cancers. The present invention provides that tumor-induced immune

tolerance mediated via Tregs is a crucial determinant of the resistance of cancers to cytotoxic chemotherapeutic agents and tumor-targeted antibodies. The targeted immunostimulatory antibodies and/or fusion proteins of the invention inhibit key immunosuppressive molecules expressed by the targeted tumor cell or tumor-infiltrating Treg cells and myeloid suppressor cells (DCs or MDSC). As such, they provide the targeted ability to inhibit the development or function of Tregs within the tumor microenvironment. In another aspect, they provide the ability to counteract Treg-induced immune suppression in the tumor microenvironment.

**[0105]** The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to inhibit the development or function of Tregs and myeloid suppressor cells (DCs or MDSC) within the tumor microenvironment. Tregs (CD4+CD25+FoxP3+ cells) express an array of immunosuppressive cytokines and molecules which act in concert to induce immune tolerance and promote tumor progression and metastases. These include: (i) Cytotoxic T-lymphocyte associated protein 4 (CTLA-4; CD152), a co-inhibitory receptor that binds to the ligands CD80 (B7-1) or CD86 (B7-2) on the antigen presenting cell (APC) and inhibits co-stimulation of T cells; (ii) Programmed death-1 ligand 1 (PD-L1; B7-H1), a ligand which engages the co-inhibitory receptor Programmed death-1 (PD-1) and inhibits T cell activation and proliferation. (iii) Transforming growth factor-beta (TGF- $\beta$ ), a cytokine which regulates immune responses by restricting the maturation and antigen-presenting function of dendritic cells, inhibiting the proliferation and activation of naïve T cells, suppressing the expression of cytotoxic molecules (Granzyme A/B, FasL, Apo2L/TRAIL, IFN- $\gamma$ ) in immune effector cells, and promoting the development and function of Tregs; (iv) Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL), a ligand which engages Receptor activator of nuclear factor- $\kappa$ B (RANK) and promotes osteoclast differentiation, Treg development, and tumor metastases. In addition, Tregs express other surface molecules; (v) LAG-3, a CD4-related molecule that binds MHC class II; (vi) glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); and (vii) IL-10. The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to bind a targeted molecule expressed by Tregs or myeloid suppressor cells while concurrently sequestering and inhibiting one or more immunosuppressive molecule that promotes their development, survival or function. In one aspect, the targeted immunostimulatory antibodies and/or fusion proteins directly deplete the number of Tregs.

[0106] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule on the tumor cell or tumor microenvironment (tumor stroma, tumor vasculature, or tumor infiltrating immune cell), and the immunomodulatory moiety specifically binds an immunosuppressive molecule expressed by the targeted tumor cell or tumor-infiltrating Treg cells and myeloid suppressor cells (DC or MDSC).

[0107] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule expressed by Treg cells, myeloid suppressor cells (MDSC), or dendritic cells (DC), and the immunomodulatory moiety specifically binds an immunosuppressive molecule that promotes their development, survival or function.

[0108] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule, and the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF- $\beta$ ); (ii) Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2); (iii) Receptor activator of nuclear factor- $\kappa$ B (RANK) ligand (RANKL); (iv) Vascular endothelial growth factor (VEGF); (v) Transforming growth factor-beta receptor (TGF- $\beta$ R); (vi) Programmed death-1 (PD-1); and (vii) Receptor activator of nuclear factor- $\kappa$ B (RANK).

[0109] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. In one aspect, the targeting moiety specifically binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), CD20, Vascular endothelial growth factor (VEGF), insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, CD30, CD33, or CD40.

[0110] In one aspect, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: (i) CD4; (ii) CD25 (IL-2 $\alpha$  receptor; IL-

2 $\alpha$ R); (iii) cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); (iv) Interleukin-10 (IL-10); (v) Transforming growth factor-beta receptor (TGF- $\beta$ R); (vi) Transforming growth factor-beta (TGF- $\beta$ ); (vii) Programmed Death-1 (PD-1); (viii) Programmed death-1 ligand (PD-L1 or PD-L2); (ix) Receptor activator of nuclear factor- $\kappa$ B (RANK); (x) Receptor activator of nuclear factor- $\kappa$ B (RANK) ligand (RANKL); (xi) LAG-3; or (xii) glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18).

**[0111]** In one aspect, the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF- $\beta$ ); (ii) Programmed death-1 ligand (PD-L1 or PD-L2); (iii) Receptor activator of nuclear factor- $\kappa$ B (RANK) ligand (RANKL); or (iv) vascular endothelial growth factor (VEGF).

**[0112]** In one aspect, the immunomodulatory moiety includes a molecule that binds TGF- $\beta$ . In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Transforming growth factor-beta receptor TGF- $\beta$ RII, TGF- $\beta$ RIIb, or TGF- $\beta$ RIII. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- $\beta$ RII. In another aspect, the molecule includes a TGF- $\beta$ -binding amino acid sequence corresponding to SEQ ID NOs: 79-91. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of TGF- $\beta$ .

**[0113]** In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- $\beta$ RII. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

**[0114]** In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Programmed Death-1 (PD-1), Programmed death-1 ligand 1 (PD-L1), or Programmed death-1 ligand 2 (PD-L2). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- $\beta$ RII. In another aspect, the molecule includes PD-1

ectodomain, immunoglobulin Fc region, and TGF $\beta$ RII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

[0115] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- $\kappa$ B (RANK) or Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- $\beta$ RII. In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and TGF $\beta$ RII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

[0116] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes a PD-L1-binding amino acid sequence corresponding to SEQ ID NO: 92, 93, or 94. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Programmed death-1 ligand 1 (PD-L1).

[0117] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.

[0118] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- $\kappa$ B (RANK) or Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK). In an additional aspect, the immunomodulatory moiety includes

an extracellular ligand-binding domain of Programmed Death-1 (PD-1). In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and PD-1 ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 25 or 26.

[0119] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK). In another aspect, the molecule includes a RANKL-binding amino acid sequence corresponding to SEQ ID NO: 95, 96, 97, or 98. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL).

[0120] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36.

[0121] The present invention provides novel targeted immunosuppressive antibodies and fusion proteins that induce or promote immune tolerance by at least one of the following:

- (i) inhibiting the activation of dendritic cells, T cells, and/or B cells; and
- (ii) promoting the development and/or suppressor function of regulatory T cells and immunosuppressive myeloid DCs. These targeted immunosuppressive molecules of the invention are designed to suppress unwanted or excessive immune or inflammatory responses in order to treat autoimmune or inflammatory diseases or prevent the rejection of a transplanted cell, tissue, or organ.

[0122] Targeted immunosuppressive antibodies and/or fusion proteins: The aberrant activation of self-reactive T cells and/or breakdown of the mechanisms of immune tolerance promotes the development of autoimmunity that results in various diseases including type I diabetes

mellitus, multiple sclerosis, systemic lupus erythematosus, inflammatory bowel disease, and rheumatoid arthritis. The targeted immunosuppressive antibodies and/or fusion proteins of the invention are designed to suppress unwanted or excessive immune or inflammatory responses and restore or promote immune tolerance. Accordingly, the compositions and methods of the invention have broad clinical relevance for the treatment of diverse autoimmune or inflammatory diseases and preventing the rejection of a transplanted cell, tissue, or organ grafts.

**[0123]** The targeted immunosuppressive antibodies and/or fusion proteins of the invention provides their ability to inhibit the activity of targeted pro-inflammatory cytokines or immune cells while simultaneously promoting immune tolerance via the targeted delivery of immunosuppressive molecules that facilitate the development and/or function of regulatory T cells. These molecules of the present invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) The molecules of the invention enable targeted delivery of immunosuppressive molecules to immune cells or pro-inflammatory molecules in the milieu of the affected cell, tissue or organ; (ii) The molecules of the invention can couple the inhibition of the targeted pro-inflammatory molecule or immune cell with the simultaneous delivery of an immunosuppressive molecule that promotes immune tolerance, thereby improving the suppression of immune effector cells; and (iii) The molecules of the invention can provide a mechanism of simultaneously engaging two independent or synergistic mechanisms of immune tolerance or immune suppression.

**[0124]** Further, the targeted immunosuppressive antibodies and/or fusion proteins of the invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) To suppress unwanted or excessive immune or inflammatory responses in order to treat autoimmune or inflammatory diseases; and (ii) To prevent the rejection of a transplanted cell, tissue, or organ grafts.

**[0125]** In one aspect, the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes a PD-1-binding amino acid sequence corresponding to SEQ ID NO: 99, 100, or 101. In an additional aspect, the immunomodulatory moiety increases the function of PD-1.

[0126] In one aspect, the targeting moiety specifically binds to Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the targeting moiety includes an antibody that binds TNF- $\alpha$ , and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 37. In another aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2), and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from PD-L1. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 38 or 39.

[0127] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25, or CD4, and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 40, 41, 42, 43, 44, or 45.

[0128] In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 46 or 47.

[0129] In one aspect, the targeting moiety includes transforming growth factor- $\beta$  (TGF- $\beta$ ) and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 48 or 49.

[0130] In one aspect, the immunomodulatory moiety includes a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 99, 100, or 101. In an additional aspect, the immunomodulatory moiety activates the signaling function of transforming growth factor- $\beta$  (TGF- $\beta$ ) receptor.

[0131] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), and the immunomodulatory moiety includes a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ). In an additional aspect, the targeting moiety includes an antibody that binds TNF- $\alpha$ , and the immunomodulatory moiety includes a sequence from TGF- $\beta$ . In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 50. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 51 or 52.

[0132] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R), or CD4, and the immunomodulatory moiety includes a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 53, 54, 55, 56, 57 or 58.

[0133] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety includes a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 59 or 60.

[0134] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK) or Osteoprotegerin (OPG). In an additional aspect, the targeting moiety includes an antibody that binds TNF- $\alpha$ , and the immunomodulatory moiety includes a sequence from an extracellular RANKL-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 61. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin

Fc region, and a sequence from an extracellular RANK-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 62 or 63.

[0135] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK) or Osteoprotegerin (OPG). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

[0136] In one aspect, the targeting moiety includes includes a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ) and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

[0137] In one aspect, the targeting moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

[0138] In various aspects, the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

[0139] In another embodiment, the present invention provides a composition including the molecule of the invention and a cell, wherein the cell is a tumor cell, immune cell, or dendritic cell.

[0140] In another embodiment, the present invention provides a method of counteracting or overcoming immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

[0141] In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. In one aspect, the neoplastic disease is a non-T cell malignancy which does not express CD4 on the tumor cell. In one embodiment, the method includes administration to a subject in need thereof an antibody that targets and depletes CD4+ regulatory T cells (Tregs) in combination with a cytotoxic anticancer therapy. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In various aspects, the cytotoxic anticancer therapy includes a chemotherapeutic molecule, tumor-targeted antibody, small molecule kinase inhibitor, hormonal agent, or tumor-targeted cytotoxic agent, anti-angiogenic agent or any combination thereof. In another aspect, the cytotoxic anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

[0142] In another embodiment, the method includes administration to a subject in need thereof an antibody or molecule that targets and depletes CD4+ regulatory T cells (Tregs) in combination with an immunostimulatory antibody, fusion protein, peptide or ligand that targets CTLA-4, PD1, PD-1L, RANKL, TGF- $\beta$ , GITR, 4-1BB, OX-40, or Toll-like receptors (TLR 1-10). In one aspect, the TLR-agonist comprises an activator of TLR-8 or TLR-9. In one aspect, the TLR agonist comprises an immunostimulatory nucleic acid sequence containing CpG nucleotides. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody.

[0143] In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof one or more molecule of the invention. In various aspects, the subject is administered one or more molecule of the invention in combination with another anticancer therapy. In one aspect, the anticancer therapy includes a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent, cytotoxic agent, targeted therapeutic agent, or anti-angiogenic agent. In another aspect, the anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation. In another aspect, the subject is administered one or more molecule of the invention in combination with an antibody or molecule that inhibits the production or function of regulatory T cells (Tregs) or depletes the number of Tregs. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In another aspect, the molecule that counteracts the function of Tregs is an

antibody, fusion protein, peptide or ligand that targets CTLA-4, PD1, PD-1L, RANKL, TGF- $\beta$ , GITR. In another aspect, the molecule that counteracts the function of Tregs is an antibody or fusion protein or ligand that targets 4-1BB or OX-40. In another aspect, the molecule that counteracts the function of Tregs is an agonist of Toll-like receptors (TLR 1-10). In one aspect, the TLR-agonist comprises an activator of TLR-8 or TLR-9. In one aspect, the TLR agonist is an immunostimulatory nucleic acid sequence containing CpG nucleotides.

[0144] In one aspect the chemotherapeutic agent is a topoisomerase-interacting agent, anthracycline, doxorubicin, mitoxantrone, camptothecin, camptothecin analogue, irinotecan, epipodophyllotoxin, etoposide, alkylating agent, cyclophosphamide, cisplatin, cisplatin analogue, oxaliplatin, antimetabolite, fluoropyrimidine analogue, 5-fluorouracil, gemcitabine, azacytidine, antimicrotubule agent, taxane, paclitaxel, or docetaxel.

[0145] In another embodiment, the subject is administered one or more molecule of the invention in combination with any vaccine. In another aspect, the vaccine includes a tumor antigen, tumor-associated antigen, tumor epitope, tumor antigen-containing fusion protein, tumor cell, or dendritic cell. In another aspect, the vaccine includes a pathogen antigen, pathogen-associated antigen, pathogen epitope, or pathogen antigen-containing fusion protein. In one aspect, the vaccine includes a surrogate CD4<sup>+</sup> T cell helper epitope from tetanus toxin. In one aspect, the CD4<sup>+</sup> T helper sequence contains a domain of tetanus toxin fragment C (pDOM1). In one aspect, the pDOM sequence is fused to a cell-permeabilizing cationic polypeptide (e.g. Arginine-9). In another aspect, the Arg9-pDOM sequence is fused to a specific antigen comprising the vaccine.

[0146] In another embodiment, the present invention provides a method for treating immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treatment of a neoplastic disease. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

[0147] In another embodiment, the present invention provides a method of inducing or promoting immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

[0148] In another embodiment, the present invention provides a method of preventing or treating an autoimmune or inflammatory disease including administering to a subject in need thereof one or more molecule of the invention. In one aspect, the subject is administered one or more molecule of the invention in combination with another anti-inflammatory or immunosuppressive therapy. In another embodiment, the present invention provides a method of treatment of immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treating an autoimmune or inflammatory disease or preventing rejection of grafted cells or tissue. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

[0149] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

[0150] As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. Thus, for example, references to “the method” includes one or more methods, and/or steps of the type described herein which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0151] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described.

[0152] As used herein “immune cells” or “immune effector cells” include T lymphocytes, B lymphocytes, natural killer (NK) cells, NKT cells, monocytes, macrophages, dendritic cells (DC), antigen presenting cells (APC).

[0153] As used herein, “neoplasm” or “tumor” including grammatical variations thereof, means new and abnormal growth of tissue, which may be benign or cancerous. In a related aspect, the neoplasm is indicative of a neoplastic disease or disorder, including but not limited,

to various cancers. For example, such cancers can include prostate, pancreatic, biliary, colon, rectal, liver, kidney, lung, testicular, breast, ovarian, pancreatic, brain, and head and neck cancers, melanoma, sarcoma, multiple myeloma, leukemia, lymphoma, and the like.

**[0154]** As used herein, “subject,” including grammatical variations thereof, means a human or vertebrate animal including a dog, cat, horse, cow, pig, sheep, goat, chicken, monkey, rat, and mouse.

**[0155]** As used herein, “targeting moiety” refers to a molecule that has the ability to localize and bind to a specific molecule or cellular component. The targeting moiety can be an antibody, antibody fragment, scFv, Fc-containing polypeptide, fusion antibody, polypeptide, peptide, aptamer, ligand, nucleic acid, or any combination thereof. In one embodiment, a targeting moiety can bind to a molecule present in a cell or tissue. In one aspect, the targeting moiety can bind a molecule in a diseased cell or tissue, such as a cancer cell or tumor. In, another aspect, the targeting molecule can bind a normal cell or tissue, such as an immune cell. In another aspect, the targeting moiety can bind a cellular or extracellular molecule that modulates the immune response. In another aspect, the targeting moiety binds a growth factor receptor, growth factor, cytokine receptor, cytokine, or cell surface molecule.

**[0156]** In another embodiment, the targeting moiety is a tumor-targeting moiety. The tumor-targeting moiety can bind a component of a tumor cell or bind in the vicinity of a tumor cell (e.g., tumor vasculature or tumor microenvironment). In one embodiment, the tumor targeting moiety binds to a component of a tumor cell, tumor microenvironment, tumor vasculature, tumor-associated lymphocyte, tumor antigen, tumor-associated antigen, tumor cell surface molecule, tumor antigenic determinant, tumor antigen-containing fusion protein, tumor-associated cell, tumor-associated immune cell, or tumor vaccine.

**[0157]** For example, in various embodiments, a targeting moiety is specific for or binds to a molecule or component, which includes but is not limited to, epidermal growth factor receptor (EGFR, EGFR1, ErbB-1, HER1), ErbB-2 (HER2/neu), ErbB-3/HER3, ErbB-4/HER4, EGFR ligand family; insulin-like growth factor receptor (IGFR) family, IGF-binding proteins (IGFBPs), IGFR ligand family (IGF-1R); platelet derived growth factor receptor (PDGFR) family, PDGFR ligand family; fibroblast growth factor receptor (FGFR) family, FGFR ligand family, vascular endothelial growth factor receptor (VEGFR) family, VEGF family; HGF

receptor family: TRK receptor family; ephrin (EPH) receptor family; AXL receptor family; leukocyte tyrosine kinase (LTK) receptor family; TIE receptor family, angiopoietin 1, 2; receptor tyrosine kinase-like orphan receptor (ROR) receptor family; discoidin domain receptor (DDR) family; RET receptor family; KLG receptor family; RYK receptor family; MuSK receptor family; Transforming growth factor alpha (TGF- $\alpha$ ), TGF- $\alpha$  receptor; Transforming growth factor-beta (TGF- $\beta$ ), TGF- $\beta$  receptor; Interleukin 13 receptor alpha2 chain (IL13Ralpha2), Interleukin-6 (IL-6), IL-6 receptor, Interleukin-4, IL-4 receptor, Cytokine receptors, Class I (hematopoietin family) and Class II (interferon/IL-10 family) receptors, tumor necrosis factor (TNF) family, TNF- $\alpha$ , tumor necrosis factor (TNF) receptor superfamily (TNFRSF), death receptor family, TRAIL-receptor; cancer-testis (CT) antigens, lineage-specific antigens, differentiation antigens, alpha-actinin-4, ARTC1, breakpoint cluster region-Abelson (Bcr-abl) fusion products, B-RAF, caspase-5 (CASP-5), caspase-8 (CASP-8), beta-catenin (CTNNB1), cell division cycle 27 (CDC27), cyclin-dependent kinase 4 (CDK4), CDKN2A, COA-1, dek-can fusion protein, EFTUD-2, Elongation factor 2 (ELF2), Ets variant gene 6/acute myeloid leukemia 1 gene ETS (ETC6-AML1) fusion protein, fibronectin (FN), GPNMB, low density lipid receptor/GDP-L fucose: beta-Dgalactose 2-alpha-Lfucosyltransferase (LDLR/FUT) fusion protein, HLA-A2, arginine to isoleucine exchange at residue 170 of the alpha-helix of the alpha2-domain in the HLA-A2 gene (HLA-A\*201-R170I), MLA-A11, heat shock protein 70-2 mutated (HSP70-2M), KIAA0205, MART2, melanoma ubiquitous mutated 1,2, 3 (MUM-1, 2,3), prostatic acid phosphatase (PAP), neo-PAP, Myosin class 1, NFYC, OGT, OS-9, pml-RARalpha fusion protein, PRDX5, PTPRK, K-ras (KRAS2), N-ras (NRAS), HRAS, RBAF600, SIRT2, SNRPD1, SYT-SSX1 or -SSX2 fusion protein, Triosephosphate Isomerase, BAGE, BAGE-1, BAGE-2,3,4,5, GAGE-1,2,3,4,5,6,7,8, GnT-V (aberrant N-acetyl glucosaminyl transferase V, MGAT5), HERV-K-MEL, KK-LC, KM-HN-1, LAGE, LAGE-1, CTL-recognized antigen on melanoma (CAMEL), MAGE-A1 (MAGE-1), MAGE-A2, MAGE-A3, MAGE-A4, MAGE-AS, MAGE-A6, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-3, MAGE-B1, MAGE-B2, MAGE-B5, MAGE-B6, MAGE-C1, MAGE-C2, mucin 1 (MUC1), MART-1/Melan-A (MLANA), gp100, gp100/Pmel17 (S1LV), tyrosinase (TYR), TRP-1, HAGE, NA-88, NY-ESO-1, NY-ESO-1/LAGE-2, SAGE, Sp17, SSX-1,2,3,4, TRP2-INT2, carcino-embryonic antigen (CEA), Kallikrein 4, mammaglobin-A, OA1, prostate specific antigen (PSA), prostate specific membrane antigen, TRP-1/gp75, TRP-2, adipophilin, interferon

inducible protein absent in melanoma 2 (AIM-2), BING-4, CPSF, cyclin D1, epithelial cell adhesion molecule (Ep-CAM), EpbA3, fibroblast growth factor-5 (FGF-5), glycoprotein 250 (gp250), intestinal carboxyl esterase (iCE), alpha-feto protein (AFP), M-CSF, mdm-2, MUC1, p53 (TP53), PBF, FRAME, PSMA, RAGE-1, RNF43, RU2AS, SOX10, STEAP1, survivin (BIRC5), human telomerase reverse transcriptase (hTERT), telomerase, Wilms' tumor gene (WT1), SYCP1, BRDT, SPANX, XAGE, ADAM2, PAGE-5, LIP1, CTAGE-1, CSAGE, MMA1, CAGE, BORIS, HOM-TES-85, AF15q14, HCA66I, LDHC, MORC, SGY-1, SPO11, TPX1, NY-SAR-35, FTHL17, NXF2 TDRD1, TEX 15, FATE, TPTE, immunoglobulin idiotypes, Bence-Jones protein, estrogen receptors (ER), androgen receptors (AR), CD40, CD30, CD20, CD19, CD33, CD4, CD25, CD3, cancer antigen 72-4 (CA 72-4), cancer antigen 15-3 (CA 15-3), cancer antigen 27-29 (CA 27-29), cancer antigen 125 (CA 125), cancer antigen 19-9 (CA 19-9), beta-human chorionic gonadotropin, 1-2 microglobulin, squamous cell carcinoma antigen, neuron-specific enolase, heat shock protein gp96, GM2, sargramostim, CTLA-4, 707 alanine proline (707-AP), adenocarcinoma antigen recognized by T cells 4 (ART-4), carcinoembryonic antigen peptide-1 (CAP-1), calcium-activated chloride channel-2 (CLCA2), cyclophilin B (Cyp-B), human signet ring tumor-2 (HST-2), Human papilloma virus (HPV) proteins (HPV-E6, HPV-E7, major or minor capsid antigens, others), Epstein-Barr virus (EBV) proteins (EBV latent membrane proteins—LMP1, LMP2; others), Hepatitis B or C virus proteins, and HIV proteins. A composition of the invention can further include the foregoing as a peptide/polypeptide and/or encoding the same.

**[0158]** In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. In one aspect, the targeting moiety specifically binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), CD20, Vascular endothelial growth factor (VEGF), insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcinoembryonic antigen, Prostate-specific membrane antigen, Mucin-1, CD30, CD33, CD40, or a combination thereof.

**[0159]** Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as trastuzumab (anti-HER2/neu antibody); Pertuzumab (anti-HER2 mAb); cetuximab (chimeric monoclonal

antibody to epidermal growth factor receptor EGFR); panitumumab (anti-EGFR antibody); nimotuzumab (anti-EGFR antibody); Zalutumumab (anti-EGFR mAb); Necitumumab (anti-EGFR mAb); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-447 (humanized anti-EGF receptor bispecific antibody); Rituximab (chimeric murine/human anti-CD20 mAb); Obinutuzumab (anti-CD20 mAb); Ofatumumab (anti-CD20 mAb); Tositumumab-I131 (anti-CD20 mAb); Ibritumomab tiuxetan (anti-CD20 mAb); Bevacizumab (anti-VEGF mAb); Ramucirumab (anti-VEGFR2 mAb); Ranibizumab (anti-VEGF mAb); Aflibercept (extracellular domains of VEGFR1 and VEGFR2 fused to IgG1 Fc); AMG386 (angiopoietin-1 and -2 binding peptide fused to IgG1 Fc); Dalotuzumab (anti-IGF-1R mAb); Gemtuzumab ozogamicin (anti-CD33 mAb); Alemtuzumab (anti-Campath-1/CD52 mAb); Brentuximab vedotin (anti-CD30 mAb); Catumaxomab (bispecific mAb that targets epithelial cell adhesion molecule and CD3); Naptumomab (anti-5T4 mAb); Girentuximab (anti-Carbonic anhydrase ix); or Farletuzumab (anti-folate receptor). Other examples include antibodies such as Panorex™ (17-1A) (murine monoclonal antibody); Panorex (@ (17-1A) (chimeric murine monoclonal antibody); BEC2 (ami-idiotypic mAb, mimics the GD epitope) (with BCG); Oncolym (Lym-1 monoclonal antibody); SMART M195 Ab, humanized 13' 1 LYM-1 (Oncolym), Ovarex (B43.13, anti-idiotypic mouse mAb); 3622W94 mAb that binds to EGP40 (17-1A) pancarcinoma antigen on adenocarcinomas; Zenapax (SMART Anti-Tac (IL-2 receptor); SMART M195 Ab, humanized Ab, humanized); NovoMAb-G2 (pancarcinoma specific Ab); TNT (chimeric mAb to histone antigens); TNT (chimeric mAb to histone antigens); Gliomab-H (Monoclonals—Humanized Abs); GNI-250 Mab; EMD-72000 (chimeric-EGF antagonist); LymphoCide (humanized IL.L.2 antibody); and MDX-260 bispecific, targets GD-2, ANA Ab, SMART IDIO Ab, SMART ABL 364 Ab or ImmuRAIT-CEA. Examples of antibodies include those disclosed in US5736167, US7060808, and US5821337.

**[0160]** In one embodiment, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: CD4; CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF- $\beta$ R); Transforming growth factor-beta (TGF- $\beta$ ); Programmed Death-1 (PD-1); Programmed death-1 ligand (PD-L1 or PD-L2); Receptor

activator of nuclear factor- $\kappa$ B (RANK); Receptor activator of nuclear factor- $\kappa$ B (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); or Interleukin-4 receptor (IL-4R). In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

**[0161]** In one aspect, the targeting moiety binds a specific cytokine, cytokine receptor, co-stimulatory molecule, co-inhibitory molecule, or immunomodulatory receptor that modulates the immune system. In another aspect, the targeting moiety specifically binds one of the following molecules: tumor necrosis factor (TNF) superfamily; tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ); tumor necrosis factor receptor (TNFR) superfamily; Interleukin-12 (IL-12); IL-12 receptor; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNR4); OX40 ligand (OX40L; CD40; CD40 ligand (CD40L); CTLA-4; Programmed death-1 (PD-1); PD-1 ligand 1 (PD-L1; B7-H1); or PD-1 ligand 2 (PD-L2; B7-DC); B7 family; B7-1 (CD80); B7-2 (CD86); B7-H3; B7-H4; GITR/AITR; GITRL/AITRL; BTLA; CD70; CD27; LIGHT; HVEM; Toll-like receptor (TLR)(TLR 1,2,3,4,5,6,7,8,9, 10). In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

**[0162]** In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide, or peptide that specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a cytokine, cytokine receptor, co-stimulatory molecule, or co-inhibitory molecule that modulates the immune system. In another aspect, the targeting moiety specifically binds one of the following molecules: CD4; CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF- $\beta$ R); Transforming growth factor-beta (TGF- $\beta$ ); Programmed Death-1 (PD-1); PD-1 ligand 1 (PD-L1; B7-H1); PD-1 ligand 2 (PD-L2; B7-DC); Receptor activator of nuclear factor- $\kappa$ B (RANK); Receptor activator of nuclear factor- $\kappa$ B (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); Interleukin-4 receptor (IL-4R); tumor necrosis factor (TNF) superfamily; tumor

necrosis factor- $\alpha$  (TNF- $\alpha$ ); tumor necrosis factor receptor (TNFR) superfamily; Interleukin-12 (IL-12); IL-12 receptor; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNFR4); OX40 ligand (OX40L; CD40; CD40 ligand (CD40L); CTLA-4; B7 family; B7-1 (CD80); B7-2 (CD86); B7-H3; B7-H4; GITR/AITR; GITRL/AITRL; BTLA; CD70; CD27; LIGHT; or HVEM. In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

**[0163]** Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as Zanolimumab (anti-CD4 mAb), Keliximab (anti-CD4 mAb); Ipilimumab (MDX-101; anti-CTLA-4 mAb); Tremilimumab (anti-CTLA-4 mAb); (Daclizumab (anti-CD25/IL-2R mAb); Basiliximab (anti-CD25/IL-2R mAb); MDX-1106 (anti-PD1 mAb); antibody to GITR; GC1008 (anti-TGF- $\beta$  antibody); metelimumab/CAT-192 (anti-TGF- $\beta$  antibody); Ierdelimumab/CAT-152 (anti-TGF- $\beta$  antibody); ID11 (anti-TGF- $\beta$  antibody); Denosumab (anti-RANKL mAb); BMS-663513 (humanized anti-4-1BB mAb); SGN-40 (humanized anti-CD40 mAb); CP870,893 (human anti-CD40 mAb); Infliximab (chimeric anti-TNF mAb); Adalimumab (human anti-TNF mAb); Certolizumab (humanized Fab anti-TNF); Golimumab (anti-TNF); Etanercept (Extracellular domain of TNFR fused to IgG1 Fc); Belatacept (Extracellular domain of CTLA-4 fused to Fc); Abatacept (Extracellular domain of CTLA-4 fused to Fc); Belimumab (anti-B Lymphocyte stimulator); Muromonab-CD3 (anti-CD3 mAb); Otelixizumab (anti-CD3 mAb); Teplizumab (anti-CD3 mAb); Tocilizumab (anti-IL6R mAb); REGN88 (anti-IL6R mAb); Ustekinumab (anti-IL-12/23 mAb); Briakinumab (anti-IL-12/23 mAb); Natalizumab (anti- $\alpha$ 4 integrin); Vedolizumab (anti- $\alpha$ 4  $\beta$ 7 integrin mAb); T1h (anti-CD6 mAb); Epratuzumab (anti-CD22 mAb); Efalizumab (anti-CD11a mAb); and Atacicept (extracellular domain of transmembrane activator and calcium-modulating ligand interactor fused with Fc).

**[0164]** In one embodiment, the present invention provides a molecule including a targeting moiety fused with an “immunomodulatory moiety”. As used herein, “immunomodulatory moiety” refers to a ligand, peptide, polypeptide, or Fc-containing polypeptide that binds a specific component of a regulatory T cell, myeloid suppressor cell, or dendritic cell and modulates the number or function of Tregs or myeloid suppressor cells. In an additional

aspect, the “immunomodulatory moiety” specifically binds a cytokine, cytokine receptor, co-stimulatory molecule, or co-inhibitory molecule that modulates the immune system. In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: Transforming growth factor-beta receptor (TGF- $\beta$ R); Transforming growth factor-beta (TGF- $\beta$ ); Programmed Death-1 (PD-1); PD-1 ligand 1 (PD-L1; B7-H1); PD-1 ligand 2 (PD-L2; B7-DC); Receptor activator of nuclear factor- $\kappa$ B (RANK); or Receptor activator of nuclear factor- $\kappa$ B (RANK) ligand (RANKL); or vascular endothelial growth factor (VEGF). In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR; TNFRSF18); GITRL/AITRL; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNR4); OX40 ligand (OX40L); B7-H3; B7-H4; BTLA; CD40; CD40 ligand (CD40L); CD70; CD27; LIGHT; or HVEM. In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ); Interleukin-12 (IL-12); IL-12R; Interleukin-10 (IL-10); IL-10R. In another aspect, the immunomodulatory moiety comprises an extracellular domain of CTLA-4. In one aspect, the immunomodulatory moiety is an agonist that increases the function of the bound molecule. In another aspect, the immunomodulatory moiety is an antagonist that inhibits the function of the targeted molecule.

[0165] In another aspect, the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF- $\beta$ RII, TGF- $\beta$ RIIb, or TGF- $\beta$ RIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor- $\kappa$ B (RANK); vascular endothelial growth factor receptor (VEGFR1 or VEGFR2); or IL-10R. In another aspect, the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: tumor necrosis factor receptor 2 (TNFR2); 4-1BB (CD137); OX40 (CD134; TNR4); CD40; IL-12R; or glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR; TNFRSF18). In an additional aspect, the extracellular domain of the specific receptor binds the cognate ligand and inhibits the interaction of the ligand with its native receptor.

[0166] In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: Transforming growth factor-beta (TGF- $\beta$ ); PD-1 ligand 1 (PD-L1); PD-1 ligand 2 (PD-L2); or IL-10. In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL.

[0167] In another aspect, the immunomodulatory moiety is fused to the C-terminus of the targeting moiety. In another aspect, the immunomodulatory moiety is fused to the N-terminus of the targeting moiety. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is the targeting moiety, Fc is an immunoglobulin Fc region, and Y is the immunomodulatory moiety. In another aspect, the fusion molecule is represented by Y-Fc-X, wherein X is the targeting moiety, and Y is the immunomodulatory moiety. In one aspect, the targeting moiety may additionally be an immunomodulatory moiety.

[0168] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell, and the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF- $\beta$ RII, TGF- $\beta$ RIIb, or TGF- $\beta$ RIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor- $\kappa$ B (RANK); vascular endothelial growth factor receptor (VEGFR1 or VEGFR2); or IL-10R.

[0169] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell, and the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL.

[0170] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds a specific component of a regulatory T cell, myeloid suppressor cell, or dendritic cell, and the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors:

Transforming growth factor-beta receptor (TGF- $\beta$ RII, TGF- $\beta$ RIIb, or TGF- $\beta$ RIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor- $\kappa$ B (RANK); or IL-10R. In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL. In another aspect, the specific targeted component of a regulatory T cell, myeloid suppressor cell, or dendritic cell is one of the following molecules: CD4; CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta (TGF- $\beta$ ); Programmed Death-1 (PD-1); Programmed death-1 ligand (PD-L1 or PD-L2); Receptor activator of nuclear factor- $\kappa$ B (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); or Interleukin-4 receptor (IL-4R).

[0171] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds one of the following: CTLA-4; 4-1BB (CD137); OX40 (CD134; TNR4); CD40; IL-12R; or glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR; TNFRSF18); and the immunomodulatory moiety comprises a different molecule selected from the following: (i) an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF- $\beta$ RII, TGF- $\beta$ RIIb, or TGF- $\beta$ RIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor- $\kappa$ B (RANK); or IL-10R; or (ii) Fc-containing polypeptide or ligand that binds one of the following: CTLA-4; 4-1BB (CD137); OX40 (CD134; TNR4); CD40; IL-12R; or GITR (AITR; TNFRSF18).

[0172] In another aspect, the targeting moiety and immunomodulatory moiety are two different molecules selected from any of the following: an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds TGF- $\beta$ , CTLA-4, PD-1, 4-1BB (CD137), OX40 (CD134; TNR4), CD40; IL-12R, or GITR/AITR (TNFRSF18), or Toll-like receptor (TLR); an extracellular domain or ligand-binding sequence of Transforming growth factor-beta receptor (TGF- $\beta$ RII, TGF- $\beta$ RIIb, or TGF- $\beta$ RIII), Programmed Death-1 (PD-1), Receptor activator of nuclear factor- $\kappa$ B (RANK), or IL-10R. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is an immunomodulatory targeting moiety and Y is a different immunomodulatory moiety.

[0173] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds one of the following molecules: CD4; CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R); or CD20; and the immunomodulatory moiety comprises one of the following ligands or active ligand fragments: Transforming growth factor-beta (TGF- $\beta$ ); PD-1 ligand 1 (PD-L1); PD-1 ligand 2 (PD-L2); or IL-10.

[0174] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), Interleukin-12 (IL-12), IL-6R, B-lymphocyte stimulator, CD11a, CD6, or CD22; and the immunomodulatory moiety comprises one of the following: (i) ligands or active ligand fragments of Transforming growth factor-beta (TGF- $\beta$ ), PD-1 ligand 1 (PD-L1), or IL-10; or (ii) an extracellular domain or ligand-binding fragment of RANK, 4-1BB (CD137), OX40 (CD134; TNR4), CD40, IL-12R or GITR/AITR (TNFRSF18).

[0175] In another aspect, the targeting moiety comprises the extracellular domain of CTLA-4 fused to immunoglobulin Fc, and the immunomodulatory moiety comprises one of the following: (i) ligands or active ligand fragments of Transforming growth factor-beta (TGF- $\beta$ ), PD-1 ligand 1 (PD-L1), or IL-10; or (ii) ligand-binding fragment of TNFR2, RANK, 4-1BB (CD137), OX40 (CD134; TNR4), CD40, IL-12R or GITR/AITR (TNFRSF18).

[0176] In another aspect, the targeting moiety and immunomodulatory moiety are two different molecules selected from any of the following: an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), Interleukin-12 (IL-12), IL-6R, B-lymphocyte stimulator, CD11a, CD6, or CD22; a ligand-binding fragment of TNFR2, RANK, 4-1BB (CD137), OX40 (CD134; TNR4), CD40, IL-12R or GITR/AITR (TNFRSF18); ligands or active ligand fragments of Transforming growth factor-beta (TGF- $\beta$ ), PD-1 ligand 1 (PD-L1), or IL-10; or CTLA-4-Fc. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is the immunomodulatory targeting moiety and Y is a different immunomodulatory moiety.

[0177] Antibodies: In one embodiment, the targeting moiety or fusion protein is an immunoglobulin. As used herein, the term "immunoglobulin" includes natural or artificial mono- or polyvalent antibodies including, but not limited to, polyclonal, monoclonal,

multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin ion can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2) or subclass of immunoglobulin molecule.

[0178] An antibody as disclosed herein includes an antibody fragment, such as, but not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments including either a VL or VH domain. In one embodiment, the targeting moiety is an antibody or scFv.

[0179] An antigen-binding antibody fragment, including single-chain antibody, may include the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. An antigen-binding fragment can also include any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. Also included is a Fc fragment, antigen-Fc fusion proteins, and Fc-targeting moiety. The antibody may be from any animal origin including birds and mammals. In one aspect, the antibody is, or derived from, a human, murine (e.g., mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken. Further, such antibody may be a humanized version of an antibody. The antibody may be monospecific, bispecific, trispecific, or of greater multispecificity.

[0180] The antibody herein specifically include a "chimeric" antibody in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al (1984) Proc. Natl. Acad. Sci. USA.

81:6851-6855). A chimeric antibody of interest herein includes “primatized” antibodies including variable domain antigen-binding sequences derived from a non-human primate (e.g., Old World Monkey, Ape etc) and human constant region sequences.

**[0181]** Various methods have been employed to produce antibodies. Hybridoma technology, which refers to a cloned cell line that produces a single type of antibody, uses the cells of various species, including mice (murine), hamsters, rats, and humans. Another method to prepare an antibody uses genetic engineering including recombinant DNA techniques. For example, antibodies made from these techniques include, among others, chimeric antibodies and humanized antibodies. A chimeric antibody combines DNA encoding regions from more than one type of species. For example, a chimeric antibody may derive the variable region from a mouse and the constant region from a human. A humanized antibody comes predominantly from a human, even though it contains nonhuman portions. Like a chimeric antibody, a humanized antibody may contain a completely human constant region. But unlike a chimeric antibody, the variable region may be partially derived from a human. The nonhuman, synthetic portions of a humanized antibody often come from CDRs in murine antibodies. In any event, these regions are crucial to allow the antibody to recognize and bind to a specific antigen.

**[0182]** In one embodiment, a hybridoma can produce a targeted fusion protein comprising a targeting moiety and an immunomodulatory moiety. In one embodiment, a targeting moiety comprising an antibody, antibody fragment, or polypeptide is linked or fused to an immunomodulatory moiety consisting of a polypeptide, with a linker or without a linker. The linker can be an amino acid linker. In one embodiment, a linker is (GGGGS)<sub>n</sub> wherein n is 1,2,3,4,5,6,7, or 8. For example, GGGGSGGGGSGGGGS (SEQ ID NO: 104). In another embodiment, a linker is EPKSCDK (SEQ ID NO: 105). In another embodiment, a linker is IEGRDMD (SEQ. ID. NO: 106). In various aspects, the length of the linker may be modified to optimize binding of the target moiety or the function of the immunomodulatory moiety. In various aspects, the immunomodulatory moiety is a polypeptide that is fused to the C-terminus of the Fc region of the heavy chain of a targeting antibody or Fc-containing fusion protein. In another aspect, the immunomodulatory moiety is a polypeptide that is fused to the C-terminus of the light chain of a targeting antibody. In another aspect, the fusion protein comprises an X-

Fc-Y sequence, wherein X is a targeting polypeptide and Y is an immunomodulatory polypeptide.

**[0183]** For example, a hybridoma can produce the polypeptides corresponding to SEQ. ID. NO: 1-69.

**[0184]** An antibody fragment can include a portion of an intact antibody, e.g. including the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')<sub>2</sub>, and Fv fragments; Fc fragments or Fc-fusion products; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragment(s).

**[0185]** An intact antibody is one which includes an antigen-binding variable region as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variant thereof for any other modified Fc (e.g. glycosylation or other engineered Fc).

**[0186]** The intact antibody may have one or more "effector functions" which refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region or any other modified Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor (BCR)); and cross-presentation of antigens by antigen presenting cells or dendritic cells. In one embodiment, the targeting antibody or Fc-containing fusion protein facilitates focused or preferential delivery of a immunomodulatory moiety to a target cell. In another aspect, a targeting antibody can induce death of the targeted cell or sensitize it to immune cell-mediated cytotoxicity. In another aspect, the Fc-fusion protein or antibody can facilitate delivery of the immunomodulatory moiety or immunogenic apoptotic material from antibody-bound tumor targets, or both, to an antigen presenting cells (APC) via interactions between their Fc and Fc receptors (on APC).

[0187] Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different “classes.” There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into “subclasses” (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains (that correspond to the different classes of antibodies) are called alpha ( $\alpha$ ), delta ( $\delta$ ), epsilon ( $\epsilon$ ), gamma ( $\gamma$ ), and mu ( $\mu$ ) respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0188] Peptides: In some aspects of the invention the targeting moiety or immunomodulatory moiety is a peptide or polypeptide. A peptide includes any analog, fragment or chemical derivative of a peptide whose amino acid residue sequence is shown herein. Therefore, a present peptide can be subject to various changes, substitutions, insertions, and deletions where such changes provide for certain advantages in its use. In this regard, a peptide of this invention corresponds to, rather than is identical to, the sequence of a recited peptide where one or more changes are made and it retains the ability to function as the unmodified peptide in one or more of the assays.

[0189] The term “analog” includes any peptide having an amino acid residue sequence substantially identical to a sequence specifically shown herein in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the activity as described herein. Examples of conservative substitutions include the substitution of one non-polar (hydrophobic) residue such as isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another.

[0190] The term “fragment” refers to any subject polypeptide having an amino acid residue sequence shorter than that of a polypeptide whose amino acid residue sequence is disclosed herein.

[0191] As used herein “a tumor targeting peptide” includes polymers containing fewer than 100 amino acids, where the polymer specifically binds to a cellular component of a tumor cell, tumor vasculature, and/or a component of a tumor microenvironment.

[0192] A peptide of the present invention can be synthesized by any of the techniques that are known to those skilled in “the polypeptide art, including recombinant DNA techniques. Synthetic chemistry techniques, such as a solid-phase Merrifield-type synthesis, are preferred for reasons of purity, antigenic specificity, freedom from undesired side products, ease of production and the like. An excellent summary of the many techniques available can be found in Steward et al., “Solid Phase Peptide Synthesis”\* W. H. Freeman Co., San Francisco, 1969; Bodanszky, et al., “Peptide Synthesis”, John Wiley & Sons, Second Edition, 1976; J. Meienhofer, “Hormonal Proteins and Peptides”. Vol. 2. p. 46, Academic Press (New York), 1983; Merrifield, Adv. Enzymol., 32:221-96, 1969; Fields et al., Int. J. Peptide Protein Res., 35:161-214, 1990; and U.S. Pat. No. 4,244,946 for solid phase peptide synthesis, and Schroder et al., “The Peptides”, Vol. 1, Academic Press (New York), 1965 for classical solution synthesis. Appropriate protective groups usable in such synthesis are described in the above texts and in J. F. W. McOmie, “Protective Groups in Organic Chemistry”, Plenum Press, New York, 1973.

[0193] Aptamers: In one aspect of the invention, the targeting moiety is an aptamer. In various embodiments, an aptamer is specific for a molecule on a tumor cell, tumor vasculature, and/or a tumor microenvironment. The term “aptamer” includes DNA, RNA or peptides that are selected based on specific binding properties to a particular molecule. For example, an aptamer(s) can be selected for binding a particular gene product in a tumor cell, tumor vasculature, tumor microenvironment, and/or an immune cell, as disclosed herein, where selection is made by methods known in the art and familiar to one of skill in the art. Subsequently, said aptamer(s) can be administered to a subject to modulate or regulate an immune response.

[0194] Some aptamers having affinity to a specific protein, DNA, amino acid and nucleotides have been described (e.g., K. Y. Wang, et al., Biochemistry 32:1899-1904 (1993); Pitner et al., U.S. Pat. No. 5,691,145; Gold, et al., Ann. Rev. Biochem. 64:763-797 (1995); Szostak et al., U.S. Pat. No. 5,631,146). High affinity and high specificity binding aptamers have been

derived from combinatorial libraries (supra, Gold, et al.). Aptamers may have high affinities, with equilibrium dissociation constants ranging from micromolar to sub-nanomolar depending on the selection used, aptamers may also exhibit high selectivity, for example, showing a thousand fold, discrimination between 7-methyl G and G (Haller and Sarnow, Proc. Natl. Acad. Sci. USA 94:8521-8526 (1997)) or between D and L-tryptophan (supra, Gold et al.). An aptamer can be selected based on the particular molecule targeted (e.g., aptamer targeting EGFR or other cancer markers). Standard procedures for in vitro selection are known, such as SELEX experiments, described at Science 249 (4968) 505-510 (1990), and Nature (London), 346 (6287) 818-822 (1990) which can be followed throughout, or with modifications and improvements known in the art.

[0195] The term “therapeutically effective amount” means the amount of the subject compound that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.

[0196] By “pharmaceutically acceptable” it is meant the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

[0197] The terms “administration of” and or “administering” should be understood to mean providing a composition in a therapeutically effective amount to the individual in need of treatment. Administration can be intratumoral or systemic (intravenous) administration. Furthermore, in conjunction with vaccination of recipient with pathogen antigen vaccine (e.g. tetanus toxoid). In addition, in conjunction with agent to deplete or inactivate regulatory T cells (e.g. cyclophosphamide) or myeloid suppressor cells (e.g. gemcitabine). In a further example, *ex vivo* treatment of immune cells and tumor cells for generation of tumor reactive or pathogen antigen reactive immune cells—for adoptive cellular immunotherapy. Administration can be intradermal or subcutaneous.

[0198] Furthermore, administration can be in combination with one or more additional therapeutic agents deplete or inactivate regulatory T cells (cyclophosphamide) or myeloid suppressor cells (e.g. gemcitabine). The pharmaceutical compositions of the invention identified herein are useful for parenteral, topical, oral, nasal (or otherwise inhaled), rectal, or local administration, such as by aerosol or transdermally, for prophylactic and/or therapeutic

treatment of one or more of the pathologies/indications described herein (e.g., cancer, pathogenic infectious agents, associated conditions thereof). The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. Suitable unit dosage forms, include, but are not limited to powders, tablets, pills, capsules, lozenges, suppositories, patches, nasal sprays, injectables, implantable sustained-release formulations, lipid complexes, etc.

[0199] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, as it will be understood that modifications and variations are encompassed within the spirit and scope of the instant disclosure.

[0200] The following examples are provided to further illustrate the embodiments of the present invention, but are not intended to limit the scope of the invention. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

#### EXAMPLE 1

[0201] Counteracting Tumor Immune Tolerance via Antibody-mediated Depletion of CD4<sup>+</sup> Regulatory T Cells Facilitates the Activation of Tumor-Reactive CD8<sup>+</sup> T Cells and Enhances the *In Vivo* Antitumor Efficacy of Cytotoxic Anticancer Agents

[0202] Immunogenic death of tumor cells by chemotherapeutic agents can induce CD8<sup>+</sup> T cell-mediated antitumor immunity. In response to specific chemotherapeutic agents, tumor cells exhibit the rapid translocation of intracellular calreticulin (CRT) to the cell surface where its aggregation provides a signal for the recognition and engulfment of dying tumor cells by antigen presenting dendritic cells (DCs). Treatment of mouse MB49 or human SW780 bladder cancer cells with doxorubicin, an anthracycline chemotherapeutic agent, induced rapid surface exposure of CRT that was detected by immunofluorescence cytometry of cells stained with Dylight 488-labeled anti-CRT antibody (Fig. 1a). To determine whether *ex vivo* treatment with doxorubicin induced an immunogenic death of tumor cells, either untreated live MB49 cells or an equivalent number of MB49 cells that were pre-treated *in vitro* with doxorubicin

were injected into one flank of syngeneic immunocompetent C57BL/6 mice. Unlike mice injected with live tumor cells, mice injected with doxorubicin-treated tumor cells exhibited increased production of IFN- $\gamma$  by draining lymph node (DLN) cells in response to *in vitro* re-challenge with MB49 cell lysates (Fig. 1b). Vaccination with doxorubicin-killed MB49 cells generated a tumor-specific immune response since no corresponding increase in IFN- $\gamma$  secretion by DLN cells was observed following *in vitro* exposure to an irrelevant peptide (Hemagglutinin-HA). Injection of doxorubicin-treated MB49 tumor cells protected mice against tumor growth upon challenge with untreated live MB49 tumor cells injected into the opposite flank. (Fig. 1c). The protection against tumor growth by vaccination with doxorubicin-treated tumor cells was not observed in mice that were depleted of CD8<sup>+</sup> T cells with an anti-CD8 antibody before challenge with live tumor cells (Fig. 1c). These observations indicate that *ex vivo* treatment with chemotherapeutic agents can induce an immunogenic death of tumor cells that generates CD8<sup>+</sup> T cell-mediated adaptive antitumor immunity.

[0203] Tumor-induced immune tolerance inhibits activation of CD8<sup>+</sup> T cells in response to chemotherapy. To examine whether *in vivo* treatment with chemotherapeutic agents can activate CD8<sup>+</sup> T cell-mediated immune responses in mice with pre-established tumors, C57BL/6 mice were injected with live syngeneic MB49 tumor cells and then administered intratumoral doxorubicin at various time points following tumor inoculation. In contrast to vaccination of naïve mice with doxorubicin-killed MB49 cells, *in vivo* treatment of mice with established MB49 tumors at d10 following tumor inoculation failed to induce a corresponding increase in IFN- $\gamma$  secretion by DLN cells in response to *in vitro* re-challenge with MB49 cell lysates (Fig. 1b). Whereas treatment with doxorubicin on d3 following tumor inoculation was able to arrest tumor growth, delayed administration of the same dose of doxorubicin on d10 failed to inhibit the progressive growth of established MB49 tumors (Fig. 1d). These results indicate that tumor-induced immune tolerance in the microenvironment of established cancers counteracts the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death.

[0204] Regulatory T cells (Treg) accumulate in the tumor microenvironment and counteract the ability of chemotherapy to activate CD8<sup>+</sup> T cell mediated antitumor immunity. To investigate whether FoxP3<sup>+</sup> Tregs are involved in enforcing immune tolerance in the tumor

microenvironment, we examined the percentage of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> cells (Tregs) among CD4<sup>+</sup> T lymphocytes in the spleen, draining lymph nodes (DLN), and tumors of immunocompetent C57BL/6 mice at d0 and d14 after tumor inoculation. Whereas tumor-bearing mice exhibited only a minor increase in the percentage of Tregs among CD4<sup>+</sup> T cells in the spleen and DLN at d14 following tumor inoculation, a majority of tumor-infiltrating CD4<sup>+</sup> T cells at this time were CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> cells (Fig. 1e). To investigate whether Tregs infiltrating the tumor microenvironment can suppress the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death, CD4<sup>+</sup>CD25<sup>+</sup> cells isolated from tumors and DLN of tumor-bearing mice were adoptively transferred into syngeneic C57BL/6 naïve mice before vaccination with doxorubicin-killed MB49 cells. The adoptive transfer of tumor-infiltrating CD4<sup>+</sup>CD25<sup>+</sup> cells into naïve mice inhibited the ability of subsequent *in vivo* vaccination with doxorubicin-treated MB49 tumor cells to increase production of IFN- $\gamma$  by draining lymph node (DLN) cells in response to *in vitro* re-challenge with MB49 cell lysates (Fig. 1f). Consistent with the ability of tumor-infiltrating CD4<sup>+</sup>CD25<sup>+</sup> cells to suppress the tumor-specific immune response, the adoptive transfer of these cells counteracted the protection conferred by vaccination with doxorubicin-treated MB49 cells against tumor growth upon challenge with untreated live MB49 tumor cells (Fig. 1g). These results indicate that the tumor microenvironment fosters the accumulation of FoxP3<sup>+</sup> Tregs which counteract the activation of CD8<sup>+</sup> T cell mediated antitumor immunity in response to chemotherapy-induced tumor cell death.

**[0205]** Inhibition of TGF- $\beta$  in the tumor microenvironment reduces tumor-infiltrating FoxP3<sup>+</sup> regulatory T cells and enhances the antitumor efficacy chemotherapy. TGF- $\beta$  induces FoxP3 expression in naïve peripheral CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> T cells and facilitates their conversion into 'adaptive' FoxP3<sup>+</sup> Tregs that share the immunosuppressive ability of natural FoxP3<sup>+</sup> Tregs generated in the thymus. Since human cancers frequently become refractory to the growth-inhibitory effect of TGF- $\beta$  and acquire an ability to increase expression and secretion of TGF- $\beta$ , we investigated whether this switch enables tumor cells to increase the number of adaptive Tregs in the tumor microenvironment. Examination of serum levels of TGF- $\beta$  in mice at d0, d14, and d28 following inoculation of live MB49 tumor cells demonstrated that tumor growth resulted in a progressive increase in the level of serum TGF- $\beta$  (Fig. 2a). To assess the precise source of TGF- $\beta$  in tumor-bearing mice, the total amount of TGF- $\beta$  in supernatants of tumor

cells or draining lymph node cells isolated from tumor-bearing mice were measured following *ex vivo* culture in serum-free medium for 24h. Measurement of the level of TGF- $\beta$ /10<sup>6</sup> cells showed that tumor cells were the dominant source of the increased level of TGF- $\beta$  in tumor-bearing mice (Fig. 2b). In addition to tumor cell-autonomous expression of TGF- $\beta$ , T cells from tumor-bearing mice also expressed higher levels of TGF- $\beta$  compared to their counterparts from tumor-free mice (Fig. 2b). To determine whether the elevation of TGF- $\beta$  is responsible for the upregulation of Tregs in the tumor microenvironment, tumor-bearing mice were treated with a soluble chimeric protein comprising the extracellular domain of TGF $\beta$ RII and the Fc portion of the murine IgG1 heavy chain (TGF $\beta$ RII:Fc). This fusion protein interferes with the binding of TGF- $\beta$  to endogenous TGF $\beta$ RII and functions as a stable TGF- $\beta$  antagonist. ELISA assays confirmed the ability of TGF $\beta$ RII:Fc to sequester TGF- $\beta$  in supernatants of MB49 tumor cells in a concentration-dependent manner (Fig. 2c). At 5d following inoculation of MB49 tumor cells, mice were either left untreated or treated with TGF $\beta$ RII:Fc (1 $\mu$ g intratumoral; twice weekly) for 3 weeks followed by flow cytometric analyses of intracellular FoxP3 expression in CD4<sup>+</sup>CD25<sup>+</sup> T cells infiltrating the tumors. *In vivo* treatment of tumors with TGF $\beta$ RII:Fc resulted in a significant decline in FoxP3 expression in tumor-infiltrating CD4<sup>+</sup> T cells (Fig. 2d) and a dramatic reduction of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> Tregs in tumor tissue (Fig. 2e). To determine whether inhibition of TGF- $\beta$  in the tumor microenvironment can improve the antitumor efficacy of chemotherapy, MB49 tumor-bearing mice were administered doxorubicin (5mg/kg i.p. weekly x 3) with or without twice weekly treatment with TGF $\beta$ RII:Fc (1 $\mu$ g intratumoral). In contrast to treatment with either doxorubicin or TGF $\beta$ RII:Fc alone, combined treatment with both agents was able to arrest the growth of MB49 tumors. These results indicate that tumor cell autonomous expression of TGF- $\beta$  in the tumor microenvironment induces 'adaptive' FoxP3<sup>+</sup> Tregs and that counteracting tumor-induced TGF- $\beta$ -mediated immune tolerance enhances the antitumor efficacy of chemotherapy.

[0206] Anti-CD4 antibody-mediated depletion of CD4<sup>+</sup> regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive CD8<sup>+</sup> T cells and enhances the antitumor efficacy of chemotherapy. To determine whether depletion of CD4<sup>+</sup> regulatory T cells can improve the antitumor efficacy of chemotherapy by enhancing the activity of CD8<sup>+</sup> T cells in the tumor microenvironment, immunocompetent mice bearing syngeneic tumors were

administered an anti-CD4 antibody (Clone GK1.5) to deplete CD4<sup>+</sup> T cells or an anti-CD8 antibody (Clone GK2.43) to deplete CD8<sup>+</sup> T cells and then treated with specific chemotherapeutic agents. Flow cytometric analyses of peripheral blood mononuclear cells from MB49 tumor-bearing mice at d7 following administration of anti-CD4 antibody or anti-CD8 antibody confirmed the target-specific depletion of either CD4<sup>+</sup> T cells or CD8<sup>+</sup> T cells, respectively (Fig. 3a). Mice treated with anti-CD4 antibody showed loss of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> T cells in the peripheral blood as well as among tumor-infiltrating cells (Fig. 3a, b). To determine whether antibody-mediated depletion of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> cells facilitates chemotherapy-induced activation of tumor-reactive CD8<sup>+</sup> T cells in the tumor microenvironment, we evaluated the expression of IFN- $\gamma$  in CD8<sup>+</sup> T cells extracted from the tumor and draining lymph node of MB49 tumor-bearing mice that were left untreated or treated with doxorubicin (with or without anti-CD4 antibody). Flow cytometric analyses showed that CD8<sup>+</sup> T cells from untreated mice did not express IFN- $\gamma$  in response to *in vitro* re-challenge with MB49 cell lysates (Fig. 3c). Whereas IFN- $\gamma$ <sup>+</sup>CD8<sup>+</sup> T cells became evident in mice treated with doxorubicin alone, antibody-mediated depletion of CD4<sup>+</sup> T cells further enhanced the percentage of tumor-reactive CD8<sup>+</sup> T cells that expressed IFN- $\gamma$  in doxorubicin-treated animals (Fig. 3c). To directly evaluate whether the activation of tumor-reactive CD8<sup>+</sup> T cells determines the *in vivo* antitumor efficacy of chemotherapy, we examined the effect of antibody-mediated depletion of CD8<sup>+</sup> T cells or CD4<sup>+</sup> T cells on the response of MB49 tumor-bearing mice to systemic treatment with doxorubicin (5mg/kg). Treatment with doxorubicin alone inhibited the growth of MB49 tumors but failed to arrest tumor progression. Whereas depletion of CD8<sup>+</sup> T cells completely impaired the *in vivo* antitumor efficacy of doxorubicin, depletion of CD4<sup>+</sup> T cells enhanced the response to doxorubicin and resulted in tumor regression (Fig. 3d).

[0207] Anti-CD4 antibody-mediated depletion of CD4<sup>+</sup> regulatory T cells augments and sustains the antitumor effect of chemotherapy by enabling activation of adaptive antitumor immunity. Whereas tumor cells treated with anthracyclins, such as doxorubicin, are particularly effective in eliciting an antitumor immune response, other chemotherapeutic agents are less effective in inducing immunogenic tumor cell death. The surface exposure of calreticulin is a key determinant of the immunogenicity of tumor cell death in response to chemotherapeutic agents. Compared to the efficient translocation of CRT to the cell surface in

response to treatment with doxorubicin (Fig. 1a), treatment of MB49 tumor cells with equitoxic doses of either cisplatin or the combination of cisplatin and gemcitabine was less effective in increasing CRT exposure (Fig. 4a). Whereas tumor-reactive IFN- $\gamma$ <sup>+</sup>CD8<sup>+</sup> T cells were evident in tumors of MB49 tumor-bearing mice treated with doxorubicin (Fig. 3c), treatment with cisplatin was unable to induce a corresponding elevation of IFN- $\gamma$  expression in CD8<sup>+</sup> T cells in response to *in vitro* re-challenge with MB49 cell lysates (Fig 4b). To examine whether counteraction of Treg-mediated immune tolerance enables the activation of antitumor immunity by cisplatin, immunocompetent tumor-bearing mice were treated with cisplatin following depletion of Tregs with anti-CD4 antibody. Antibody-mediated depletion of CD4<sup>+</sup> T cells enhanced the percentage of tumor-reactive IFN- $\gamma$ <sup>+</sup>CD8<sup>+</sup> T cells as well as CD8<sup>+</sup>CD62L<sup>-</sup> T cells in cisplatin-treated animals (Fig. 4b,c). Treatment of MB49 tumor-bearing mice with cisplatin partially inhibited tumor growth but failed to arrest tumor progression. Whereas depletion of CD8<sup>+</sup> T cells completely negated the *in vivo* antitumor effect of cisplatin, depletion of CD4<sup>+</sup> T cells enhanced the response to cisplatin and arrested tumor growth (Fig. 4d). Although treatment of tumor-bearing mice with the combination of cisplatin and gemcitabine was also able to arrest tumor growth, tumor growth rapidly resumed following termination of therapy with none of the animals (0/8) being tumor-free at d50 following tumor inoculation (Fig. 4e). In contrast, mice depleted of CD4<sup>+</sup> T cells exhibited a more sustained response to either single agent or combination chemotherapy, with 7/16 mice exhibiting complete tumor regression. The complete regression of tumors was attended with establishment of adaptive antitumor immunity since none of the cured mice (7/7) developed tumors when re-challenged with live MB49 tumor cells in the opposite flank.

[0208] Chemotherapy-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD4<sup>+</sup> regulatory T cells to stimulate CD8<sup>+</sup> T cell-mediated tumor regression. NKG2D (NK group 2, member D) is a lectin-like type II transmembrane stimulatory receptor used by NK cells,  $\gamma\delta$ -TCR<sup>R+</sup> T cells and  $\alpha\beta$ -TCR<sup>R+</sup> T cells for immune surveillance of tumors. Expression of mouse and human ligands for NKG2D is upregulated in transformed epithelial cell lines in response to genotoxic stress or stalled DNA replication, via activation of a DNA damage checkpoint pathway initiated by ATM (ataxia telangiectasia, mutated) or ATR (ATM- and Rad3-related) protein kinases. Treatment of CT26 mouse colon cancer cells with genotoxic chemotherapeutic agents resulted in upregulation of mouse NKG2D ligands of the

retinoic acid inducible gene family (Rae1)(Fig. 5a). RT-PCR showed that Rae1 mRNA was induced in CT26 cells by 2-4h, reached a plateau after 16-24h, and began to decline after 48h of treatment with either the irinotecan or oxaliplatin (Fig. 5a). Flow cytometric analysis demonstrated that cell surface expression of human NKG2D ligands (MHC-I-related A and B molecules – MICA, MICB) was also upregulated on human colorectal cancer cells (HCT116) in response to treatment with irinotecan (Fig. 1b). Isogenic HCT116 cells that differ only in their p53 status demonstrated that p53 is not required for irinotecan-induced upregulation of MICA/B (Fig. 1b). To examine whether the induction of NKG2D ligands contributes to the antitumor effect of chemotherapy *in vivo*, immunocompetent Balb/C mice inoculated with syngeneic CT26 tumor cells were treated with irinotecan (50 mg/kg i.p) with or without pre-treatment with an NKG2D blocking antibody (200 µg i.p.). Whereas treatment with irinotecan alone inhibited the growth of CT26 tumors, the antitumor effect of irinotecan was negated by pre-treatment with the NKG2D blocking antibody (Fig. 1c). Since engagement of NKG2D by its ligands provides a costimulatory signal for the activation of CD<sup>8+</sup> T cells, we investigated whether DNA damage-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD<sup>4+</sup> regulatory T cells to stimulate CD<sup>8+</sup> T cell-mediated tumor regression. Balb/C mice bearing CT26 tumors were administered an anti-CD4 antibody (Clone GK1.5) to deplete CD<sup>4+</sup> T cells and/or an anti-CD8 antibody (Clone GK2.43) to deplete CD<sup>8+</sup> T cells and then treated with irinotecan. Flow cytometric analyses confirmed the loss of CD<sup>4+</sup>CD2<sup>5+</sup>FoxP<sup>3+</sup> T cells in the spleen and draining lymph node of mice treated with anti-CD4 antibody (Fig. 5d). Antibody-mediated depletion of CD<sup>4+</sup> T cells enhanced the percentage of tumor-reactive IFN- $\gamma$ <sup>+</sup>CD<sup>8+</sup> T cells in irinotecan-treated animals (Fig. 5e). Whereas treatment of CT26 tumor-bearing mice with irinotecan only slowed tumor growth, depletion of CD<sup>4+</sup> T cells enhanced the response to irinotecan and arrested tumor growth (Fig. 5f). The ability of CD<sup>4+</sup> T cell depletion to augment the antitumor efficacy of irinotecan was mediated by CD<sup>8+</sup> T cells since antibody-mediated depletion of CD<sup>8+</sup> T cells completely negated the *in vivo* antitumor effect of chemotherapy in CD<sup>4+</sup> T cell-depleted mice (Fig. 5f).

[0209] These data provide the following insights: (i) activation of tumor-reactive CD<sup>8+</sup> T cells in response to immunogenic tumor cell death is a crucial determinant of the antitumor efficacy of chemotherapy *in vivo*; (ii) tumor-induced Tregs impair the antitumor efficacy of chemotherapy by inhibiting the activation of CD<sup>8+</sup> T cells in the tumor microenvironment; and

(iii) Counteracting tumor-induced immune tolerance via antibody-mediated depletion of CD4<sup>+</sup> regulatory T cells facilitates chemotherapy-induced activation of antitumor immunity with memory, thereby enhancing the antitumor efficacy of chemotherapy; (iv) Strategies to decrease the number or function of CD4<sup>+</sup> regulatory T cells in the tumor microenvironment can increase the activation of CD8<sup>+</sup> T cells and improve the response of tumors to cytotoxic anticancer agents (chemotherapy, tumor-targeted antibodies, targeted therapeutics, kinase inhibitors) or chemoimmunotherapy (combination of chemotherapeutic agent with immunotherapeutic agents).

## EXAMPLE 2

### [0210] Exemplary Targeted Immunomodulatory Antibodies & Fusion Proteins

[0211] A targeting moiety, including an antibody, can be coupled to an immunomodulatory moiety including a polypeptide derived from the extracellular domain of TGFBR2. Crosslinkers or activating agents for such coupling or conjugation are well known in the art. Alternatively, the fusion proteins of the invention can be synthesized using recombination DNA technology well known in the art where the coding sequences of various portions of the fusion proteins can be linked together at the nucleic acid level. Subsequently the fusion proteins of the invention can be produced using a host cell well known in the art. Examples of targeted immunomodulatory antibodies and fusion proteins are shown in Figures 1-33 and briefly described below.

[0212] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety specifically binds to Transforming growth factor-beta (TGF- $\beta$ ). SEQ ID NO: 1 provides a fusion protein including anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD) (Figure 2). SEQ ID NO: 2 provides a fusion protein including anti-EGFR1 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD) (Figure 3). SEQ ID NO: 3 provides a fusion protein including anti-CD20 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD) (Figure 4). SEQ ID NO: 4 provides a fusion protein including anti-VEGF antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain

(ECD) (Figure 5). SEQ ID NO: 5 provides a fusion protein including anti-human CTLA-4 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD) (Figure 6). SEQ ID NO: 6 provides a fusion protein including IL-2, Fc, and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD) (Figure 7). SEQ ID NO: 7 provides a fusion protein including Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD), Fc, and IL-2 (Figure 7). SEQ ID NO: 8 provides a fusion protein including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD) (Figure 8A). SEQ ID NO: 9 provides a fusion protein including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD) (Figure 8B). SEQ ID NO: 10 provides a fusion protein including anti-CD4 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD) (Figure 9). SEQ ID NO: 11 provides a fusion protein including PD-1 Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ectodomain) (PD-1 ectodomain + Fc + TGF $\beta$ RII ectodomain; Figure 10). SEQ ID NO: 12 provides a fusion protein including Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ectodomain), Fc, and PD-1 Ectodomain (TGF $\beta$ RII ectodomain + Fc + PD-1 ectodomain; Figure 10). SEQ ID NO: 13 provides a fusion protein including RANK Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ectodomain) (RANK ectodomain + Fc + TGF $\beta$ RII ectodomain; Figure 11). SEQ ID NO: 14 provides a fusion protein including Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ectodomain), Fc, and RANK Ectodomain (TGF $\beta$ RII ectodomain + Fc + RANK ectodomain; Figure 11).

[0213] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety is a molecule that specifically binds to Programmed Death-1 ligand 1 (PD-L1 or B7-H1) or Programmed Death-1 ligand 2 (PD-L2 or B7-DC). SEQ ID NO: 15 provides a fusion protein including anti-HER2/neu antibody and PD-1 Ectodomain (Figure 13). SEQ ID NO: 16 provides a fusion protein including anti-EGFR1 antibody and PD-1 Ectodomain (Figure 14). SEQ ID NO: 17 provides a fusion protein including anti-CD20 antibody and PD-1 Ectodomain (Figure 15). SEQ ID NO: 18 provides a fusion protein including anti-VEGF antibody and PD-1

Ectodomain (Figure 16). SEQ ID NO: 19 provides a fusion protein including anti-human CTLA-4 antibody and PD-1 Ectodomain (Figure 17). SEQ ID NO: 20 provides a fusion protein including anti-CD25 antibody and PD-1 Ectodomain (Figure 18A). SEQ ID NO: 21 provides a fusion protein including anti-CD25 antibody and PD-1 Ectodomain (Figure 18B). SEQ ID NO: 22 provides a fusion protein including IL-2, Fc, and PD-1 ectodomain (IL-2 + Fc + PD-1 ectodomain; Figure 19). SEQ ID NO: 23 provides a fusion protein including PD-1 ectodomain, Fc, and IL-2 (PD-1 ectodomain + Fc + IL-2; Figure 19). SEQ ID NO: 24 provides a fusion protein including anti-CD4 antibody and PD-1 Ectodomain (Figure 20). SEQ ID NO: 25 provides a fusion protein including RANK Ectodomain, Fc, and PD-1 ectodomain (RANK ectodomain + Fc + PD-1 ectodomain; Figure 21). SEQ ID NO: 26 provides a fusion protein including PD-1 ectodomain, Fc, and RANK Ectodomain (PD-1 ectodomain + Fc + RANK ectodomain; Figure 21).

**[0214]** In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety is a molecule that specifically binds to Receptor activator of NF- $\kappa$ B ligand (RANKL). SEQ ID NO: 27 provides a fusion protein including anti-HER2/neu antibody and RANK Ectodomain (Figure 23). SEQ ID NO: 28 provides a fusion protein including anti-EGFR1 antibody and RANK Ectodomain (Figure 24). SEQ ID NO: 29 provides a fusion protein including anti-CD20 antibody and RANK Ectodomain (Figure 25). SEQ ID NO: 30 provides a fusion protein including anti-VEGF antibody and RANK Ectodomain (Figure 26). SEQ ID NO: 31 provides a fusion protein including anti-human CTLA-4 antibody and RANK Ectodomain (Figure 27). SEQ ID NO: 32 provides a fusion protein including anti-CD25 antibody and RANK Ectodomain (Figure 28A). SEQ ID NO: 33 provides a fusion protein including anti-CD25 antibody and RANK Ectodomain (Figure 28B). SEQ ID NO: 34 provides a fusion protein including IL-2, Fc, and RANK ectodomain (IL-2 + Fc + RANK ectodomain; Figure 29). SEQ ID NO: 35 provides a fusion protein including RANK ectodomain, Fc, and IL-2 (RANK ectodomain + Fc + IL-2; Figure 29). SEQ ID NO: 36 provides a fusion protein including anti-CD4 antibody and RANK Ectodomain (Figure 30).

[0215] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety includes a molecule that specifically binds to Programmed death-1 (PD-1). SEQ ID NO: 37 provides a fusion protein including anti-tumor necrosis factor (TNF $\alpha$ ) antibody and PD-1 ligand 1 (Figure 32). SEQ ID NO: 38 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand: (TNFR2 ECD + IgG C $\gamma$ 1 + PD-L1; Figure 33). SEQ ID NO: 39 provides a fusion protein including PD-1 ligand, Fc, and TNFR2 Extracellular ligand binding domain: (PD-L1 + IgG C $\gamma$ 1 -TNFR2 ECD; Figure 33). SEQ ID NO: 40 provides a fusion protein including anti-CD20 antibody and PD-1 ligand 1 (PD-L1) (Figure 34). SEQ ID NO: 41 provides a fusion protein including anti-CD25 antibody and PD-1 ligand 1 (PD-L1) (Figure 35A). SEQ ID NO: 42 provides a fusion protein including anti-CD25 antibody and PD-1 ligand 1 (PD-L1) (Figure 35B). SEQ ID NO: 43 provides a fusion protein including PD-1 ligand 1 (PD-L1), Fc, and IL-2 (PD-L1 – Fc – IL2; Figure 36). SEQ ID NO: 44 provides a fusion protein including IL-2, Fc, and PD-1 ligand 1 (PD-L1) (IL-2 – Fc – PD-L1; Figure 36). SEQ ID NO: 45 provides a fusion protein including anti-CD4 antibody and PD-1 ligand 1 (PD-L1) (Figure 37). SEQ ID NO: 46 provides a fusion protein including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from PD-1 ligand (PD-L1) (Oncostatin M signal peptide + CTLA-4 ECD + IgG C $\gamma$ 1 + PD-L1; Figure 38). SEQ ID NO: 47 provides a fusion protein including the extracellular domain of PD-1 ligand (PD-L1), immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from the extracellular domain of CTLA-4: (PD-L1 + IgG C $\gamma$ 1 + CTLA-4 ECD; Figure 38). SEQ ID NO: 48 provides a fusion protein including Transforming growth factor- $\beta$  (TGF- $\beta$ ), immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from PD-1 ligand 1 (PD-L1) (TGF $\beta$ -1 + Fc + PD-L1; Figure 39). SEQ ID NO: 49 provides a fusion protein including a sequence from PD-1 ligand 1 (PD-L1), immunoglobulin Fc (IgG C $\gamma$ 1), and Transforming growth factor beta (TGF- $\beta$ ) (PD-L1 + Fc + TGF $\beta$ -1; Figure 39).

[0216] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety includes a molecule that specifically binds to Transforming growth factor-beta receptor (TGF- $\beta$ R). SEQ ID NO:

50 provides a fusion protein including an antibody that binds TNF- $\alpha$ , and a sequence from Transforming growth factor- $\beta$  (TGF- $\beta$ ) (Figure 41). SEQ ID NO: 51 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and a sequence from Transforming growth factor- $\beta$  (TGF- $\beta$ ) (TNFR2 ECD + IgG C $\gamma$ 1 + TGF- $\beta$ ; Figure 42). SEQ ID NO: 52 provides a fusion protein including a sequence from Transforming growth factor- $\beta$  (TGF- $\beta$ ), Fc, and TNFR2 Extracellular ligand binding domain: (TGF- $\beta$  + IgG C $\gamma$ 1 + TNFR2 ECD; Figure 42). SEQ ID NO: 53 provides a fusion protein including anti-CD20 antibody and a sequence from Transforming growth factor- $\beta$  (TGF- $\beta$ ) (Figure 43). SEQ ID NO: 54 provides a fusion protein including anti-CD25 antibody and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ) (Figure 44A). SEQ ID NO: 55 provides a fusion protein including anti-CD25 antibody and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ) (Figure 44B). SEQ ID NO: 56 provides a fusion protein including a sequence from Transforming growth factor- $\beta$  (TGF- $\beta$ ), Fc, and IL-2 (TGF- $\beta$  + Fc + IL-2; Figure 45). SEQ ID NO: 57 provides a fusion protein including IL-2, Fc, and Transforming growth factor- $\beta$  (TGF- $\beta$ )(IL-2 + Fc + TGF- $\beta$ ; Figure 45). SEQ ID NO: 58 provides a fusion protein including anti-CD4 antibody and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ) (Figure 46). SEQ ID NO: 59 provides a fusion protein including the extracellular domain of CTLA-4, immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ) (Oncostatin M signal peptide + CTLA-4 ECD + IgG C $\gamma$ 1 + TGF- $\beta$ 1; Figure 47). SEQ ID NO: 60 provides a fusion protein including a sequence from Transforming growth factor- $\beta$  (TGF- $\beta$ ), immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from the extracellular domain of CTLA-4: (TGF- $\beta$ 1 + IgG C $\gamma$ 1 + CTLA-4 ECD) (Figure 47).

[0217] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety is a molecule that specifically binds to Receptor activator of NF- $\kappa$ B ligand (RANKL). SEQ ID NO: 61 provides a fusion protein including an antibody that binds TNF- $\alpha$ , and a sequence from RANK Ectodomain (Figure 48). SEQ ID NO: 62 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and a sequence from RANK Ectodomain (TNFR2 ECD + IgG C $\gamma$ 1 + RANK Ectodomain; Figure 49). SEQ ID NO: 63 provides a fusion protein including a sequence from RANK Ectodomain, Fc, and TNFR2 Extracellular ligand binding

domain: (RANK Ectodomain + IgG C $\gamma$ 1 + TNFR2 ECD; Figure 49). SEQ ID NO: 64 provides a fusion protein including the extracellular domain of CTLA-4, immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from a sequence from RANK Ectodomain (Oncostatin M signal peptide + CTLA-4 ECD + IgG C $\gamma$ 1 + RANK Ectodomain; Figure 50). SEQ ID NO: 65 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from the extracellular domain of CTLA-4: (RANK Ectodomain + IgG C $\gamma$ 1 + CTLA-4 ECD) (Figure 50). SEQ ID NO: 66 provides a fusion protein including a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ), immunoglobulin Fc region (IgG C $\gamma$ 1), and an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK) (TGF- $\beta$  + IgG C $\gamma$ 1 + RANK Ectodomain; Figure 51). SEQ ID NO: 67 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ): (RANK Ectodomain + IgG C $\gamma$ 1 + TGF- $\beta$ ) (Figure 51). SEQ ID NO: 68 provides a fusion protein including a sequence from Programmed death-1 ligand 1 (PD-L1), immunoglobulin Fc region (IgG C $\gamma$ 1), and an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK) (PD-L1 + IgG C $\gamma$ 1 + RANK Ectodomain; Figure 52). SEQ ID NO: 69 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from Programmed death-1 ligand 1 (PD-L1): (RANK Ectodomain + IgG C $\gamma$ 1 + PD-L1) (Figure 52).

**[0218]** Although the invention has been described with reference to the above example, it will be understood that modifications and variations are encompassed within the spirit and scope of the invention. Accordingly, the invention is limited only by the following claims.

What is claimed is:

1. A molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:
  - (a) the targeting moiety specifically binds a target molecule, and
  - (b) the immunomodulatory moiety specifically binds to a molecule selected from:
    - (i) Transforming growth factor-beta (TGF- $\beta$ ); Transforming growth factor-beta receptor (TGF- $\beta$ R); Programmed death-1 ligand 1 (PD-L1; B7-H1) or Programmed death-1 ligand 2 (PD-L2; B7-DC); Programmed death-1 (PD-1); Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL); or Receptor activator of nuclear factor- $\kappa$ B (RANK); and
    - (ii) 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNFR4); OX40 ligand (OX40L); glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR); GITR ligand (GITRL); HVEM; LIGHT; CD27; or CD70.
2. The molecule of claim 1, wherein the targeting moiety comprises an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell.
3. The molecule of claim 2, wherein the immunomodulatory moiety comprises a molecule that binds to TGF- $\beta$
4. The molecule of claim 3, wherein the immunomodulatory moiety comprises an extracellular ligand-binding domain of Transforming growth factor-beta receptor TGF- $\beta$ RII, TGF- $\beta$ RIIb, or TGF- $\beta$ RIII.
5. The molecule of claim 4, wherein the immunomodulatory moiety comprises an extracellular ligand-binding domain of TGF- $\beta$ RII.

6. The molecule of claim 5, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, epidermal growth factor receptor (EGFR1), CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R), or CD4.
7. The molecule of claim 5, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.
8. The molecule of claim 5, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to Programmed Death-1 (PD-1), Programmed death-1 ligand 1 (PD-L1), or Programmed death-1 ligand 2 (PD-L2).
9. The molecule of claim 8, wherein the targeting moiety comprises an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1).
10. The molecule of claim 9, wherein the molecule comprises PD-1 ectodomain, immunoglobulin Fc region, and TGF $\beta$ RII ectodomain.
11. The molecule of claim 10, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 11 or 12.
12. The molecule of claim 5, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- $\kappa$ B (RANK) or Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL).
13. The molecule of claim 12, wherein the targeting moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK).
14. The molecule of claim 13, wherein the molecule comprises RANK ectodomain, immunoglobulin Fc region, and TGF $\beta$ RII ectodomain.
15. The molecule of claim 14, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

16. The molecule of claim 1, wherein the immunomodulatory moiety comprises a molecule that specifically binds to Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2).
17. The molecule of claim 16, wherein the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1).
18. The molecule of claim 17, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, Epidermal growth factor receptor (EGFR1), CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R), or CD4.
19. The molecule of claim 18, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.
20. The molecule of claim 16, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- $\kappa$ B (RANK) or Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL).
21. The molecule of claim 20, wherein the targeting moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK).
22. The molecule of claim 21, wherein the molecule comprises RANK ectodomain, immunoglobulin Fc region, and PD-1 ectodomain.
23. The molecule of claim 22, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 25 or 26.
24. The molecule of claim 1, wherein the immunomodulatory moiety comprises a molecule that specifically binds to Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL).

25. The molecule of claim 24, wherein the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK).
26. The molecule of claim 25, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, Epidermal growth factor receptor (EGFR1), CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R), or CD4.
27. The molecule of claim 26, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36.
28. The molecule of claim 1, wherein the immunomodulatory moiety comprises an antibody or ligand that specifically binds to 4-1BB, OX40, or GITR/AITR.
29. The molecule of claim 28, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, Epidermal growth factor receptor (EGFR1), CD20, vascular endothelial growth factor (VEGF), CD25, CD4, cytotoxic T-lymphocyte antigen-4 (CTLA-4), Transforming growth factor-beta (TGF- $\beta$ ), Programmed Death-1 (PD-1), Programmed death-1 ligand (PD-L1 or PD-L2), Receptor activator of nuclear factor- $\kappa$ B (RANK) ligand (RANKL), glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18), 4-1BB, OX40, or Interleukin-4 receptor (IL-4R).
30. The molecule of claim 1, wherein the targeting moiety specifically binds to Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), and the immunomodulatory moiety comprises a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2).
31. The molecule of claim 30, wherein the targeting moiety comprises an antibody that binds TNF- $\alpha$  or an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2), and the immunomodulatory moiety comprises a sequence from PD-1 ligand 1 (PD-L1 or B7-H1).
32. The molecule of claim 31, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 37, 38, or 39.

33. The molecule of claim 1, wherein the targeting moiety comprises an antibody, antibody fragment, or a peptide that specifically binds to CD20, CD25, or CD4, and the immunomodulatory moiety comprises a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2).
34. The molecule of claim 33, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 40, 41, 42, 43, or 44.
35. The molecule of claim 1, wherein the targeting moiety comprises an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety comprises a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2).
36. The molecule of claim 35, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 46 or 47.
37. The molecule of claim 1, wherein the targeting moiety comprises transforming growth factor- $\beta$  (TGF- $\beta$ ) and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety comprises a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2).
38. The molecule of claim 37, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 48 or 49.
39. The molecule of claim 1, wherein the immunomodulatory moiety comprises a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ).
40. The molecule of claim 39, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), CD20, CD25 (IL-2 $\alpha$  receptor; IL-2 $\alpha$ R), or CD4, and the immunomodulatory moiety comprises a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ).
41. The molecule of claim 40, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 50, 51, 52, 53, 54, 55, 56, 57, or 58.

42. The molecule of claim 39, wherein the targeting moiety comprises an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety comprises a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ).
43. The molecule of claim 42, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 59 or 60.
44. The molecule of claim 25, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), and the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK).
45. The molecule of claim 44, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 61, 62, or 63.
46. The molecule of claim 25, wherein the targeting moiety comprises an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK).
47. The molecule of claim 46, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 64 or 65.
48. The molecule of claim 25, wherein the targeting moiety comprises a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ) and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK).
49. The molecule of claim 48, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

50. The molecule of claim 25, wherein the targeting moiety comprises a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG C $\gamma$ 1), and the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- $\kappa$ B (RANK).
51. The molecule of claim 50, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 68 or 69.
52. The molecule of claim 1, wherein the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.
53. A composition comprising the molecule of claim 1 and a cell, wherein the cell is a tumor cell, immune cell, or dendritic cell.
54. A method of counteracting or overcoming immune tolerance comprising: administering to a subject in need thereof one or more molecule of claims 1-29.
55. A method of preventing or treating a neoplastic disease comprising: administration to a subject in need thereof one or more molecule of claims 1-29.
56. The method of claim 55, wherein the subject is administered in combination with another anticancer therapy.
57. The method of claim 55, wherein the anticancer therapy comprises a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent, ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.
58. The method of claim 55, wherein the subject is administered in combination with a vaccine.
59. The method of claim 55, wherein the subject is human.
60. A method for treating immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of claims 1-29.

61. A method of treatment of a neoplastic disease comprising:  
administering to a subject in need thereof a composition comprising immune cells  
contacted with a molecule of claims 1-29.
62. A method of treatment of a neoplastic disease comprising:  
administering to a subject in need thereof an antibody or Fc-containing fusion protein  
that targets CD4 in combination with another therapy, wherein the neoplastic disease is  
not a T cell malignancy, and the therapy comprises one or more of the following:  
(i) an immunostimulatory molecule;  
(ii) a tumor-targeted antibody; or  
(iii) a cytotoxic anticancer agent.
63. The method of claim 62, wherein the immunostimulatory molecule comprises an  
immunostimulatory antibody or fusion protein of claims 1-29.
64. The method of claim 62, wherein the immunostimulatory molecule comprises an  
antibody, antibody fragment, Fc-containing polypeptide, peptide or ligand that  
specifically binds to cytotoxic T-lymphocyte antigen-4 (CTLA4), Programmed death-1  
(PD-1), Programmed death-1 ligand 1 (PD-1L1; B7-H1), Programmed death-1 ligand 2  
(PD-1L2; B7-DC), OX40 (CD134; TNFR4), 4-1BB (CD137), CD40, or glucocorticoid-  
induced tumor necrosis factor receptor family-related gene (GITR; AITR;  
TNFRSF18), or transforming growth factor- $\beta$  (TGF- $\beta$ ).
65. The method of claim 62, wherein the tumor-targeted antibody comprises an antibody,  
antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a  
component of a tumor cell, tumor antigen, tumor vasculature, tumor  
microenvironment, or tumor-infiltrating immune cell.
66. The method of claim 62, wherein the cytotoxic anticancer agent comprises a  
chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent  
or ionizing radiation.

67. A method of preventing or treating an autoimmune or inflammatory disease comprising:  
administering to a subject in need thereof one or more molecule of claims 30-51.

1. Amino acid sequences of transforming growth factor beta receptor type II (TGF- $\beta$ -RII) or TGF- $\beta$ -RIIB or a fragment thereof:

(i) Transforming growth factor beta receptor type II (TGF- $\beta$ -RII) (SEQ ID NO: 79):

MGRGLLRGLW PLHIVLWTRI *ASTIPPHVQK* *SVNNDMIVTD* *NNGAVKFPQL* *CKFCDVRFST*  
*CDNQKSCMSN* *CSITSICEKP* *QEVCAVWRK* *NDENITLET* *CHDPKLPYHD* *FILEDAASPK*  
*CIMKEKKKPG* *ETFFMCSCSS* *DECNDNIIFS* *EEYNTSNPDL* *LLVIFQVTGI* *SLLPPLGVAI*  
SVIIIFYCYR VNRQQKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT  
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK  
HENILQFLTA EERKTELKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH  
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQGQVG  
TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE  
HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC WDHDPEARLT AQCVAERFSE  
LEHLDRLSGR SCSEEKIPED GSLNTTK

[Italic – Extracellular domain of Transforming growth factor beta Receptor II (TGF $\beta$  RII)]  
[Underlined = TGF $\beta$  RII Extracellular domain (ECD) region that binds TGF- $\beta$ ]

(ii) Transforming growth factor beta receptor type IIB (TGF- $\beta$ -RIIB) (SEQ ID NO: 80):

MGRGLLRGLW PLHIVLWTRI *ASTIPPHVQK* *SDVEMEAQKD* *EIICPSCNRT* *AHPLRHINND*  
*MIVTDNNGAV* *KFPQLCKFCD* *VRFSTCDNQK* *SCMSNCSITS* *ICEKPQEVCAVWRKNDENI*  
*TLETVCHDPK* *LPYHDFILED* *AASPKCIMKE* *KKKPGETFFM* *CSCSSDECND* *NIIFSEEYNT*  
*SNPDL* *LLVIF* *QVTGISLLPP* *LGVAISVIII* *FYCYRVNRQQ* *KLSSTWETGK* *TRKLMEFSEH*  
CAIILEDERS DISSTCANNI NHNTELLPIE LDTLVGKGRF AEVYKAKLKQ NTSEQFETVA VKI FP  
YEEYASWKTE KDIFSDINLK HENILQFLTA EERKTELKQ YWLITAFHAK GNLQEYLTRH  
VISWEDLRKL GSSLARGIAH LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL  
SLRLDPTLSV DDLANSQGQVG TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR  
CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC  
WDHDPEARLT AQCVAERFSE LEHLDRLSGR SCSEEKIPED GSLNTTK

[Italic – Extracellular domain of Transforming growth factor beta Receptor IIB (TGF $\beta$  RIIB)]  
[Underlined = TGF $\beta$  RIIB Extracellular domain (ECD) region that binds TGF- $\beta$ ]

2. Truncated mutants of Transforming growth factor beta Receptor II (TGF- $\beta$ -RII) or TGF- $\beta$ -RIIB comprising the Extracellular domain (ECD) region that binds TGF- $\beta$

(i) TGF- $\beta$  R-II ( $\Delta$ C terminus): TGF $\beta$  RII lacking the last 38 amino acids from the C-terminus (SEQ ID NO: 81):

MGRGLLRGLW PLHIVLWTRI *ASTIPPHVQK* *SVNNDMIVTD* *NNGAVKFPQL* *CKFCDVRFST*  
*CDNQKSCMSN* *CSITSICEKP* *QEVCAVWRK* *NDENITLET* *CHDPKLPYHD* *FILEDAASPK*  
*CIMKEKKKPG* *ETFFMCSCSS* *DECNDNIIFS* *EEYNTSNPDL* *LLVIFQVTGI* *SLLPPLGVAI*  
SVIIIFYCYR VNRQQKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT  
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK  
HENILQFLTA EERKTELKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH  
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQGQVG  
TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE  
HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC WDHDPEARLT

Fig. 1A

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TGF- $\beta$  R-IIB ( $\Delta$ C terminus): TGF $\beta$  RII lacking the last 38 aa from the C-terminus (SEQ ID NO: 82):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND  
 MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS ICEKPQEVAVWRKNDENI  
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT  
SNPDLVVIF QVTGISLLPP LGVAISVIII FYCYRVNRQQ KLSSTWETGK TRKLMEFSEH  
 CAIILEDERS DISSTCANNI NHNTELLPIE LDTLVGKGRF AEVYKAKLKQ NTSEQFETVA VKI FP  
 YEEYASWKTE KDIFSDINLK HENILQPLTA BERKTELKQ YWLITAFHAK GNLQEYLTRH  
 VISWEDLRKL GSSLARGIAH LHSHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL  
 SLRLDPTLSV DDLANSQGVG TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR  
 CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC  
 WDHDPEARL

(ii) TGF- $\beta$  R-II ( $\Delta$ cyt): TGF $\beta$  RII lacking the kinase domain & juxtamembrane region (SEQ ID NO: 83):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST  
CDNQKSCMSN CSITSICEKP QEVAVWRKNDENITLET CHDPKLPYHD FILEDAASPK  
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD LLVIFQVTGI SLLPPLGVAI  
 SVIIIFYCYR VNRQQKLSS

TGF- $\beta$  R-IIB ( $\Delta$ cyt): TGF $\beta$  RII lacking the kinase domain & juxtamembrane region (SEQ ID NO: 84):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND  
 MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS ICEKPQEVAVWRKNDENI  
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT  
SNPDLVVIF QVTGISLLPP LGVAISVIII FYCYRVNRQQ KLSS

(iii) TGF- $\beta$  R-II containing the N-terminus region including the extracellular domain (SEQ ID NO: 85):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST  
CDNQKSCMSN CSITSICEKP QEVAVWRKNDENITLET CHDPKLPYHD FILEDAASPK  
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD LLVIFQ

TGF- $\beta$  R-IIB containing the N-terminus region including the extracellular domain (SEQ ID NO: 86):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND  
 MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS ICEKPQEVAVWRKNDENI  
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT  
SNPDLVVIF Q

(iv) TGF- $\beta$  R-II containing the extracellular domain that binds TGF- $\beta$  (SEQ ID NO: 87):

TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP  
QEVAVWRKNDENITLET CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS  
DECNDNIIFS EYNTSNPD

TGF- $\beta$  R-IIB containing the extracellular domain that binds TGF- $\beta$  (SEQ ID NO: 88):

TIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND MIVTDNNGAV KFPQLCKFCD  
VRFSTCDNQK SCMSNCSITS ICEKPQEVAVWRKNDENI TLETVCHDPK LPYHDFILED  
AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT SNPD

(v) TGF- $\beta$  R-II containing the region of the extracellular domain that binds TGF- $\beta$  (SEQ ID NO: 89):

PQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVAVWRKNDENITLET CHDPKLPYHD  
FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

Fig. 1B

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3. Kinase-deficient mutants, deletion mutants, or point mutants of Transforming growth factor beta Receptor II (TGF $\beta$ -RII) or TGF $\beta$ -RIIB or a fragment thereof which binds TGF- $\beta$

(i) Transforming growth factor beta Receptor II containing point mutations:

Amino acid sequence of TGF- $\beta$  R-II (K277R) contains a point mutation in its ATP-binding site and is inactive as a kinase (SEQ ID NO: 90):

```
MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPDL LLVIFQVTGI SLLPPLGVAI
SVIIIFYCYR VNRQQKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVRIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQQVG
TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
LEHLDRLSGR SCSEKIPED GSLNNTTK
```

(ii) Transforming growth factor beta Receptor II containing deletions in the amino acid sequence (deletion mutants): Transforming growth factor beta Receptor II ( $\Delta$ i)

TGF- $\beta$  R-II ( $\Delta$ i2) contains a deletion of amino acids 498 to 508 and is inactive as a kinase (SEQ ID NO: 91):

```
MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPDL LLVIFQVTGI SLLPPLGVAI
SVIIIFYCYR VNRQQKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQQVG
TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
HPCVESMKDA SGIQMVCELT TECWDHDPEA RLTAQCVAER FSELEHLDR LSGRSCSEKI
PEDGSLNNTTK
```

4. Amino acid sequences of transforming growth factor beta receptor type III (TGF- $\beta$ -RIII) or or a fragment thereof.
5. Hybrid or fusion proteins containing amino acid sequences of transforming growth factor beta receptor type II (TGF- $\beta$ -RII) and TGF- $\beta$ -RIII.
6. Hybrid or fusion proteins containing amino acid sequences of transforming growth factor beta receptor type IIB (TGF- $\beta$ -RIIB) and TGF- $\beta$ -RIII.

Linker sequence used in fusion proteins (SEQ ID NO: 104):

GGGGSGGGSGGGGS

Fig. 1C

**Fusion proteins comprising Anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD).**

Anti-HER2/neu heavy chain + TGF $\beta$ -RII ECD fusion amino acid sequence

(SEQ ID NO: 1):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKG  
RFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGPSVFPLA  
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT  
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC  
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA  
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT  
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK  
**GGGGSGGGSGGGGS** **TIPPHVQK** **SVNNDMIVTD** **NNGAVKFPQL** **CKFCDVRFST**  
**CDNQKSCMSN** **CSITSICEKP** **QEVCAVWRK** **NDENITLETV** **CHDPKLPYHD** **FILEDAASPK**  
**CIMKEKKKPG** **ETFFMCSCSS** **DECNDNIIFS** **EEYNTSNPD**

Anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFVPSRFS  
SGTDFTLTITSSLPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV  
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSKADYEKHKVYACEVTH  
QGLSSPVTKSFNRGEC

Underlined: Anti-HER2 antibody variable region

Plain: Anti-HER2 antibody constant region

*Italic: Linker*

**Bold: TGF $\beta$ RII ectodomain**

Fig. 2

**Fusion proteins comprising Anti-EGFR1 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD).**

Anti-EGFR1 heavy chain + TGF $\beta$ -RII ECD fusion amino acid sequence

(SEQ ID NO: 2):

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPFTSR  
LSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYDYEFAYWGQGLTVTVSAASTKGPSVFPLAP  
 SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ  
 TYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV  
 VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL  
 PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT  
 PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK  
 GGGGSGGGGSGGGGS TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST  
 CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDNITLETV CHDPKLPYHD FILEDAASPK  
 CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

Anti-EGFR1 light chain amino acid sequence (SEQ ID NO: 71):

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSG  
SGTDFTLSINSVESEDIADYYCQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASV  
 VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSKADYEEKHKVYACEVTH  
 QGLSSPVTKSFNRGEC

Underlined: Anti-EGFR antibody variable region

Plain: Anti-EGFR antibody constant region

*Italic: Linker*

**Bold: TGF $\beta$ RII ectodomain**

Fig. 3

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**Fusion proteins comprising Anti-CD20 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD).**

Anti-CD20 heavy chain+ TGF $\beta$ -RII ECD fusion amino acid sequence

(SEQ ID NO: 3):

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKG  
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPL  
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG  
 TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT  
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK  
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK  
 GGGGSGGGGSGGGGS **TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST**  
 CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK  
 CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

Anti-CD20 light chain amino acid sequence (SEQ ID NO: 72):

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVFVRFSGSGS  
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV  
 CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEEKHKVYACEVTHQ  
 GLSSPVTKSFNRGEC

Underlined: Anti-CD20 antibody variable region

Plain: Anti-CD20 antibody constant region

*Italic: Linker*

**Bold: TGF $\beta$ RII ectodomain**

Fig. 4

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**Fusion proteins comprising anti-VEGF antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD).**

Anti-VEGF heavy chain+ TGF $\beta$ -RII ECD fusion amino acid sequence

(SEQ ID NO: 4):

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFKR  
RFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYGSSHWYFDVWGQGTLLTVSSASTKGPSVF  
 PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSS  
 LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE  
 VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV  
 NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN  
 YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK  
 GGGGSGGGGSGGGGS **TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST**  
 CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK  
 CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

Anti-VEGF Light chain sequence (SEQ ID NO: 73):

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSRFSGSG  
SGTDFTLTISLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV  
 VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEKHKVYACEVTH  
 QGLSSPVTKSFNRGEC

Underlined: Anti-VEGF antibody variable region

Plain: Anti-VEGF antibody constant region

*Italic: Linker*

**Bold: TGF $\beta$ RII ectodomain**

Fig. 5

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**Fusion proteins comprising anti-human CTLA-4 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD).**

Anti-CTLA-4 heavy chain + TGF $\beta$ -RII Extracellular domain fusion amino acid sequence (SEQ ID NO: 5):

QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYTMHWVRQA PGKGLEWVTF ISYDGNNKYY  
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGO GTLVTVSSAS  
 TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL  
 YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPELLGGPS  
 VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST  
 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT  
 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPPVLD SDGSFFLYSK LTVDKSRWQQ  
 GNVFSCSVMH EALHNHYTQK SLSLSPGK **GGGSGGGSGGGGS** **TIPPHVQK** **SVNNDMIVTD**  
**NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV**  
**CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD**

Anti-CTLA-4 light chain (SEQ ID NO: 74):

EIVLTQSPGT LSLSPGERAT LSCRASQSVG SSYLAWYQQK PGQAPRLLIY GAFSRATGIP  
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPWTFG QGTKVEIKRT VAAPSVFIFP  
 PSDEQLKSGT ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYSLSTL  
 TLSKADYEKH KVIACEVTHQ GLSSPVTKSF NRGEC

Underlined: Anti-CTLA-4 antibody heavy chain variable region

Plain: Anti-CTLA-4 antibody heavy chain constant region

*Italic: Linker*

**Bold: TGF $\beta$ RII ectodomain**

Fig. 6

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**Fusion proteins comprising IL-2, Fc, and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD).**IL-2 + Fc + TGF $\beta$ -RII Extracellular domain (SEQ ID NO: 6):

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL  
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL  
T *GGGSGGGSGGGGS*  
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS* **TIPPHVQK SVNNDMIVTD**  
**NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV**  
**CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFSEEYNTSNPD**

TGF $\beta$ -RII Extracellular domain + Fc + IL-2 (SEQ ID NO: 7):

**TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP**  
**QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS**  
**DECNDNIIFS EEYNTSNPD** *GGGSGGGSGGGGS*  
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*  
APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL  
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL  
T

**Bold: TGF $\beta$ RII ectodomain***Italic: Linker (Optional; other linker sequences)*

Plain: Fc

Underlined: IL-2 fragment(Note: Can replace linker *GGGSGGGSGGGGS* SEQ ID NO: 104 with *EPKSCDK* SEQ ID NO: 105)(Note: Can replace underlined aa in Fc: E with D and M with L in Fc)

Fig. 7

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**Fusion proteins comprising anti-CD25 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD).**

Anti-CD25 (Daclizumab) heavy chain and TGF $\beta$ -RII Extracellular domain  
(SEQ ID NO: 8):

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY  
NQKFQDKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LVTVSSASTK  
GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS  
LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF  
LFPPKPKDTL MISRTPEVTC VVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR  
VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN  
QVSLTCLVKG FYPSDIAVEW ESNQGPENNY KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN  
VFSCSVMEHA LHNHYTQKSL SLSPGK GGGGSGGGGSGGGGS **TIPPHVQK SVNNDMIVTD**  
**NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCAVVRK NDENITLETV**  
**CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD**

Anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75):

DIQMTQSPST LSASVGDRVT ITCSASSSIS YMHWYQQKPG KAPKLLIYTT SNLASGVPAR  
FSGSGSGTEF TLTISSLQPD DFATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS  
DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLT  
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

Plain: Anti-CD25 antibody

*Italic: Linker*

**Bold: TGF $\beta$ RII ectodomain**

Fig. 8A

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Anti-CD25 (Basiliximab) heavy chain and TGF $\beta$ -RII Extracellular domain

(SEQ ID NO: 9):

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ  
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDIYGY YFDFWGQGTT LTVSSASTKG  
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL  
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL  
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV  
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYITLP PSRDELTKNQ  
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV  
FSCSVMEAL HNHYTQKSL SLPKG *GGGGSGGGSGGGGS* **TIPPHVQK SVNNDMIVTD**  
**NNGAVKFPQL CKFCDVRFST** **CDNQKSCMSN CSITSICEKP** **QEVCAVVRK NDENITLETV**  
**CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEEYNTSNPD**

Anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76):

QIVSTQSPAI MSASPGEKVT MTCSASSRS YMQWYQQKPG TSPKRWIYDT SKLASGVPAR  
FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE  
QLKSGTASVV CLLNFPYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK  
ADYEKHKVYA CEVTHQGLSS PVTKSFNRGEC

Plain: Anti-CD25 antibody

*Italic: Linker*

**Bold: TGF $\beta$ RII ectodomain**

Fig. 8B

**Fusion proteins comprising anti-CD4 antibody and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ECD).**

Anti-CD4 heavy chain and TGF $\beta$ -RII Extracellular domain (SEQ ID NO: 10):

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN  
NRVSISIDTSKNLFSCLKRSVTAADTAVYYCASNILKYLHWLLYWGQGVLVTVSSASTKGPSVFPL  
 APSSKSTSGGTAALGLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG  
 TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT  
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK  
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK  
 GGGGSGGGGSGGGGS **TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRF**  
**STCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMK**  
**EKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPD**

Anti-CD4 light chain (SEQ ID NO: 77):

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS  
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA  
 SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV  
 THQGLSSPVTKSFNRGEC

Plain: Anti-CD4 antibody (variable region – underlined)

*Italic: Linker*

**Bold: TGF $\beta$ RII ectodomain**

Fig. 9

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**Fusion proteins comprising PD-1 Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGF $\beta$ -RII) Extracellular domain (ectodomain).**PD-1 ectodomain + Fc + TGF $\beta$ RII ectodomain (SEQ ID NO: 11):

PGWFLDSPDRPWNPTFSPALLVVTEDGNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR  
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGYLCAISLAPKAQIKESLRAELRVTERRAEVP  
TAHPSPSPRPAGQFQTLV *GGGSGGGSGGGGS*  
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVMHREALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*  
 TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVW  
 RKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYN  
 TSNPD

TGF $\beta$ RII ectodomain + Fc + PD-1 ectodomain (SEQ ID NO: 12):

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVW  
 RKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYN  
 TSNPD *GGGSGGGSGGGGS*  
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVMHREALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*  
PGWFLDSPDRPWNPTFSPALLVVTEDGNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR  
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGYLCAISLAPKAQIKESLRAELRVTERRAEVP  
TAHPSPSPRPAGQFQTLV

Underlined: PD-1 ectodomain*Italic: Linkers*

Plain: IgG1 Fc

**Bold: TGF $\beta$ RII ectodomain**

(Note: The first linker sequence *GGGSGGGSGGGGS* is optional and may be replaced with *EPKSCDK* SEQ ID NO: 105 or deleted)

Fig. 10

**Fusion proteins comprising Transforming growth factor-beta receptor II (TGF $\beta$ -RII) ectodomain, Fc, and Receptor activator of nuclear factor- $\kappa$ B (RANK) Ectodomain:**

TGF $\beta$ RII ectodomain – Fc - RANK ectodomain (SEQ. ID. NO: 13):

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVW  
 RKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKKPGETFFMCSCSSDECNDNIIFSEEYN  
 TSNPDGGGGSGGGSGGGSGTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH  
 EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK  
 TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK GGGGSGGGSGGGGS QI  
APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK  
VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV  
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

RANK ectodomain – Fc - TGF $\beta$ RII ectodomain (SEQ. ID. NO: 14):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK  
VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV  
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG  
GGGGSGGGSGGGSGTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV  
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF  
LYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGSGTIPPHVQKS  
VNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITL  
ETVCHDPKLPYHDFILEDAAAPKCIMKEKKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

**Bold:** TGF $\beta$ RII ectodomain

Plain: IgG1 Fc

*Italics:* Linker

Underlined: RANK ectodomain

(optional linker 1 may be deleted or replaced with another linker such as IEGRDMD (SEQ. ID. NO:106) or EPKSCDK (SEQ.ID. NO: 105))

Fig. 11

## (i) Full-length PD-1 or fragment thereof (SEQ ID NO: 92):

1 MQIPQAPWV VWAFLQLGWR PGWFLDSPDR PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS  
 61 ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT  
 121 YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV VGVVGGLLGS  
 181 LVLLVWVLAV ICSRAARGTI GARRTGQPLK EDPSAVPVFS VDYGELDFQW REKTPEPPVP  
 241 CVPEQTEYAT IVFPSGMGTS SPARRGSADG PRSAQPLRPE DGHCSWPL

*Italic: PD-1 ectodomain*

*Underlined Italic: ligand-binding domain*

## (ii) PD-1 extracellular domain (ectodomain) or fragment thereof (SEQ ID NO: 93):

PGWFLDSPDR PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA  
AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA  
ELRVTERRAE VPTAHPSPSP RPAGQFQTLV

## (iii) PD-1 extracellular domain (ectodomain) ligand-binding region (SEQ ID NO: 94):

DSPDR PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA  
AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA  
ELRVTERRAE VPTAHPSPSP RPAGQFQ

## (iv) Mutant of PD-1 or a fragment thereof which binds Programmed Death-1 ligand [PD-L1 (B7-H1) or PD-L2 (B7-DC)]

Fig. 12

**Fusion proteins comprising Anti-HER2/neu antibody and PD-1 Ectodomain.**

Anti-HER2/neu heavy chain + PD-1 ectodomain fusion amino acid sequence  
(SEQ ID NO: 15):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKG  
RFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLA  
PSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT  
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC  
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA  
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT  
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK  
**GGGGSGGGSGGGGS** **PGWFLDSPDR** **PWNPPTFSPA** **LLVVTEGDNA** **TFTCSFSNTS**  
**ESFVLNWYRM** **SPSNQTDKLA** **AFPEDRSQPG** **QDCRFRVTQL** **PNGRDFHMSV** **VRARRNDSGT**  
**YLCGAISLAP** **KAQIKESLRA** **ELRVTERRAE** **VPTAHPSPSP** **RPAGQFQTLV**

Anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFRFSGSR  
SGTDFTLTTISSLPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV  
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSSTLTLSKADYEKHKVYACEVTH  
QGLSSPVTKSFNRGEC

Underlined: Anti-HER2/neu Variable region

Plain: Anti-HER2/neu Constant region

*Italic: Linker*

**Bold: PD-1 ectodomain**

Fig. 13

**Fusion proteins comprising Anti-EGFR1 antibody and PD-1 Ectodomain.**

Anti-EGFR heavy chain + PD-1 ectodomain fusion amino acid sequence

(SEQ ID NO: 16):

QVQLKQSGPGLVQPSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGNTDYNTPFTSR  
LSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYDYEFAYWGQGTLVTVSAASTKGPSVFPLAP  
SSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQ  
TYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV  
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL  
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT  
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCVMHEALHNHYTQKSLSLSPGK  
GGGGSGGGSGGGGS PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS  
ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT  
YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV

Anti-EGFR light chain amino acid sequence (SEQ ID NO: 71):

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGSG  
SGTDFTLSINSVESEDIADYYCQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASV  
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH  
QGLSSPVTKSFNRGEC

Underlined: Anti-EGFR Variable region

Plain: Anti-EGFR Constant region

*Italic: Linker***Bold: PD-1 ectodomain**

Fig. 14

**Fusion proteins comprising Anti-CD20 antibody and PD-1 Ectodomain.**

Anti-CD20 heavy chain + PD-1 ectodomain fusion amino acid sequence

(SEQ ID NO: 17):

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKG  
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPL  
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG  
 TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT  
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK  
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK  
**GGGGSGGGSGGGGS** **PGWFLDSPDR PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS**  
**ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT**  
**YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPPSP RPAGQFQTLV**

Anti-CD20 light chain amino acid sequence (SEQ ID NO: 72):

QIVLSQSPAILSASPGEKVTMTTCRASSSVSYIHWFOQKPGSSPKPWIYATSNLASGVPVRFSGSGS  
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV  
 CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEEKHKVYACEVTHQ  
 GLSSPVTKSFNRGEC

Underlined: Anti-CD20 Variable region

Plain: Anti-CD20 Constant region

*Italic: Linker*

**Bold: PD-1 ectodomain**

Fig. 15

**Fusion proteins comprising Anti-VEGF antibody and PD-1 Ectodomain.**

Anti-VEGF heavy chain + PD-1 ectodomain fusion amino acid sequence  
(SEQ ID NO: 18):

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFKR  
RFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWFVDVWGQGTLLVTVSSASTKGPSVF  
 PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSS  
 LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE  
 VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV  
 NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN  
 YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK  
 GGGGSGGGGSGGGGS PGWFLDSPDR PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS  
 ESFVLNWMYR SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT  
 YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPPSP RPAGQFQTLV

Anti-VEGF Light chain sequence (SEQ ID NO: 73):

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSRFSGSG  
SGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV  
 VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSKADYEEKHKVYACEVTH  
 QGLSSPVTKSFNRGEC

Underlined: Anti-VEGF Variable region

Plain: Anti-VEGF Constant region

*Italic: Linker*

**Bold: PD-1 ectodomain**

Fig. 16

**Fusion proteins comprising anti-human CTLA-4 antibody and PD-1 Ectodomain.**

Anti-(human CTLA-4) (human  $\gamma$ 1-chain)-PD-1 ectodomain fusion protein, disulfide with human  $\kappa$ -chain, dimer

Anti-CTLA-4 heavy chain + PD-1 ectodomain fusion amino acid sequence

(SEQ ID NO: 19):

QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYTMHWVRQA PGKGLEWVTF ISYDGNNKYY  
 ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWQQ GTLVTVSSAS  
 TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL  
 YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPELLGGPS  
 VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST  
 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT  
 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ  
 GNVFSCSVMH EALHNHYTQKSLSLSPGK **GGGSGGGSGGGGS** **PGWFLDSPDR**  
 PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG  
 QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA ELRVTERRAE  
 VPTAHPSPPSP RPAGQFQTLV

Anti-CTLA-4 light chain (SEQ ID NO: 74):

EIVLTQSPGT LSLSPGERAT LSCRASQSVG SSYLAWYQQK PGQAPRLLIY GAFSRATGIP  
 DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPWTFG QGTKVEIKRT VAAPSVFIFP  
 PSDEQLKSGT ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYLSSTL  
 TLSKADYEKH KVYACEVTHQ GLSSPVTKSF NRGEC

Underlined: Anti-CTLA-4 Variable region

Plain: Anti-CTLA-4 Constant region

*Italic: Linker*

**Bold: PD-1 ectodomain**

Fig. 17

**Fusion proteins comprising anti-CD25 antibody and PD-1 Ectodomain.**

Anti-CD25 (Daclizumab) heavy chain and PD-1 ectodomain (SEQ ID NO: 20):

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY  
 NQKFQDKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LVTVSSASTK  
 GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS  
 LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF  
 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR  
 VVSVLTVLHQ DWLNGKEYKC KVSNAKLPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN  
 QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN  
 VFSCSVMHEA LHNHYTQKSL SLSPGK **GGGSGGGSGGGGS**  
**PGWFLDSPDRPWNPTTFSPALLVTEGDNATFTCSFSNT**  
**SESFVLNWYRMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLPNGRDFHMSVVRAR**  
**RNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSRPAGQFQTLV**

Anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75):

DIQMTQSPST LSASVGDRVT ITCSASSSIS YMHWYQQKPG KAPKLLIYTT SNLASGVPAR  
 FSGSGSGTEF TLTISSLQPD DFATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS  
 DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLT  
 SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

Plain: Anti-CD25 antibody

*Italic: Linker*

**Bold: PD-1 ectodomain**

Fig. 18A

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Anti-CD25 (Basiliximab) heavy chain and PD-1 ectodomain (SEQ ID NO: 21):

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ  
 KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDIYGY YFDFWQGGTT LTVSSASTKG  
 PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL  
 SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL  
 FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV  
 VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYITLP PSRDELTKNQ  
 VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV  
 FSCSVMHEAL HNHYTQKSLS LSPGK GGGGSGGGGSGGGGS  
 PGWFLDSPDRPWNPPPTFSPALLVTEGDNATFTCSFSNT  
 SESFVLNWRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRAR  
 RNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLV

Anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76):

QIVSTQSPAI MSASPGEKVT MTCSASSSRS YMQWYQQKPG TSPKRWIYDT SKLASGVPAR  
 FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE  
 QLKSGTASVV CLLNMFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK  
 ADYEEKHKVYA CEVTHQGLSS PVTKSFNRGEC

Plain: Anti-CD25 antibody

*Italic: Linker*

**Bold: PD-1 ectodomain**

Fig. 18B

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**Fusion proteins comprising IL-2, Fc, and PD-1 ectodomain.**

IL-2 + Fc + PD-1 ectodomain (SEQ ID NO: 16):

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL  
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL  
T *GGGSGGGSGGGGS*  
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVMH~~E~~ALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*  
 PGWFLDSPDRPWNPTTFSPALLVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPEDR  
 SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGYLCAISLAPKAQIKESLRAELRVTERRAEVP  
 TAHPSPSRPAGQFQTLV

PD-1 ectodomain + Fc + IL-2 (SEQ ID NO: 23):

PGWFLDSPDRPWNPTTFSPALLVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPEDR  
 SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGYLCAISLAPKAQIKESLRAELRVTERRAEVP  
 TAHPSPSRPAGQFQTLV *GGGSGGGSGGGGS*  
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVMH~~E~~ALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*  
APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL  
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL  
T

Underlined: IL-2 fragment*Italic: Linker*

Plain: Fc

**Bold: human PD-1 ectodomain**

(Note: Can replace optional linker 1 *GGGSGGGSGGGGS* SEQ ID NO: 104 with  
*EPKSCDK* SEQ ID NO: 105)

(Note: Can replace underlined aa in Fc: E with D and M with L)

Fig. 19

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**Fusion proteins comprising anti-CD4 antibody and PD-1 Extracellular domain**

Anti-CD4 heavy chain and PD-1 Extracellular domain (SEQ ID NO: 24):

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN  
NRVSISIDTSKNLFSCLKRSVTAADTAVYYCASNILKYLHWLLYWQGVLTVSSASTKGPSVFPL  
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG  
 TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT  
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK  
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK  
*GGGGSGGGSGGGGS*  
 PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPFEDR  
 SQPGQDCFRFRTQLPNGRDFHMSVVRARRNDSTYLCAISLAPKAQIKESLRAELRVTERRAEVP  
 TAHPSPSPRPAGQFQTLV

Anti-CD4 light chain (SEQ ID NO: 77):

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS  
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA  
 SVVCLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEV  
 THQGLSSPVTKSFNRGEC

Plain: Anti-CD4 antibody (Variable region – underlined)

*Italic: Linker***Bold: PD-1 ectodomain**

Fig. 20

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**Fusion proteins comprising PD-1 Ectodomain, Fc, and Receptor activator of nuclear factor- $\kappa$ B (RANK) Extracellular domain (ectodomain):**

RANK ectodomain – Fc - PD-1 ectodomain (SEQ. ID. NO: 25):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK  
VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV  
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG  
GGGSGGGSGGGGS  
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQG  
 NVFSCSVMH EALHNHYTQKSLSLSPGK GGGSGGGSGGGGS  
 PGWFLDSPDRPWNPTTFSPALLVTEGDNATFTCSFSNTSESFVLNWMSPSNQTDKLAAPFEDR  
 SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSTYLCAISLAPKAQIKESLRAELRVTERRAEVP  
 TAHPSPSRPAGQFQTLV

PD-1 ectodomain – Fc - RANK ectodomain (SEQ. ID. NO: 26):

PGWFLDSPDRPWNPTTFSPALLVTEGDNATFTCSFSNTSESFVLNWMSPSNQTDKLAAPFEDR  
 SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSTYLCAISLAPKAQIKESLRAELRVTERRAEVP  
 TAHPSPSRPAGQFQTLVGGGSGGGSGGGGS  
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQG  
 NVFSCSVMH EALHNHYTQKSLSLSPGK GGGSGGGSGGGGS QI APPCTSEKHY EHLGRCCNKC  
EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR  
RACTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR  
PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

**Bold: PD-1 ectodomain**

*Italics: Linker (optional: may delete linker 1 or replace with IEGRDMD or EPKSCDK)*

Plain: IgG1 Fc

Underlined: RANK ectodomain

Fig. 21

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**Moieties that bind Receptor activator of nuclear factor- $\kappa$ B (RANK) ligand**(i) Full-length RANK (TNFRSF11A) or fragment thereof (SEQ. ID. NO. 95):

1 MAPRARRRRP LFALLLLCAL LARLQVALQI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC  
 61 TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW  
 121 SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK  
 181 RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPGLIILLLF ASVALVA AII FGVCYRKKGK  
 241 ALTANLWHWI NEACGRLSGD KBSSGDS CVS THTANFGQQG ACEGVLLLTLE EKTTFPEDMC  
 301 YPDQGGVCQG TCVGGGPYAQ GEDARMLSLV SKTEIEEDSF RQMPTED EYM DRPSQPTDQL  
 361 LFLTEPGSKS TPPFSEPLEV GENDSLSQCF TGTQSTVGSE SCNCTEPLCR TDWTPMSSEN  
 421 YLQKEVDSGH CPHWAASPSP NWADVCTGCR NPPGEDCEPL VGSPKRGPLP QCAYGMGLPP  
 481 EEEASRTEAR DQPEDGADGR LPSSARAGAG SGSSPGGQSP ASGNVTGNSN STFISSGQVM  
 541 NPKGDIIVVY VSQTSQEGAA AAAEPMGRPV QEETLARRDS FAGNGPRFPD PCGGPEGLRE  
 601 PEKASRPVQE QGGAKA

**Bold: RANK extracellular domain (RANK ligand-binding domain)**(ii) RANK extracellular domain (ectodomain) or fragment thereof (SEQ. ID. NO. 96):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA  
 VVAGNSTTPR RACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR  
 PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

(iii) Peptide sequences in RANKL binding domains of RANK or peptides containing key RANKL-binding residues of RANK (SEQ. ID. NO. 97):

1 MAPRARRRRP LFALLLLCAL LARLQVALQI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC  
 61 TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW  
 121 SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK  
 181 RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

**Bold: RANK extracellular domain (RANK ligand-binding domain)**

RANK peptide sequences (Underlined) and residues (Italics) involved in binding RANKL.

(iv) Mutant of RANK or a fragment thereof which binds Receptor activator of nuclear factor- $\kappa$ B (RANK) ligand (RANKL)(v) Full-length Osteoprotegerin (OPG, TNFRSF11B) or fragment thereof (SEQ. ID. NO. 98):

1 MNKLLCCALV FLDISIKWTT QETFPKYLH YDEETSHQLL CDKCPPGTYL KQHCTAKWKT  
 61 VCAPCPDHY TDSWHTSDEC LYCSPVCKEL QYVKQECNRT HNRVCECKEG RYLEIEFCLK  
 121 HRSCPPGFGV VQAGTPERN T VCKRCPDGFF SNETSSKAPC RKHTNCSVFG LLLTQKGNAT  
 181 HDNICS GNSE STQKCGIDVT LCBEAFFRFA VPTKFTPNWL SVLVDNLP GT KVNAESVERI  
 241 KRQHSSQEQT FQLKLWKHQ NKAQDIVKKI IQDIDLCENS VQRHIGHANL TFEQLRSLME  
 301 SLPGKKVGAE DIEKTIKACK PSDQILKLLS LWRIKNGDQD TLKGLMHALK HSKTYHFPKT  
 361 VTQSLKKTIR FLHSFTMYKL YQKLFLEMIG NQVQSVKISC L

**Bold: RANK ligand-binding domain**

Fig. 22

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**Fusion proteins comprising Anti-HER2/neu antibody and Receptor activator of nuclear factor- $\kappa$ B (RANK) Extracellular domain (ECD).**

Anti-HER2/neu heavy chain+ RANK ECD fusion amino acid sequence (SEQ. ID. NO: 27):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISA  
DTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA  
 LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK  
 KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH  
 NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE  
 MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCVMH  
 EALHNHYTQKSLSLSPGK **GGGGSGGGSGGGGS** **QI** **APPCTSEKHY** **EHLGRCCNKC** **EPGKYMSSKC**  
**TTTSDSVCLP** **CGPDEYLDW** **NEEDKCLLHK** **VCDTGKALVA** **VVAGNSTTPR** **RCACTAGYHW**  
**SQDCECCRN** **TECAPGLGAQ** **HPLQLNKDTV** **CKPCLAGYFS** **DAFSSTDKCR** **PWTNCTFLGK**  
**RVEHHGTEKS** **DAVCSSSLPA** **RKPPNEPHVY** **LPG**

Anti-HER2/neu light chain amino acid sequence (SEQ. ID. NO: 70):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSSGSRSGTDFT  
LTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA  
 KVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Underlined: Anti-HER2 antibody variable region

Plain: Anti-HER2 antibody constant region

*Italics: Linker*

**Bold: RANK ectodomain**

Fig. 23

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**Fusion proteins comprising Anti-EGFR1 antibody and Receptor activator of nuclear factor- $\kappa$ B (RANK) Extracellular domain (ECD).**

Anti-EGFR1 heavy chain + RANK ECD fusion amino acid sequence (SEQ. ID. NO: 28):

QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPTTSRLSINKD  
NSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTVTVSAASTKGPSVFPLAPSSKSTSGGTAAL  
 GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVFSSSLGTQTYICNVNHKPSNTKVDKR  
 VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN  
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL  
 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHE  
 ALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**  
**TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR R**ACTAGYHW  
**SQDCECCRRN** **TECAPGLGAQ** **HPLQLNKDTV** **CKPCLAGYFS** **DAFSSTDKCR** **PWTNCTFLGK**  
**RVEHHGTEKS** **DAVCSSSLPA** **RKPPNEPHVY** **LPG**

Anti-EGFR1 light chain amino acid sequence (SEQ. ID. NO: 71):

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFT  
LSINSVESEDIADYYCQONNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREA  
 KVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Underlined: Anti-EGFR antibody variable region

Plain: Anti-EGFR antibody constant region

Italics: Linker

**Bold: RANK ectodomain**

Fig. 24

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**Fusion proteins comprising Anti-CD20 antibody and Receptor activator of nuclear factor- $\kappa$ B (RANK) Extracellular domain (ECD).**

Anti-CD20 heavy chain+ RANK ECD fusion amino acid sequence (SEQ. ID. NO: 29):

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKGKATLTA  
DKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNWVGAGTTVTVSAASTKGPSVFPLAPSSKSTSGGTA  
 ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVD  
 KKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV  
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD  
 ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSV  
 HEALHNHYTQKSLSLSPGK **GGGGSGGGSGGGGS** **QI** **APPCTSEKHY** **EHLGRCCNKC** **EPGKYMSSKC**  
**TTTSDSVCLP** **CGPDEYLDW** **NEEDKCLLHK** **VCDTGKALVA** **VVAGNSTTPR** **RCACTAGYHW**  
**SQDCECCRRN** **TECAPGLGAQ** **HPLQLNKDTV** **CKPCLAGYFS** **DAFSSTDKCR** **PWTNCTFLGK**  
**RVEHHGTEKS** **DAVCSSSLPA** **RKPPNEPHVY** **LPG**

Anti-CD20 light chain amino acid sequence (SEQ. ID. NO: 72):

QIVLSQSPAILLSASPGEKVTMTCRASSSVSYIHWFOQKPGSSPKPWYATSNLASGVPVRFSGSGSGTSYSL  
TISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK  
 VQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Underlined: Anti-CD20 antibody variable region

Plain: Anti-CD20 antibody constant region

*Italics: Linker*

**Bold: RANK ectodomain**

Fig. 25

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**Fusion proteins comprising anti-VEGF antibody and Receptor activator of nuclear factor- $\kappa$ B (RANK) Extracellular domain (ECD).**

Anti-VEGF heavy chain+ RANK ECD fusion amino acid sequence (SEQ. ID. NO: 30):

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADF  
KRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWFYFDVWGQGTLLTVSSASTKG  
 PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP  
 SSSLTGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT  
 EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK  
 CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP  
 ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK  
*GGGGSGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP**  
 CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCTAGYHW SQDCECCRRN  
 TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS  
 DAVCSSSLPA RKPPNEPHVY LPG

Anti-VEGF Light chain sequence (SEQ. ID. NO: 73):

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQOKPGKAPKVLIYFTSSLHSGVPSRFS  
SGSGSGTDFTLTISSLQPEDFATYYCQOYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV  
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYEKHKVYACEVTHQGL  
 SSPVTKSFNRGEC

Underlined: Anti-VEGF antibody variable region

Plain: Anti-VEGF antibody constant region

Italics: Linker

**Bold: RANK ectodomain**

Fig. 26

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**Fusion proteins comprising anti-human CTLA-4 antibody and Receptor activator of nuclear factor- $\kappa$ B (RANK) Extracellular domain (ECD).**

Anti-CTLA-4 heavy chain + RANK ECD fusion sequence (SEQ. ID. NO: 31):

QVQLVESGGG VVQPGSLRL SCAASGFTFS SYTMHWRQA PGKGLEWTF ISYDGNNKYY  
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGQ GTLVTVSSAS  
 TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL  
 YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPELLGGPS  
 VFLFPPKPKD TLMISRTPEV TCVVVDVSH EDEPKFKNWYV DGVEVHNAKT KPREEQYNST  
 YRVVSVLTVL HQDWLNKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT  
 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ  
 GNVFSCSVMH EALHNHYTQK SLSLSPGK **GGGGSGGGSGGGGS** **QI** **APPCTSEKHY** **EHLGRCCNKC**  
**EPGKMSSKC** **TTTSDSVCLP** **CGPDEYLD****SW** **NEEDKCLLHK** **VCDTGKALVA** **VVAGNSTTPR**  
**RC****ACTAGYHW** **SQDCECCRRN** **TECAPGLGAQ** **HPLQLNKDTV** **CKPCLAGYFS** **DAFSSTDKCR**  
**PWTNCTFLGK** **RVEHHGTEKS** **DAVCSSSLPA** **RKPPNEPHVY** **LPG**

Anti-CTLA-4 light chain (SEQ. ID. NO: 74):

EIVLTQSPGT LSLSPGERAT LSCRASQSVG SSYLAWYQQK PGQAPRLLIY GAFSRATGIP  
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPWTFG QGTKVEIKRT VAAPSVFIFP  
 PSDEQLKSGT ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYLSSTL  
 TLSKADYEKH KVIACEVTHQ GLSSPVTKSF NRGEK

Underlined: Anti-CTLA-4 antibody variable region

Plain: Anti-CTLA-4 antibody constant region

*Italics: Linker*

**Bold: RANK ectodomain**

Fig. 27

**Fusion proteins comprising anti-CD25 antibody and Receptor activator of nuclear factor- $\kappa$ B (RANK) Extracellular domain (ECD).**

Anti-CD25 and RANK ectodomain - Heavy chain (SEQ. ID. NO: 32):

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY  
 NQKFQDKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LVTVSSASTK  
 GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS  
 LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF  
 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR  
 VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN  
 QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN  
 VFSCSVMEHA LHNHYTQKSL SLSPGK GGGGSGGGGSGGGGS QI APPCTSEKHY EHLGRCCNKC  
 EPGKYMSSKC TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR  
 RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR  
 PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Anti-CD25 - Light chain (SEQ. ID. NO. 75):

DIQMTQSPST LSASVGDRVIT ITCSASSSIS YMHWYQQKPG KAPKLLIYTT SNLASGVPAR  
 FSGSGSGTEF TLTISSLQPD DFATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS  
 DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTSL  
 SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

Plain: Anti-CD25 antibody

*Italics: Linker*

**Bold: RANK ectodomain**

Fig. 28A

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**Fusion proteins comprising anti-CD25 antibody and Receptor activator of nuclear factor- $\kappa$ B (RANK) Extracellular domain (ECD).**

Anti-CD25 and RANK ectodomain - Heavy chain (SEQ. ID. NO: 33):

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ  
 KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDIYGY YDFWQGQGT LTVSSASTKG  
 PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL  
 SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL  
 FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV  
 VSVLTVLHQD WLNKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLPSRDELTKNQ  
 VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV  
 FSCSVMEAL HNHYTQKSLS LSPGK *GGGGSGGGSGGGGS* **QI** APPCTSEKHY EHLGRCCNKC  
 EPGKYMSSKC TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR  
 RCTAGYHW SQDCECCRN TECAPGLAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR  
 PWTNCTFLGK RVEHGGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Anti-CD25 – Light chain (SEQ. ID. NO: 76):

QIVSTQSPAI MSASPGEKVT MTCSASSRS YMQWYQQKPG TSPKRWIYDT SKLASGVPAR  
 FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE  
 QLKSGTASVV CLLNNFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK  
 ADYEKHKVYA CEVTHQGLSS PVTCSFNRGEC

Plain: Anti-CD25 antibody

*Italics: Linker*

**Bold: RANK ectodomain**

Fig. 28B

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**Fusion proteins comprising IL-2, Fc, and Receptor activator of nuclear factor- $\kappa$ B (RANK) Extracellular domain (ECD).**

IL2 – Fc – RANK ECD (SEQ. ID. NO: 34):

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL  
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGSG  
 GGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK  
 PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ  
 VSLTCLVKGFPYSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHN  
 HYTQKSLSLSPGK GGGGSGGGSGGGGS **QI** **APPCTSEKHY** **EHLGRCCNKC** **EPGKYMSSKC**  
**TTTSDSVCLP** **CGPDEYLDW** **NEEDKCLLHK** **VCDTGKALVA** **VVAGNSTTPR** **RCACTAGYHW**  
**SQDCECCRRN** **TECAPGLGAQ** **HPLQLNKDTV** **CKPCLAGYFS** **DAFSSTDKCR** **PWTNCTFLGK**  
**RVEHHGTEKS** **DAVCSSSLPA** **RKPPNEPHVY** **LPG**

RANK ECD – Fc – IL-2 (SEQ. ID. NO: 35):

**QI** **APPCTSEKHY** **EHLGRCCNKC** **EPGKYMSSKC** **TTTSDSVCLP** **CGPDEYLDW** **NEEDKCLLHK**  
**VCDTGKALVA** **VVAGNSTTPR** **RCACTAGYHW** **SQDCECCRRN** **TECAPGLGAQ** **HPLQLNKDTV**  
**CKPCLAGYFS** **DAFSSTDKCR** **PWTNCTFLGK** **RVEHHGTEKS** **DAVCSSSLPA** **RKPPNEPHVY** **LPG**  
 GGGSGGGSGGGSGSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV  
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL  
 PPSREEMTKNQVSLTCLVKGFPYSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV  
 FSCVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGGSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKN  
PKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMC  
EYADETATIVEFLNRWITFCQSIISTLT

**Bold: RANK ectodomain**

*Italics: Linker* (Optional; any linker sequence)

Plain: Fc

Underlined: IL2 fragment that binds CD25 (IL-2R $\alpha$ )

(Can replace linker 1 with EPKSCDK or IEGRDMD)

(Can replace underlined aa in Fc: E with D and M with L)

Fig. 29

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**Fusion protein comprising anti-CD4 antibody and Receptor activator of nuclear factor- $\kappa$ B (RANK) Extracellular domain (ECD)**

Anti-CD4 heavy chain and RANK Extracellular domain (SEQ ID NO: 36):

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN  
NRVSIISIDTSKNLFSCLKRSVTAADTAVYYCASNILKYLHWLLYWGQGVLTVTSSASTKGPSVFPL  
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG  
 TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT  
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK  
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK  
 GGGGSGGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP**  
**CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR R**CACTAGYHW **SQDCECCRRN**  
**TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS**  
**DAVCSSSLPA RKPPNEPHVY LPG**

Anti-CD4 light chain (SEQ ID NO: 77):

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS  
GNTATLTISGVEAGDEADYVCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA  
 SVVCLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEV  
 THQGLSSPVTKSFNRGEC

Underlined: Anti-CD4 antibody variable region

Plain: Anti-CD4 antibody constant region

*Italic: Linker*

**Bold: PD-1 ectodomain**

Fig. 30

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- (i) Full-length human PD-1 ligand 1 (B7-H1; PDCD1L1; PDL1; CD274) protein or a fragment thereof (SEQ ID NO: 99):

MRIFAVFIFM TYWHLNNAFT VTVPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME  
DKNIIQFVHG EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG  
ADYKRITVKV NAPYNKINQR ILVVDPTSE HELTCQAEY PKAEVIWTSS DHQVLSGKTT  
TTNSKREEKL FNVSTTLRIN TTTNEIFYCT FRRLDPEENH TAEVIPPELP LAHPPNERTH  
 LVILGAILLC LGVALTFIFR LRKGRMDVK KCGIQDTNSK KQSDTHLEET

- (ii) PD-L1 extracellular binding domain (ectodomain) or fragment thereof (SEQ ID NO: 100):

AFT VTVPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG  
EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV  
NAPYNKINQR ILVVDPTSE HELTCQAEY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL  
FNVSTTLRIN TTTNEIFYCT FRRLDPEENH TAEVI

- (iii) Human PD-1 ligand 2 [PD-L2 (B7-DC)] or a fragment thereof.
- (iv) Mutant of Programmed Death-1 ligand [PD-L1 (B7-H1) or PD-L2 (B7-DC)] or a fragment thereof which binds Programmed Death-1 (PD-1).

Sequence used in fusion proteins (SEQ ID NO: 101):

RIFAVFIFM TYWHLNNAFT VTVPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME  
DKNIIQFVHG EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG  
ADYKRITVKV NAPYNKINQR ILVVDPTSE HELTCQAEY PKAEVIWTSS DHQVLSGKTT  
TTNSKREEKL FNVSTTLRIN TTTNEIFYCT FRRLDPEENH TAEVIPPELP LAHPPNERTH  
 LVILGAILLC LGVALTFIFR LRKGRMDVK KCGIQDTNSK KQSDTHLEET

**Bold: PD-L1 extracellular domain (ectodomain)**

**Underlined Bold: Ligand Binding domain**

Fig. 31

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**Fusion proteins comprising anti-tumor necrosis factor (TNF $\alpha$ ) antibody and PD-1 ligand 1 or PD-1 ligand 2**

Anti-TNF $\alpha$  heavy chain + PD-L1 (SEQ ID NO: 37):

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSVEG  
RFTISRDNAKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGTLVTVSSASTKGPSVFPL  
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG  
 TQTYICNVNHKPSNTKVD**KKAE**PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT  
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK  
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

*GGGGSGGGGSGGGGS*

RIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF  
 VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP  
 YNKINQRILVVDPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTNSKREEKLFNVTSTLRI  
 NTTTNEIFYCTFRRLDPEENHTAELVIPPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR  
 MMDVKKCGIQDTNSKKQSDTHLEET

Anti-TNF $\alpha$  light chain (SEQ ID NO: 78):

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLPGKAPKLLIYAASTLSGVPSRFSSGSG  
SGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV  
 VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSLSSTLTLSKADYEEKHKVYACEVTH  
 QGLSSPVTKSFNRGEC

Underlined: anti-TNF $\alpha$  antibody variable region

Plain: anti-TNF $\alpha$  antibody constant region

*Italic: Linker*

**Bold: human PD-1 ligand 1 (PD-L1)**

(Note: Fc region **KKAE** SEQ ID NO: 106 can be replaced with **KRVE** SEQ ID NO: 107  
 or **KKVE** SEQ ID NO: 108)

Fig. 32

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**Fusion proteins comprising TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand: (TNFR2 ECD + IgG C $\gamma$ 1 + PD-L1)**

TNFR2 ECD + IgG C $\gamma$ 1 + PD-L1 (SEQ ID NO: 38):

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN  
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKEGCRCLCAPLRKCRPGFGVARPGT  
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV  
STRSQHTQPTPEPSTAPSTSFLPMGPSPPAEGSTGD *EPKSCDK*  
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVSVLTVLHQQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVMEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*  
 RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF  
 VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP  
 YNKINQRILVVDVPTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI  
 NTTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR  
 MMDVKKCGIQDTNSKKQSDTHLEET

**Fusion proteins comprising PD-1 ligand, Fc, and TNFR2 Extracellular ligand binding domain: (PD-L1 + IgG C $\gamma$ 1 + TNFR2 ECD)**

PD-L1 + IgG C $\gamma$ 1 + TNFR2 ECD (SEQ ID NO: 39):

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQ  
 FVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA  
 PYNKINQRILVVDVPTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR  
 INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFR  
 LRKGRMMDVKKCGIQDTNSKKQSDTHLEET *GGGGSGGGSGGGGS* *EPKSCDK*  
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVSVLTVLHQQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVMEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*  
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN  
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKEGCRCLCAPLRKCRPGFGVARPGT  
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV  
STRSQHTQPTPEPSTAPSTSFLPMGPSPPAEGSTGD

Underlined: TNFR2 ligand-binding domain

Plain: human IgG1 heavy chain constant region (IgG C $\gamma$ 1 domain = H, CH2, CH3)

*Italic: Linker (Linker 1 is optional and may be replaced with another linker such as EPKSCDK or GGGSGGGSGGGGS)*

**Bold: human PD-1 ligand 1 or extracellular binding domain of PD-L1**

Fig. 33

**Fusion proteins comprising anti-CD20 antibody and PD-1 ligand 1 (PD-L1).**

Anti-CD20 heavy chain + PD-L1 sequence (SEQ ID NO: 40):

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKG  
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTTVTSAASTKGPSVFPL  
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG  
 TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT  
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK  
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK  
 GGGGSGGGGSGGGGS  
 RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF  
 VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP  
 YNKINQRILVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI  
 NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERNTHLVILGAILLCLGVALTFIFRLRKGR  
 MMDVKKCGIQDTNSKKQSDTHLEET

Anti-CD20 light chain sequence (SEQ ID NO: 72):

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGS  
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV  
 CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEEKHKVYACEVTHQ  
 GLSSPVTKSFNRGEC

Underlined: anti-CD20 antibody variable region

Plain: anti-CD20 antibody constant region

*Italic:* Linker**Bold:** human PD-1 ligand 1 or extracellular binding domain of PD-L1

Fig. 34

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**Fusion proteins comprising anti-CD25 antibody and PD-1 ligand 1 (PD-L1).**

Anti-CD25 (Daclizumab) heavy chain and PD-L1 (SEQ ID NO: 41):

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY  
 NQKFQDKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LVTVSSASTK  
 GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS  
 LSSVTVTPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF  
 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR  
 VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN  
 QVSLTCLVKG FYPSDIAVEW ESNQGPENNY KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN  
 VFSCSVMEHA LHNHYTQKSL SLSPGK GGGSGGGSGGGGS  
RIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECK  
FPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNA  
ALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHEL  
TCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYC  
TFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR  
MMDVKKCGIQDTNSKKQSDTHLEET

Anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75):

DIQMTQSPST LSASVGDRVT ITCSASSSIS YMHWYQQKPG KAPKLLIYTT SNLASGVPAR  
 FSGSGSGTEF TLTISSLQPD DFATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS  
 DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL  
 SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

Plain: anti-CD25 antibody

*Italic: Linker* (optional and may be replaced)**Bold: human PD-1 ligand 1 or extracellular binding domain of PD-L1 (underlined)**

Fig. 35A

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Anti-CD25 (Basiliximab) heavy chain and PD-1 ectodomain (SEQ ID NO: 42)

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ  
 KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDIYGY YFDFWGQGT LTVSSASTKG  
 PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL  
 SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL  
 FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV  
 VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLPSRDELTKNQ  
 VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV  
 FSCSVMHEAL HNHYTQKSLS LSPGK GGGGSGGGSGGGGS

RIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECK

FPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNA

ALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDVPTSEHEL

TCQAEQYPAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYC

TFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR

MMDVKKCGIQDTNSKKQSDTHLEET

Anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76):

QIVSTQSPAI MSASPGEKVT MTCSASSRS YMQWYQQKPG TSPKRWIYDT SKLASGVPAR  
 FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE  
 QLKSGTASVV CLLNNFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSY SLSSTLTLSK  
 ADYEEKHKVYA CEVTHQGLSS PVTKSFNRRGE

Plain: anti-CD25 antibody

*Italic: Linker*

**Bold: human PD-1 ligand 1 or extracellular binding domain of PD-L1 (underlined)**

Fig. 35B

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**Fusion proteins comprising IL-2, Fc, and PD-1 ligand 1 (PD-L1)**

Fusion protein: hPD-1 ligand 1 + Fc + IL-2 (SEQ ID NO: 43):

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQFVHGEE  
DLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKV  
NAPYNKINQRILVVDPTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTT  
TNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFR  
 LRKGRMMDVKKCGIQDTNSKKQSDTHLEET GGGGSGGGSGGGGS  
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT  
 CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSVHEALHNHYTQ  
 KSLSLSPGK GGGGSGGGSGGGGS  
APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLCLEELKPLEEVNL  
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

Fusion protein: IL-2 + Fc + PD-1 ligand 1 (SEQ ID NO: 44):

APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLCLEELKPLEEVNL  
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT  
 GGGGSGGGSGGGGS  
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT  
 CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSVHEALHNHYTQ  
 KSLSLSPGK GGGGSGGGSGGGGS  
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQFVHGEE  
LKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD  
PVTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE  
ENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEE  
 T

Underlined: IL-2 fragment

*Italic: Linker (optional)*

Plain: Fc

**Bold: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)**

(Note: Can replace linker 1 GGGGSGGGSGGGGS SEQ ID NO: 104 with

EPKSCDK SEQ ID NO: 105)

(Note: Can replace underlined aa in Fc: E with D and M with L)

Fig. 36

**Fusion protein comprising anti-CD4 antibody and PD-1 ligand 1 (PD-L1)**

Anti-CD4 heavy chain and PD-1 ligand 1 (SEQ ID NO: 45)

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN  
NRVSIISIDTSKNLFSCLKRSVTAADTAVYYCASNILKYLHWLLYWQGVLVTVSSASTKGPSVFPL  
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG  
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT  
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK  
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK  
*GGGSGGGSGGGGS*

RIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQF  
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP  
YNKINQRILVVDVPTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI  
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVLGAILLCLGVALTFIFRLRKGR  
MMDVKKCGIQDTNSKKQSDTHLEET

Anti-CD4 light chain (SEQ ID NO: 77)

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS  
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA  
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEEKHKVYACEV  
THQGLSSPVTKSFNRGEC

Underlined: Anti-CD4 antibody variable region

Plain: Anti-CD4 antibody constant region

*Italic: Linker***Bold: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)**

Fig. 37

**Fusion proteins comprising the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from PD-1 ligand (PD-L1)**

Oncostatin M signal peptide + CTLA-4 ECD + IgG C $\gamma$ 1 + PD-L1 (SEQ ID NO: 46):

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLROA  
DSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLGI  
GNGTQIYVIDPEPCPDS QEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM  
 ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE  
 YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN  
 GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK  
 GGGGSGGGGSGGGGS  
 RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQF  
 VHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP  
 YNKINQRILVVDVPTSEHELTCQAEQYPAEVIWTSDDHQLVSGKTTTTNSKREEKLFNVTSTLR  
 NTTTNEIFYCTFRRLDPEENHTAELVIPPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR  
 MMDVKKCGIQDTNSKKQSDTHLEET

**Fusion proteins comprising the extracellular domain of PD-1 ligand (PD-L1), Immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from the extracellular domain of CTLA-4.**

PD-L1 + IgG C $\gamma$ 1 + CTLA-4 ECD (SEQ ID NO: 47):

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQ  
FVHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA  
PYNKINQRILVVDVPTSEHELTCQAEQYPAEVIWTSDDHQLVSGKTTTTNSKREEKLFNVTSTLR  
INTTTNEIFYCTFRRLDPEENHTAELVIPPELPLAHPNERTHLVILGAILLCLGVALTFIFR  
LRKGRMMDVKKCGIQDTNSKKQSDTHLEET GGGGSGGGGSGGGGS  
QEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD  
 GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP  
 QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV  
 DKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS  
AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLROADSQVTEVCAATYMMGNELTFLDDSI  
CTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLGIGNGTQIYVIDPEPCPDS

Underlined Italic: Oncostatin M Signal Peptide (-25 to -1)

Underlined: CTLA-4 extracellular domain (1-125)

Plain: human IgG1 heavy chain constant region (IgG C $\gamma$ 1 domain = H, CH2, CH3)

*Italic:* Linker (optional)

**Underlined:** human PD-1 ligand 1 (PD-1L) or extracellular binding domain of PD-1L

(Note: Optional C to S conversion in IgG sequence (bold underlined))

(Note: The linker QEPKSCDK SEQ ID NO: 110 can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence)

Fig. 38

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**Fusion proteins comprising a sequence of transforming growth factor- $\beta$  (TGF- $\beta$ ), Immunoglobulin Fc (IgG C $\beta$ 1), and a sequence of PD-1 ligand (PD-L1)**

**TGF $\beta$ -1 + Fc + PD-L1 (SEQ ID NO: 48):**

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN  
 QHNPASAAAPCCVPQALEPLPIVYVGRKPKVEQLSNMIVRSCKCS GGGSGGGSGGGGS  
 THTCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVMEALHNHYTQKSLSLSPGK GGGSGGGSGGGGS  
 RIFAVFIFMTYWHLLNAFTVTVPKDLVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQF  
 VHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA  
 YNKINQRIILVVDVPTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR  
 NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR  
 MMDVKKCGIQDTNSKKQSDTHLEET

**Fusion protein comprising a sequence of PD-1 ligand (PD-L1), Immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence of Transforming growth factor beta (TGF- $\beta$ )**

**PD-L1 + Fc + TGF $\beta$ -1 (SEQ ID NO: 49):**

MRIFAVFIFMTYWHLLNAFTVTVPKDLVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQ  
 FVHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA  
 PYNKINQRIILVVDVPTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR  
 INTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR  
 RMDVKKCGIQDTNSKKQSDTHLEET GGGSGGGSGGGGS  
 THTCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVMEALHNHYTQKSLSLSPGK GGGSGGGSGGGGS  
 ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN  
 QHNPASAAAPCCVPQALEPLPIVYVGRKPKVEQLSNMIVRSCKCS

Underlined: TGF $\beta$ -1

*Italic: Linker (optional)*

Plain: Fc

**Bold: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)**

(Note: Can replace linker GGGSGGGSGGGGS SEQ ID NO: 104 with

*EPKSCDK* SEQ ID NO: 105)

(Note: Can replace underlined aa in Fc: E with D and M with L)

Fig. 39

- (i) Transforming growth factor-beta (TGF- $\beta$ 1, TGF- $\beta$ 2, or TGF- $\beta$ 3 or a fragment thereof:

TGF- $\beta$ 1 full sequence (SEQ ID NO: 102):

```
1  MPPSGLRLLP LLLPLLRLLV LTPGRPAAGL STCKTIDMEL VKRKRIEAIR GQILSKLRLS
61  SPSPQGEVPP VPLPEAVLAL YNSTRDRVAG ESAEPEPEPE ADYYAKEVTR VLMVENTNKI
121 YEKVKKSPHS IYMLFNTSEL REAVPEPVLL SRAELRLLRL KLKAEQHVEL YQKYSNDSWR
181 YLSNRL LAPS DTPEWLSFDV TGVVRQWLSH GGEVEGFRLS AHCSCDSKDN TLQVDINGFS
241 SSRRGDLATI HGMNRPFLLL MATPLERAQH LHSSRQRRAL DTNYCFSSTE KNCCVRQLYI
301 DFRKDLGWKW IHEPKGYHAN FCLGPCPYIW SLDTQYSKVL ALYNQHNPQA SAAPCCVPQA
361 LEPLPIVYYV GRKPKVEQLS NMIVRSCKCS
```

Underlined: Mature (active) TGF- $\beta$ 1 sequence (Ala 279 – Ser 390; 112 aa).

- (ii) Mature (active) TGF- $\beta$ 1 sequence (Ala 279 – Ser 390; 112 aa)  
(SEQ ID NO: 103):

```
AL DTNYCFSSTE KNCCVRQLYI DFRKDLGWKW IHEPKGYHAN FCLGPCPYIW
SLDTQYSKVL ALYNQHNPQA SAAPCCVPQA LEPLPIVYYV GRKPKVEQLS NMIVRSCKCS
```

Fig. 40

**Fusion proteins comprising an antibody that binds TNF- $\alpha$ , and a sequence of transforming growth factor- $\beta$  (TGF- $\beta$ ).**

Anti-TNF $\alpha$  heavy chain + TGF- $\beta$ 1 (SEQ ID NO: 50):

EVQLVESGGGLVQPGRSRLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSVEG  
RFTISRDNKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGTTLVTVSSASTKGPSVFPL  
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG  
 TQTYICNVNHKPSNTKVD**KKAE**PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT  
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK  
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

*GGGGSGGGSGGGGS*

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN  
 QHNPGASAAPCCVPQALEPLPIVYYYVGRKPKVEQLSNMIVRSCKCS

Anti-TNF $\alpha$  light chain (SEQ ID NO: 77):

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLPKGAPKLLIYAASLTQSGVPSRFS  
SGTDFTLTISSLPEDVATYYCQRYNRAPYTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASV  
 VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTH  
 QGLSSPVTKSFNRGEC

Underlined: anti-TNF $\alpha$  antibody variable region

Plain: anti-TNF $\alpha$  antibody constant region

*Italic: Linker*

**Bold: Mature (active) human TGF- $\beta$ 1**

(Note: **KKAE** SEQ ID NO: 107 can be replaced with **KRVE** SEQ ID NO: 108 or **KKVE** SEQ ID NO: 109)

Fig. 41

**Fusion proteins comprising TNFR2 Extracellular ligand binding domain, Fc, and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ )**

TNFR2 ECD + IgG C $\gamma$ 1 + TGF- $\beta$ 1 (SEQ ID NO: 51):

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN  
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT  
ETSDVVCCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV  
STRSQHTQPTPEPSTAPSTSFLPMGPSPPAEGSTGD *EPKSCDK*  
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 R**E****E**MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVSMHEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*  
 ALD**T**NYCFS**S**TEKNCCVRQ**L**YIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN  
 QHNP**G**ASAA**P**CCVP**Q**ALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

**Fusion proteins comprising a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ), Fc, and TNFR2 Extracellular ligand binding domain:**

TGF- $\beta$ 1 + IgG C $\gamma$ 1 + TNFR2 ECD (SEQ ID NO: 52):

ALD**T**NYCFS**S**TEKNCCVRQ**L**YIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN  
 QHNP**G**ASAA**P**CCVP**Q**ALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS *GGGGSGGGSGGGGS*  
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 R**E****E**MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVSMHEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*  
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN  
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT  
ETSDVVCCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV  
STRSQHTQPTPEPSTAPSTSFLPMGPSPPAEGSTGD

Underlined: TNFR2 ligand-binding domain

Plain: human IgG1 heavy chain constant region (IgG C $\gamma$ 1 domain = H, CH2, CH3)

*Italic: Linker*

**Bold: Mature (active) human TGF- $\beta$ 1**

(Note: Can replace linker 1 with *GGGGSGGGSGGGGS* SEQ ID NO: 104 or *EPKSCDK* SEQ ID NO: 105)

(Note: Can replace underlined aa in Fc: **E** with D and **M** with L)

Fig. 42

**Fusion proteins comprising anti-CD20 antibody and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ )**

Anti-CD20 heavy chain + mature TGF $\beta$ 1 sequence (SEQ ID NO: 53):

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKG  
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPL  
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG  
 TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT  
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK  
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

*GGGSGGGSGGGGS*

**ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN**  
**QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS**

Anti-CD20 light chain sequence (SEQ ID NO: 72):

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQOKPGSSPKPWIYATSNLASGVPVRFSGSGS  
GTSYSLTISRVEAEDAATYYCQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASV  
 CLLNFPYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQ  
 GLSSPVTKSFNRGEC

Underlined: anti-CD20 antibody variable region

Plain: anti-CD20 antibody constant region

*Italic: Linker*

**Bold: Mature (active) human TGF- $\beta$ 1**

Fig. 43

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**Fusion proteins comprising anti-CD25 antibody and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ).**

Anti-CD25 (Daclizumab) heavy chain and TGF- $\beta$ 1 (SEQ ID NO: 54):

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY  
NQKFKDKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LVTVSSASTK  
GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS  
LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF  
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR  
VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN  
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN  
VFSCSVMHEA LHNHYTQKSL SLSPGK **GGGGSGGGGSGGGGS** **ALDTNYCFSSTEKNCCVRQLY**  
**IDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPLP**  
**IVYYVGRKPKVEQLSNMIVRSCKCS**

Anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75):

DIQMTQSPST LSASVGDRVT ITCSASSSIS YMHWYQQKPG KAPKLLIYTT SNLASGVPAR  
FSGSGSGTEF TLTISSLQPD DFATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS  
DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLT  
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

Plain: anti-CD25 antibody

*Italic: Linker*

**Bold: Mature (active) human TGF- $\beta$ 1**

Fig. 44A

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Anti-CD25 (Basiliximab) heavy chain and TGF- $\beta$ 1 (SEQ ID NO: 55):

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ  
 KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDIYGY YFDFWGQGT LTVSSASTKG  
 PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL  
 SSVVTVPPSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL  
 FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV  
 VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTL PPSRDELTKNQ  
 VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFFLYSKLTV DKSRWQQGNV  
 FSCSVMEAL HNHYTQKSLS LSPGK GGGGSGGGGSGGGGS  
**ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHAN**  
**FCLGPCPYIWSLDTQYSKVLALYNQHNP GASAAPCCVPQALEPLPIVYYYVGRKPKVEQ**  
**LSNMIVRSCKCS**

Anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76):

QIVSTQSPAI MSASPGEKVT MTCSASSRS YMOWYQQKPG TSPKRWIYDT SKLASGVPAR  
 FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE  
 QLKSGTASVV CLLNMFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK  
 ADYEKHKVYA CEVTHQGLSS PVTKSFNRGEC

Plain: anti-CD25 antibody

*Italic: Linker*

**Bold: Mature (active) human TGF- $\beta$ 1**

Fig. 44B

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**Fusion proteins comprising IL-2, Fc, and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ).**

TGF- $\beta$ 1 + Fc + IL-2 (SEQ ID NO: 56):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN  
 QHNPGASAAPCCVPQALEPLPIVYVGRKPKVEQLSNMIVRSCKCS GGGGSGGGGSGGGGS  
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVMHREALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS  
APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL  
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL  
T

IL-2 + Fc + TGF- $\beta$ 1 (SEQ ID NO: 57):

APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL  
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL  
T GGGGSGGGGSGGGGS  
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVMHREALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS  
 ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN  
 QHNPGASAAPCCVPQALEPLPIVYVGRKPKVEQLSNMIVRSCKCS

Underlined: IL-2 fragment

*Italic: Linker (optional)*

Plain: Fc

**Bold: Mature (active) human TGF- $\beta$ 1**

(Note: Can replace linker 1 GGGGSGGGGSGGGGS SEQ ID NO: 104 with  
 EPKSCDK SEQ ID NO: 105)

(Note: Can replace underlined aa in Fc: E with D and M with L)

Fig. 45

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**Fusion protein comprising anti-CD4 antibody and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ).**

Anti-CD4 heavy chain and TGF- $\beta$ 1 (SEQ ID NO: 58)

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN  
NRVSISIDTSKNLFSKLRSVTAADTAVYYCASNILKYLHWLLYWGQGVLTVSSASTKGPSVFPL  
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG  
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT  
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK  
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK  
*GGGSGGGSGGGGS*  
**ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCFYIWSLDTQYSKVLALYN**  
**QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS**

Anti-CD4 light chain (SEQ ID NO: 77)

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS  
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFVGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA  
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEEKHKVYACEV  
THQGLSSPVTKSFNRGEC

Underlined: Anti-CD4 antibody variable region

Plain: Anti-CD4 antibody constant region

*Italic: Linker*

**Bold: Mature (active) human TGF- $\beta$ 1**

Fig. 46

**Fusion proteins comprising the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ )**

Oncostatin M signal peptide + CTLA-4 ECD + IgG C $\gamma$ 1 + TGF- $\beta$ 1 (SEQ ID NO: 59):

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLAASSRGIA SFVCEYASPGKATEVRVTVLRQA  
DSQVTEVCAATYMMGNELTFLDDSI CTGTSSGNQVNLT IQGLRAMDTGLYICKVELMYPPPYLGI  
GNGTQIYVIDPEPCPDSDQEPKSCDK THTCPPCPAPELLGGPSVFLFPPKPKDTLM  
 ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE  
 YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN  
 GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK  
 GGGGSGGGGSGGGGS  
 ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN  
 QHNP GASAAPCCVPQALEPLPIVYVGRKPKVEQLSNMIVRSCKCS

**Fusion proteins comprising a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ), Immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from the extracellular domain of CTLA-4**

TGF- $\beta$ 1 + IgG C $\gamma$ 1 + CTLA-4 ECD (SEQ ID NO: 60):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN  
 QHNP GASAAPCCVPQALEPLPIVYVGRKPKVEQLSNMIVRSCKCS EPKSCDK  
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFCSCVMHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS  
AMHVAQPAVVLAASSRGIA SFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSI  
CTGTSSGNQVNLT IQGLRAMDTGLYICKVELMYPPPYLGIGNGTQIYVIDPEPCPDSD

Underlined Italic: Oncostatin M Signal Peptide (-25 to -1)

Underlined: CTLA-4 extracellular domain (1-125)

Plain: human IgG1 heavy chain constant region (IgG C $\gamma$ 1 domain = H, CH2, CH3)

*Italic: Linker (optional)*

**Bold: Mature (active) human TGF- $\beta$ 1**

(Note: Optional C to S conversion in IgG sequence (bold underlined))

(Note: The linker *QEPKSCDK* SEQ ID NO: 110 can be replaced with *EPKSCDK* SEQ ID NO: 105 or another linker sequence)

Fig. 47

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**Fusion proteins comprising an antibody that binds TNF- $\alpha$ , and a RANKL-binding sequence of receptor activator of nuclear factor-kB (RANK)**

Anti-TNF $\alpha$  heavy chain + RANK ectodomain (SEQ ID NO: 61):

EVQLVESGGGLVQPGKSLRLSCAASGFTFDDYAMHWVRQAPGKLEWVSAITWNSGHIDYADSVEG  
RFTISRDNAKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGTLVTVSSASTKGPSVFPL  
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG  
 TQTYICNVNHKPSNTKVD**KKAE**PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT  
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK  
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK  
 GGGSGGGSGGGGS *QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP*  
 CGPDEYLDW *NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN*  
 TECAPGLGAQ *HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS*  
 DAVCSSSLPA *RKPPNEPHVY LPG*

Anti-TNF $\alpha$  light chain (SEQ ID NO: 78):

DIQMTQSPSSLSASVGDRVTITCRASQGI RNYLAWYQQLPGKAPKLLIYAAS  
TLQSGVPSRFS  
SGSGSGTDFTLTISSLPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV  
 VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEEKHKVYACEVTH  
 QGLSSPVTKSFNRGEC

Underlined: anti-TNF $\alpha$  antibody variable region

Plain: anti-TNF $\alpha$  antibody constant region

*Italic: Linker*

**Bold: RANK ectodomain**

(Note: **KKAE** SEQ ID NO: 107 can be replaced with **KRVE** SEQ ID NO: 108 or **KKVE** SEQ ID NO: 109)

Fig. 48

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**Fusion proteins comprising TNFR2 Extracellular ligand binding domain, Fc, and a RANKL-binding sequence of receptor activator of nuclear factor- $\kappa$ B (RANK)**  
TNFR2 ECD + IgG C $\gamma$ 1 + RANK ectodomain (SEQ ID NO: 62):

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN  
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT  
ETSDVVCCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV  
STRSQHTQPTPEPSTAPSTSFLPMGSPSPAEGSTGD *EPKSCDK*  
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
**REEM**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG  
NVFSCSVMHREALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS* **QI** APPCTSEKHY  
EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA  
VVAGNSTTPR RCTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS  
DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

**Fusion proteins comprising a RANKL-binding sequence of receptor activator of nuclear factor- $\kappa$ B (RANK), Fc, and TNFR2 Extracellular ligand binding domain:**  
RANK ectodomain + IgG C $\gamma$ 1 + TNFR2 ECD (SEQ ID NO: 63):

**QI** APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDW  
NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCTAGYHW SQDCECCRN TECAPGLGAQ  
HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA  
RKPPNEPHVY LPG  
*GGGGSGGGSGGGGS* THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED  
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT  
ISKAKGQPREPQVYTLPPS**REEM**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD  
GSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*  
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN  
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT  
ETSDVVCCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV  
STRSQHTQPTPEPSTAPSTSFLPMGSPSPAEGSTGD

Underlined: TNFR2 ligand-binding domain

Plain: human IgG1 heavy chain constant region (IgG C $\gamma$ 1 domain = H, CH2, CH3)

*Italic: Linker*

**Bold: RANK ectodomain**

(Note: Can replace linker 1 with *GGGGSGGGSGGGGS* SEQ ID NO: 104 or  
*EPKSCDK* SEQ ID NO: 105)

(Note: Can replace underlined aa in Fc: **E** with D and **M** with L)

Fig. 49

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**Fusion proteins comprising the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C $\gamma$ 1), and a RANKL-binding sequence of receptor activator of nuclear factor- $\kappa$ B (RANK)**

Oncostatin M signal peptide + CTLA-4 ECD + IgG C $\gamma$ 1 + RANK ectodomain (SEQ ID NO: 64):

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQA  
DSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLGI  
GNGTQIYVIDPEPCPDSDQEPKSCDK **THTCPPCPAPELLGGPSVFLFPPKPKDTLM**  
 ISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE  
 YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN  
 GQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK  
 GGGGSGGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP**  
**CGPDEYLD SW** **NEEDKCLLHK** **VCDTGKALVA** **VVAGNSTTPR** **RCACTAGYHW** **SQDCECCRN**  
**TECAPGLGAQ** **HPLQLNKDTV** **CKPCLAGYFS** **DAFSSTDKCR** **PWTNCTFLGK** **RVEHHGTEKS**  
**DAVCSSSLPA** **RKPPNEPHVY** **LPG**

**Fusion proteins comprising a RANKL-binding sequence of receptor activator of nuclear factor- $\kappa$ B (RANK), Immunoglobulin Fc (IgG C $\gamma$ 1), and a sequence from the extracellular domain of CTLA-4**

RANK ectodomain + IgG C $\gamma$ 1 + CTLA-4 ECD (SEQ ID NO: 65):

**QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW**  
**NEEDKCLLHK** **VCDTGKALVA** **VVAGNSTTPR** **RCACTAGYHW** **SQDCECCRN** **TECAPGLGAQ**  
**HPLQLNKDTV** **CKPCLAGYFS** **DAFSSTDKCR** **PWTNCTFLGK** **RVEHHGTEKS** **DAVCSSSLPA**  
**RKPPNEPHVY** **LPG** **EPKSCDK**  
**THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK**  
**TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS**  
**RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQG**  
**NVFSVCSVMHEALHNHYTQKSLSLSPGK** **GGGGSGGGGSGGGGS**  
AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSI  
CTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLGIGNGTQIYVIDPEPCPDSD

Underlined Italic: Oncostatin M Signal Peptide (-25 to -1)

Underlined: CTLA-4 extracellular domain (1-125)

Plain: human IgG1 heavy chain constant region (IgG C $\gamma$ 1 domain = H, CH2, CH3)

*Italic: Linker (optional)*

**Bold: RANK ectodomain**

(Note: Optional C to S conversion in IgG sequence (bold underlined))

(Note: The linker QEPKSCDK SEQ ID NO: 110 can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence)

Fig. 50

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**Fusion proteins comprising a RANKL-binding sequence of receptor activator of nuclear factor- $\kappa$ B (RANK), Fc, and a sequence from transforming growth factor- $\beta$  (TGF- $\beta$ ).**

TGF- $\beta$ 1 + Fc + RANK ectodomain (SEQ ID NO: 66):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN  
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS *GGGGSGGGSGGGGS*  
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVMHREALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS* **QI** APPCTSEKHY  
 EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA  
 VVAGNSTTPR RCTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS  
 DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

RANK ectodomain + Fc + TGF- $\beta$ 1 (SEQ ID NO: 67):

**QI** APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW  
 NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCTAGYHW SQDCECCRN TECAPGLGAQ  
 HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA  
 RKPPNEPHVY LPG *GGGGSGGGSGGGGS*  
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG  
 NVFSCSVMHREALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*  
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN  
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Underlined: Mature (active) human TGF- $\beta$ 1

*Italic: Linker (optional)*

Plain: Fc

**Bold: RANK ectodomain**

(Note: Can replace linker 1 *GGGGSGGGSGGGGS* SEQ ID NO: 104 with

*EPKSCDK* SEQ ID NO: 105)

(Note: Can replace underlined aa in Fc: E with D and M with L)

Fig. 51

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**Fusion proteins comprising a RANKL-binding sequence of receptor activator of nuclear factor- $\kappa$ B (RANK), Fc, and a sequence from PD-1 ligand 1**

PD-1L1 + Fc + RANK ectodomain (SEQ ID NO: 68):

RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEED  
LKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD  
PVTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE  
ENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMDVKKCGIQDTNSKKQSDTHLEE  
 T GGGGSGGGGSGGGGS  
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT  
 CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSVHEALHNHYTQ  
 KSLSLSPGK GGGGSGGGGSGGGGS QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP  
 CGPDEYLDW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCTAGYHW SQDCECCRN  
 TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS  
 DAVCSSSLPA RKPPNEPHVY LPG

RANK ectodomain + Fc + PD-1L1 (SEQ ID NO: 69):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDW NEEDKCLLHK  
 VCDTGKALVA VVAGNSTTPR RCTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV  
 CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG  
 GGGGSGGGGSGGGGS  
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT  
 CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSVHEALHNHYTQ  
 KSLSLSPGK GGGGSGGGGSGGGGS  
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEED  
LKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD  
PVTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE  
ENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMDVKKCGIQDTNSKKQSDTHLEE  
 T

**Bold: PD-1L1 (May use PD-1L1 extracellular domain – underlined)**

*Italic: Linker (optional)*

Plain: Fc

Underlined: RANK ectodomain

(Note: Can replace linker 1 GGGGSGGGGSGGGGS SEQ ID NO: 104 with

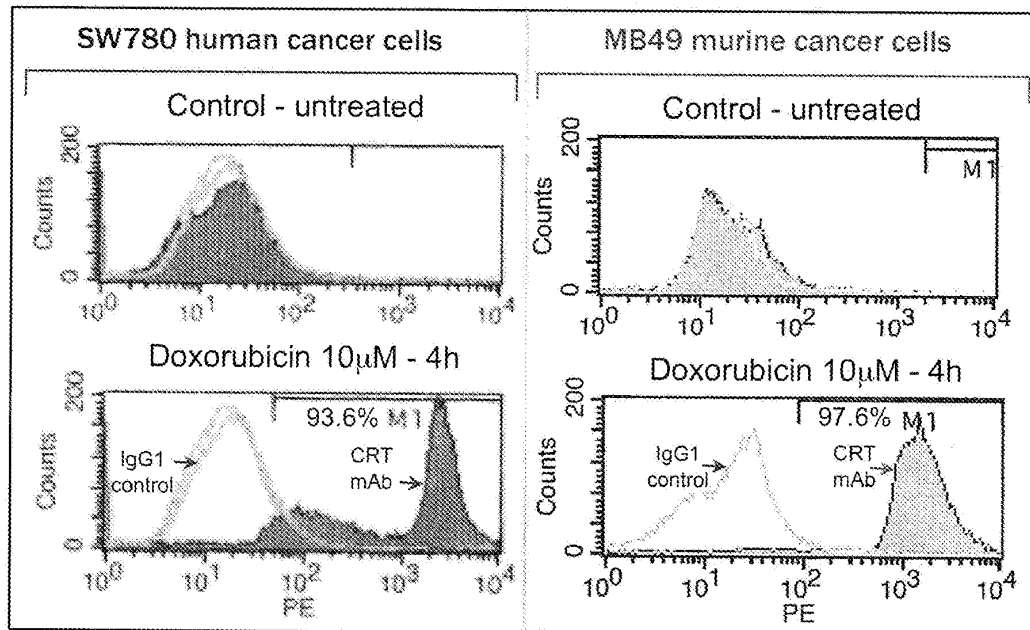
EPKSCDK SEQ ID NO: 105)

(Note: Can replace underlined aa in Fc: E with D and M with L)

Fig. 52

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A



B

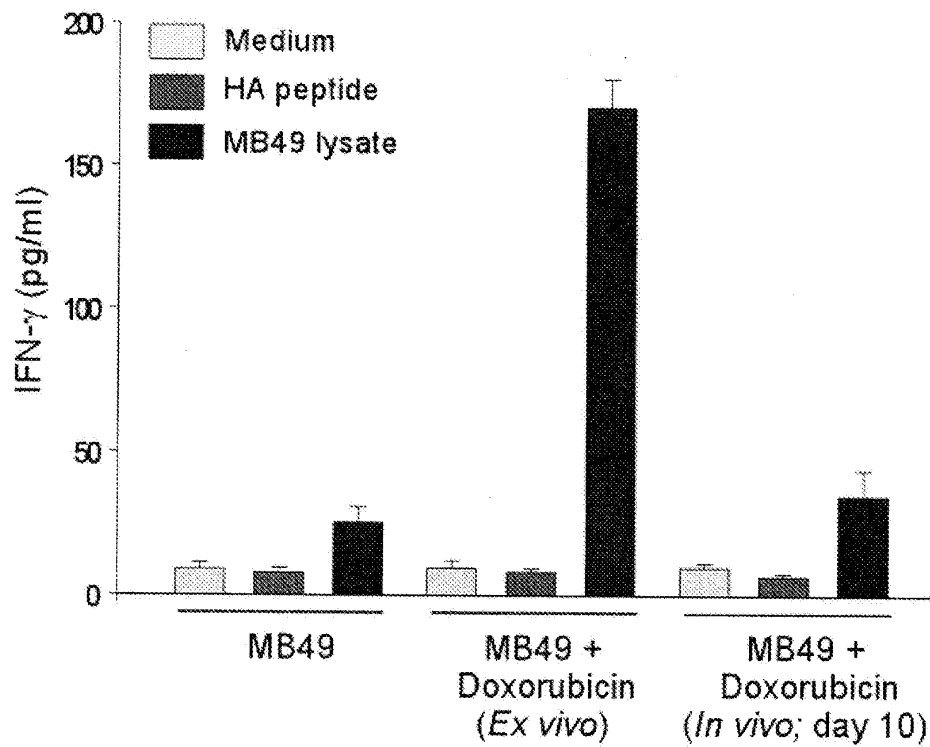


Fig. 53 A &amp; B

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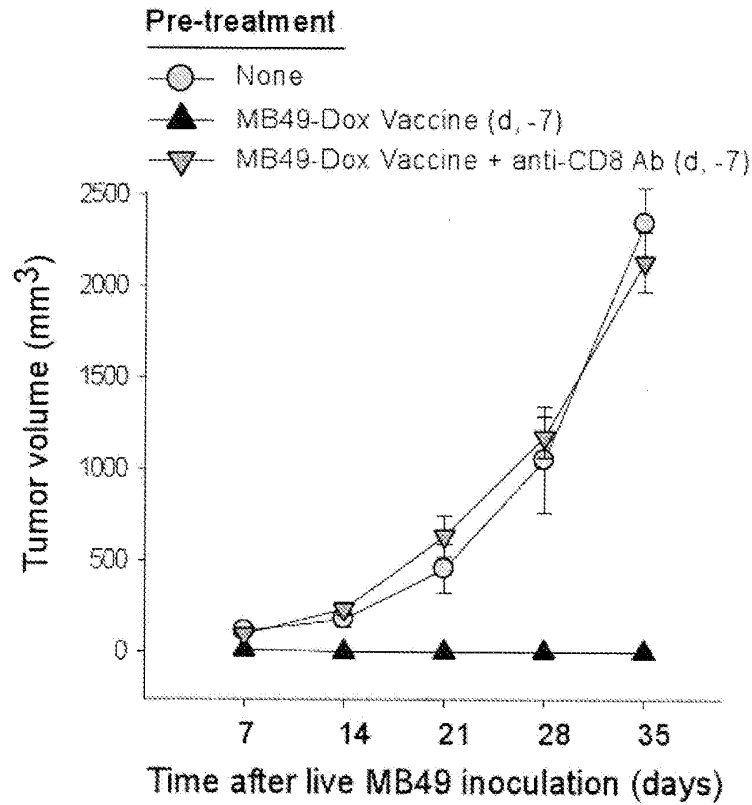
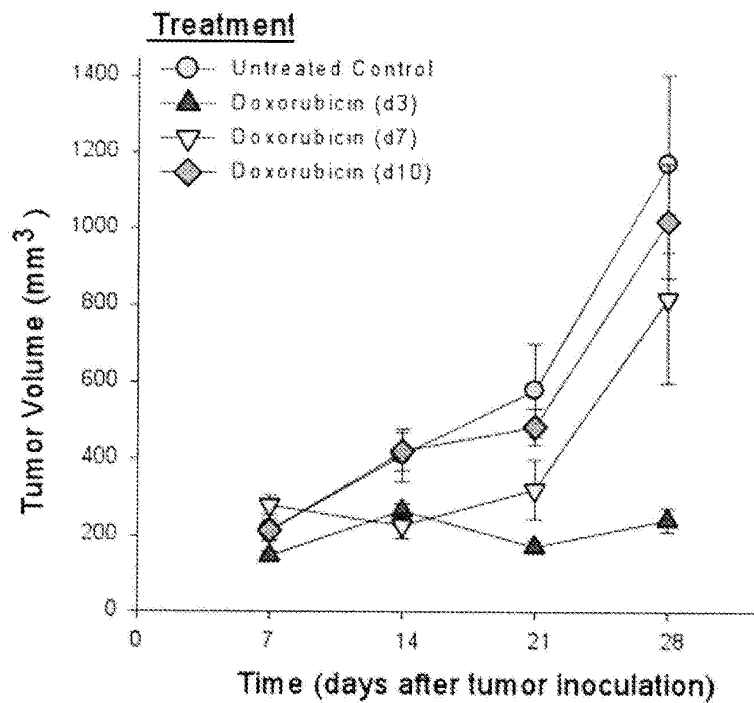
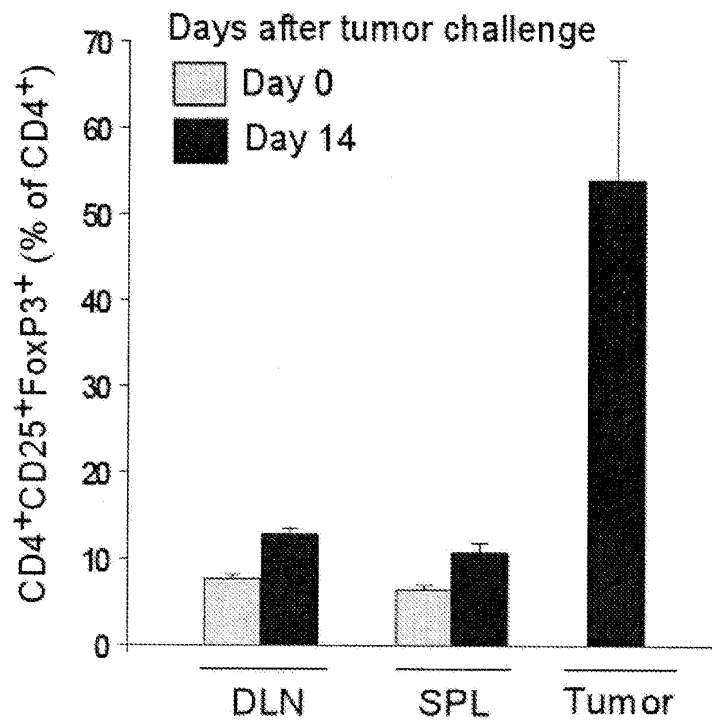
**C****D**

Fig. 53 C &amp; D

62 / 75

E



F

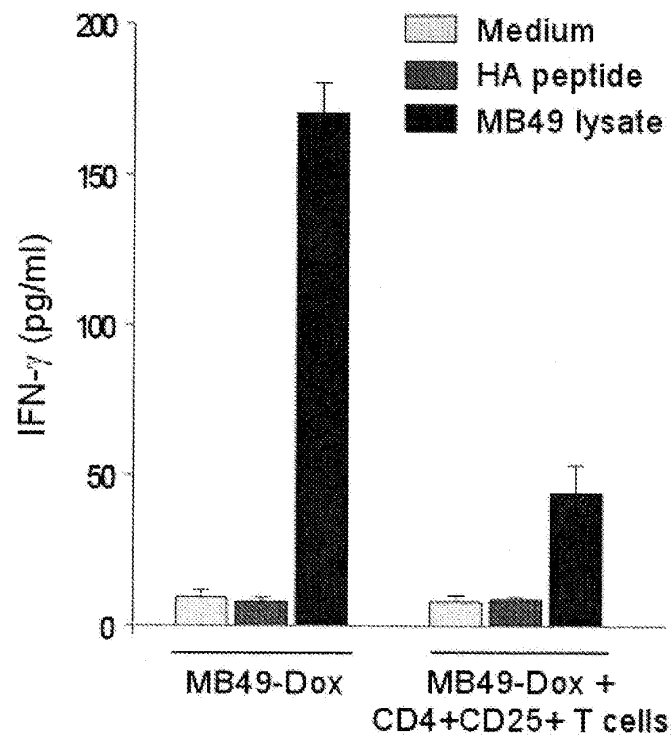


Fig. 53 E &amp; F

63 / 75

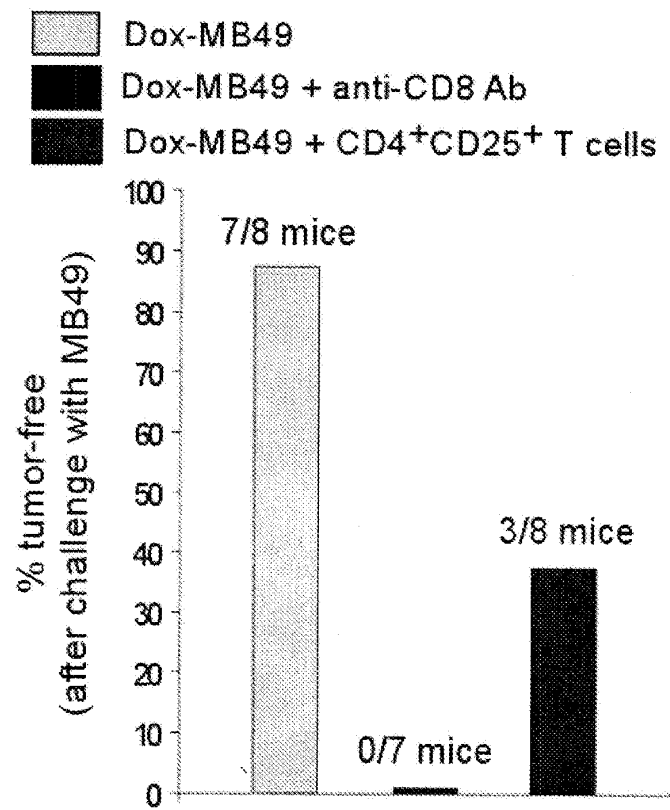
**G**

Fig. 53G

64 / 75

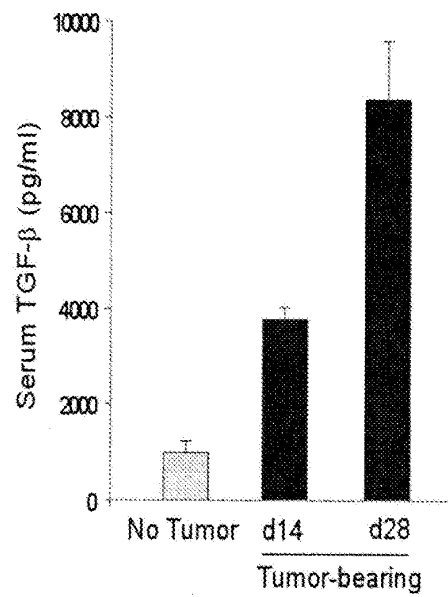
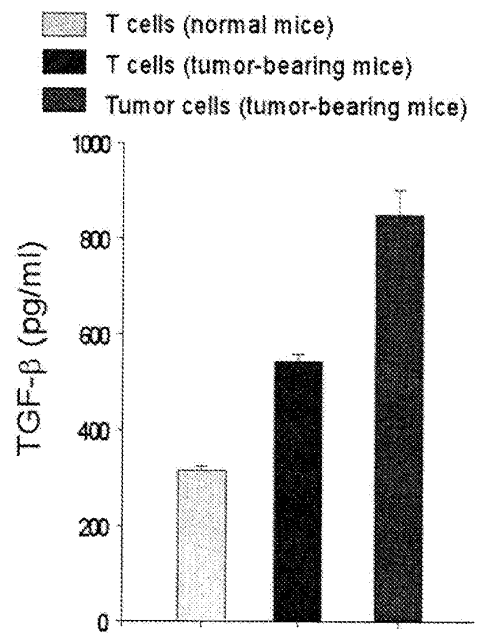
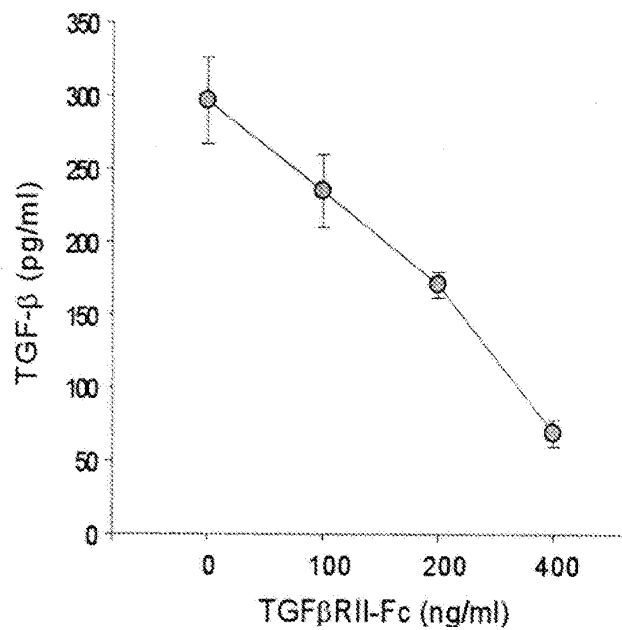
**A****B**

Fig. 54 A &amp; B

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C



D

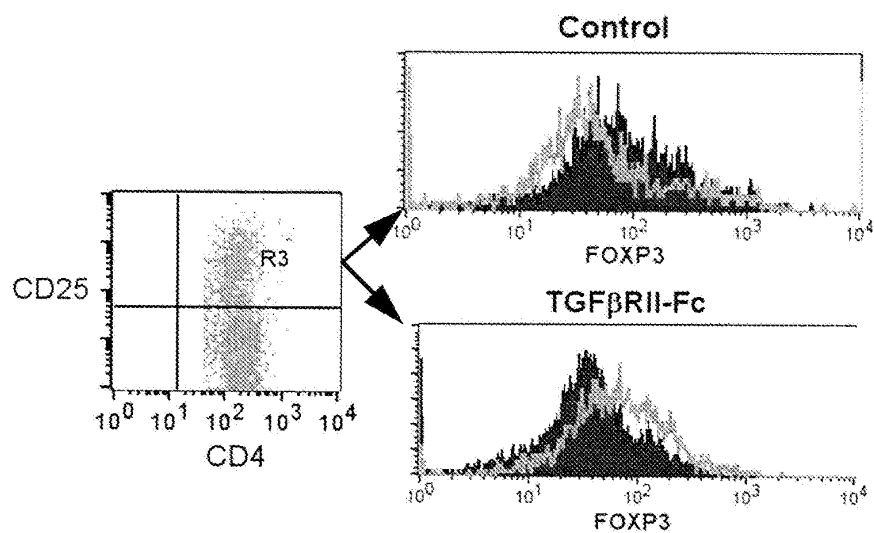


Fig. 54 C &amp; D

66 / 75

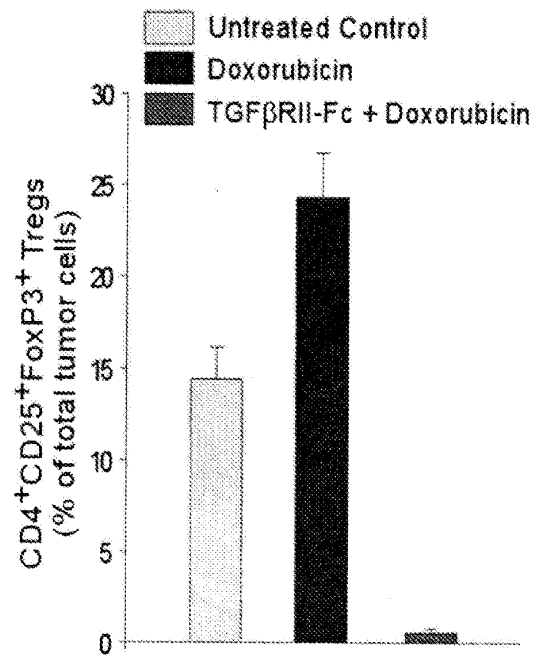
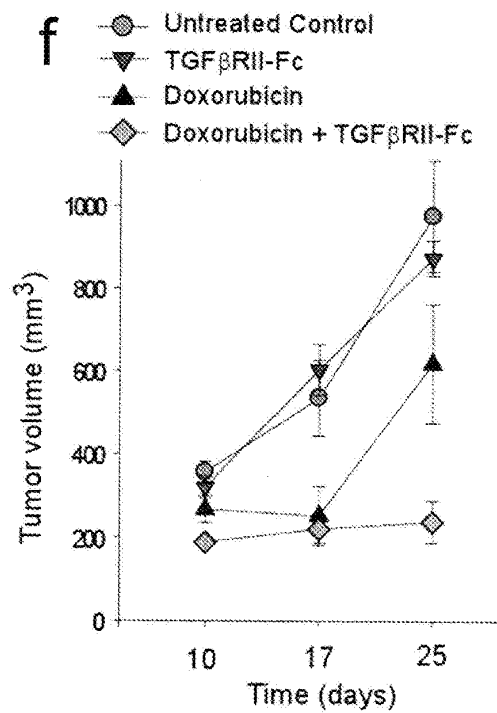
**E****F**

Fig. 54 E &amp; F

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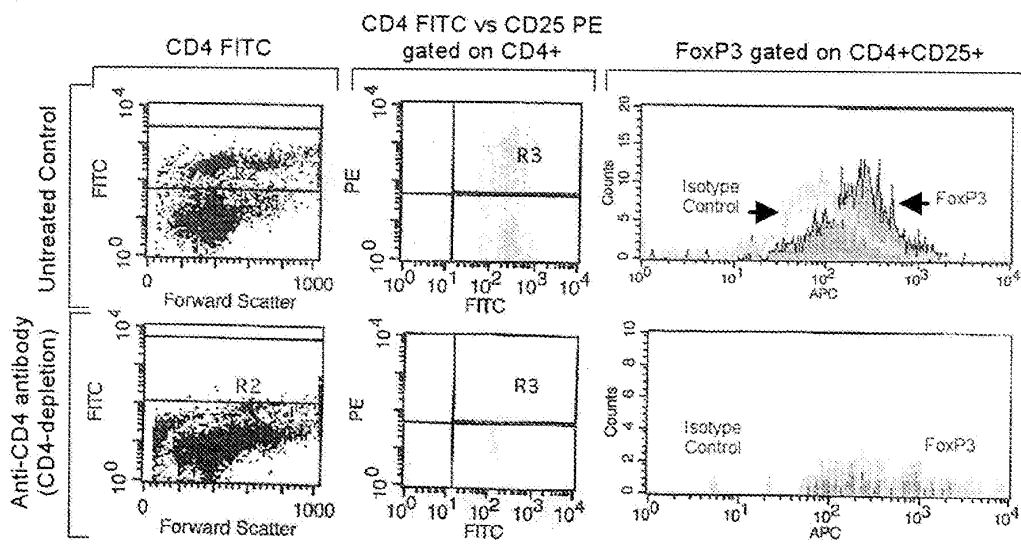
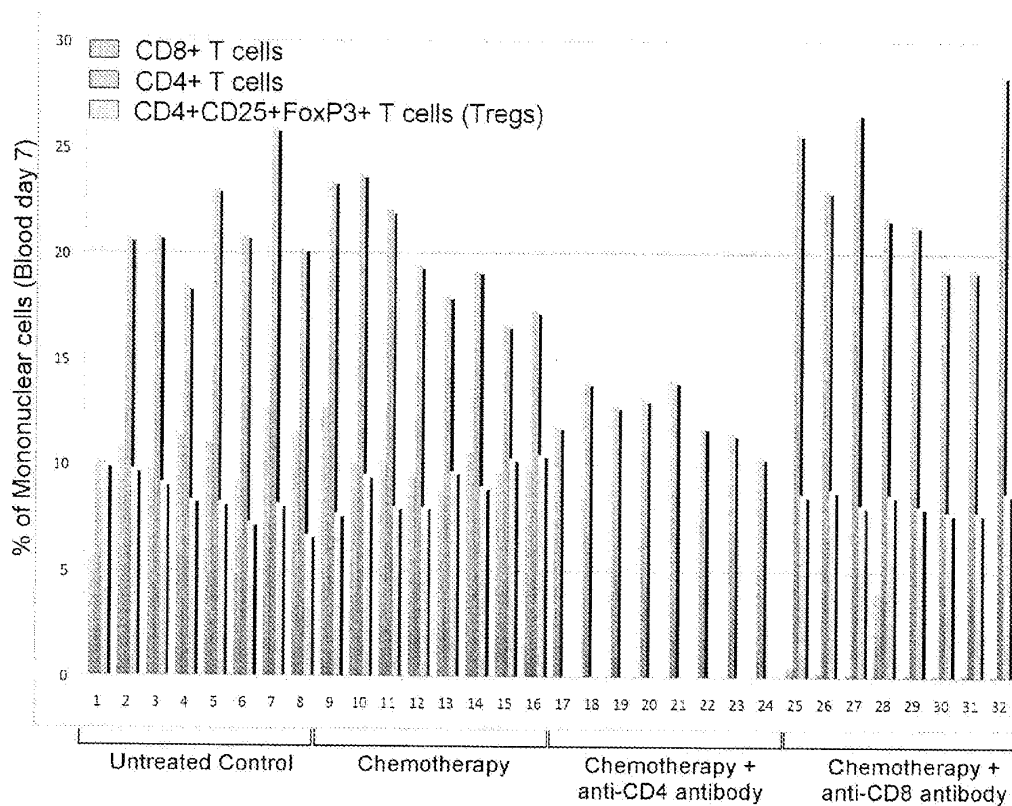
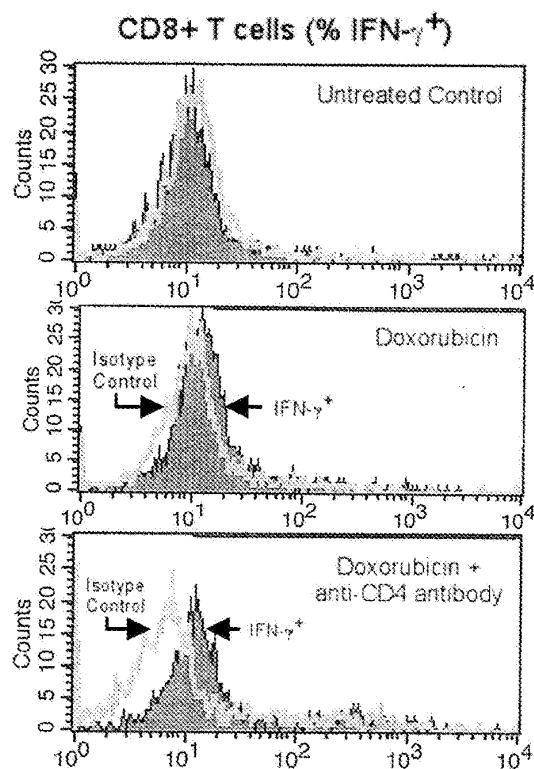
**A****B**

Fig. 55 A &amp; B

68 / 75

C



D

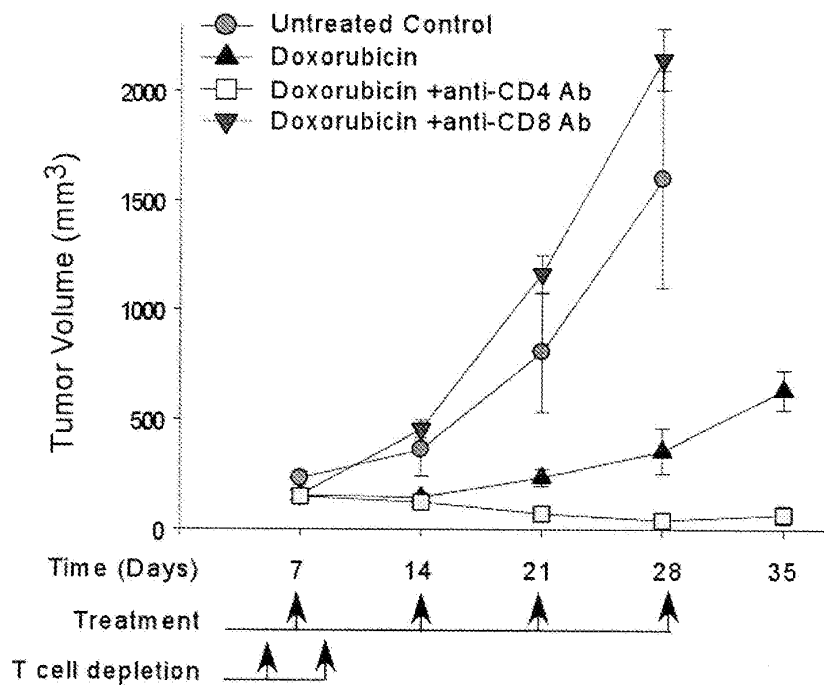
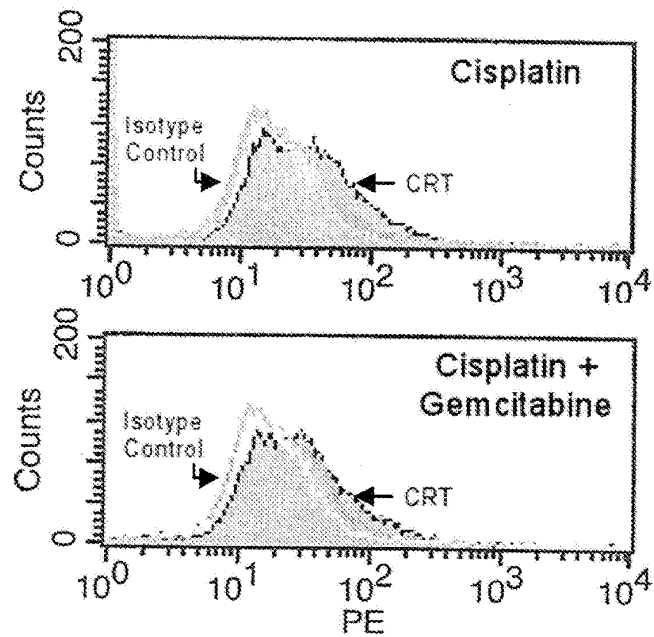


Fig. 55 C &amp; D

69 / 75

A



B

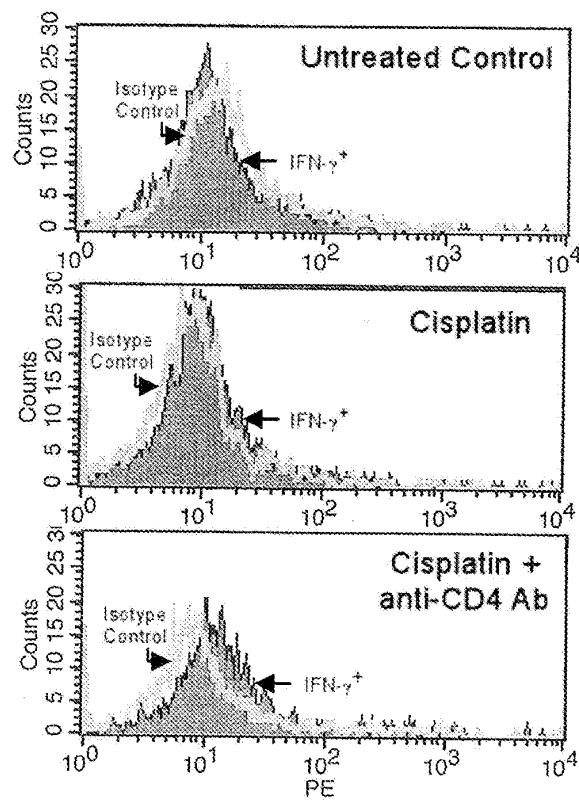
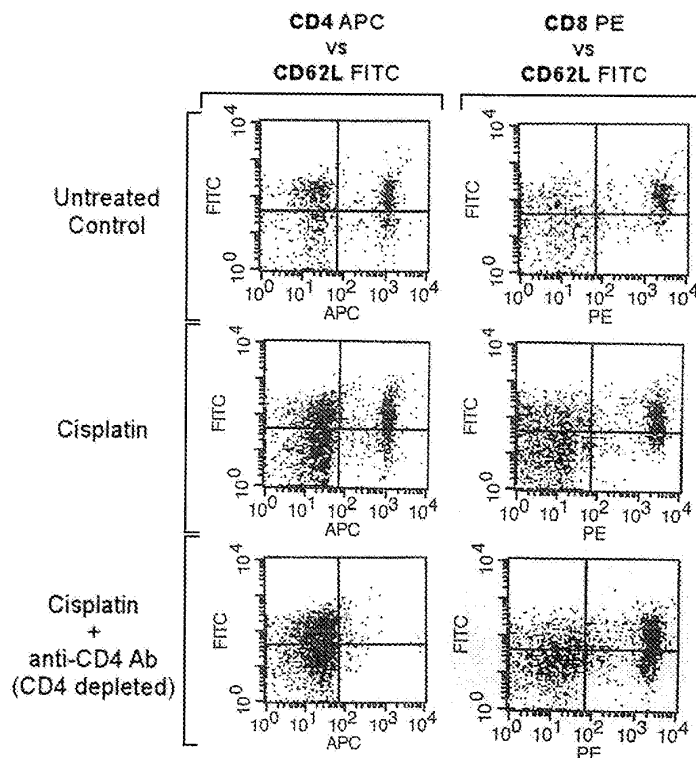


Fig. 56 A &amp; B

70 / 75

C



D

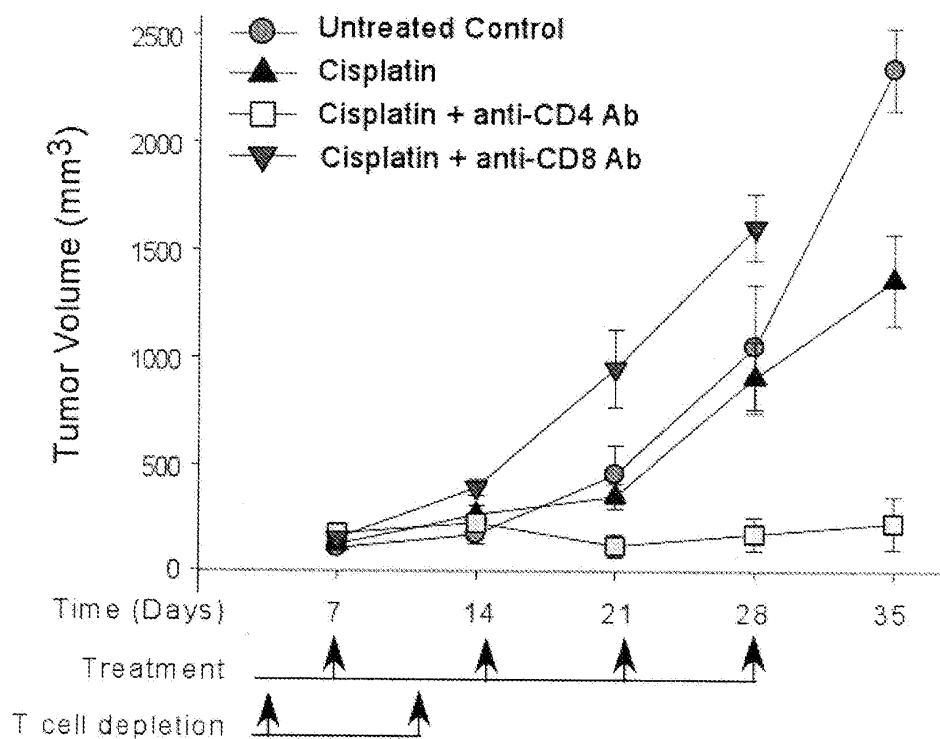


Fig. 56 C &amp; D

71 / 75

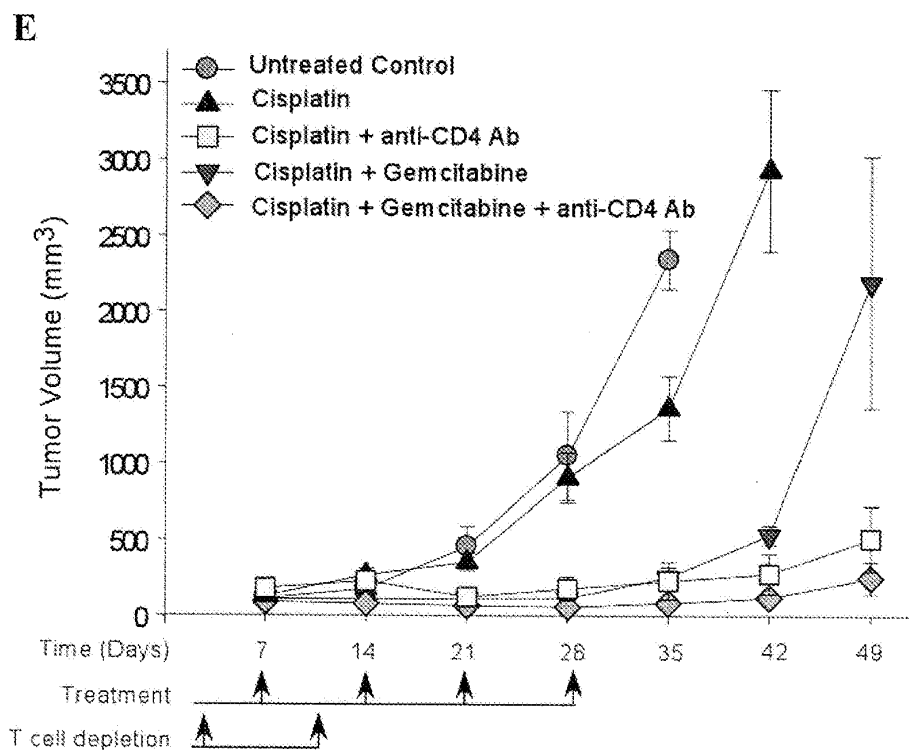
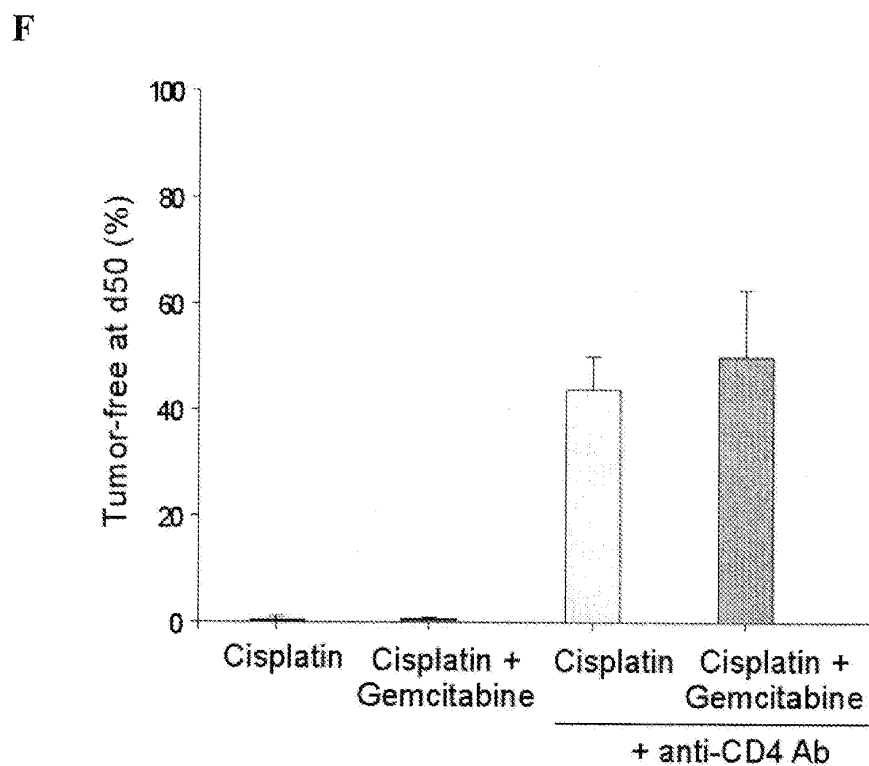


Fig. 56 E &amp; F

72 / 75

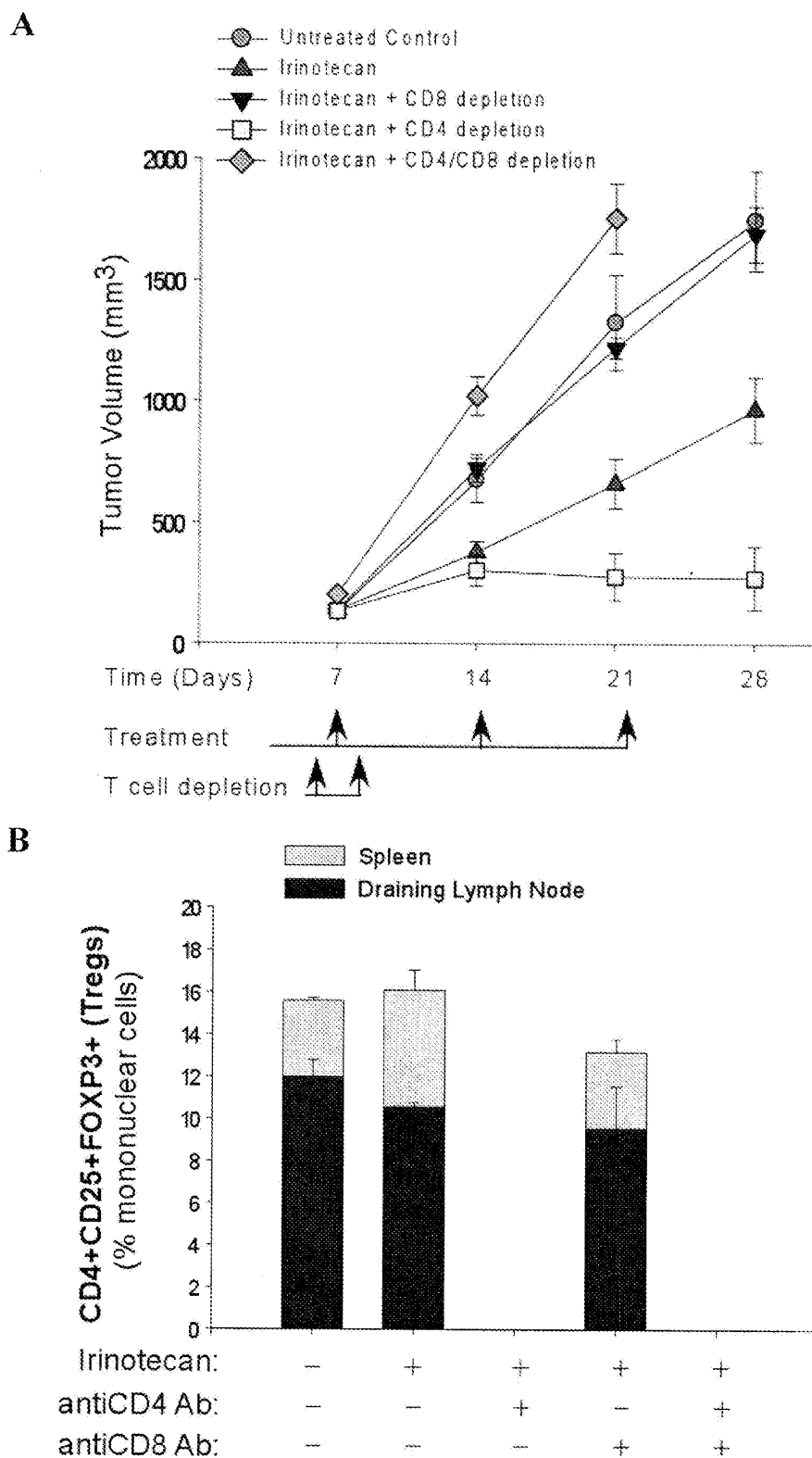


Fig. 57 A &amp; B

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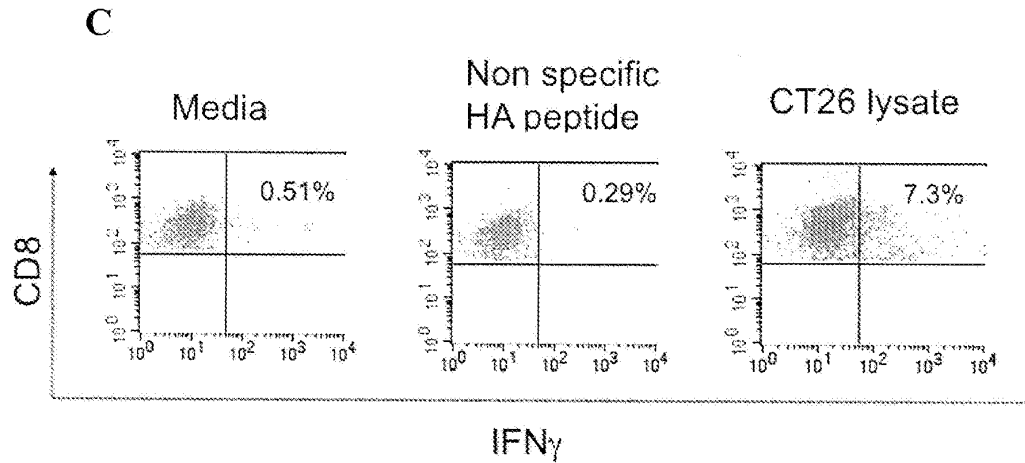
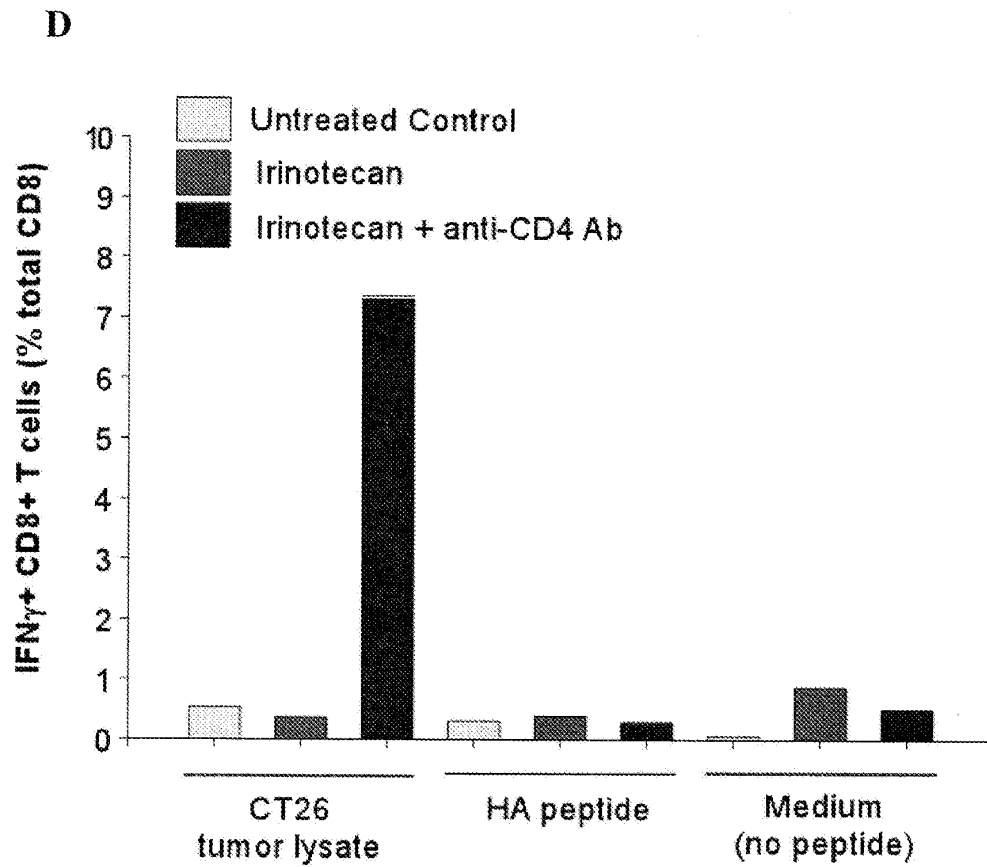
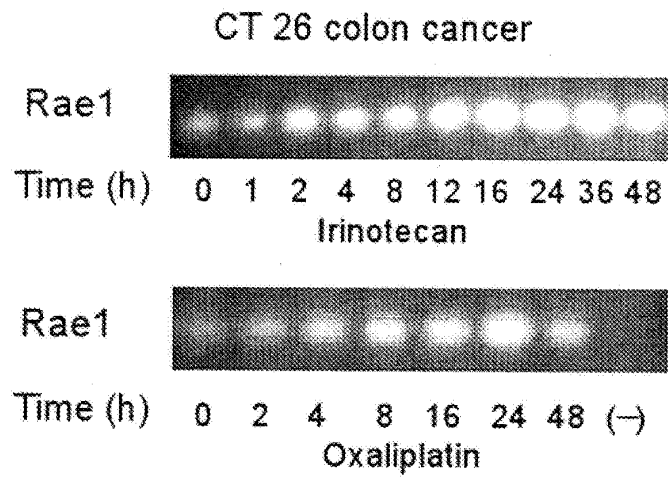


Fig. 57 C &amp; D

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E



F

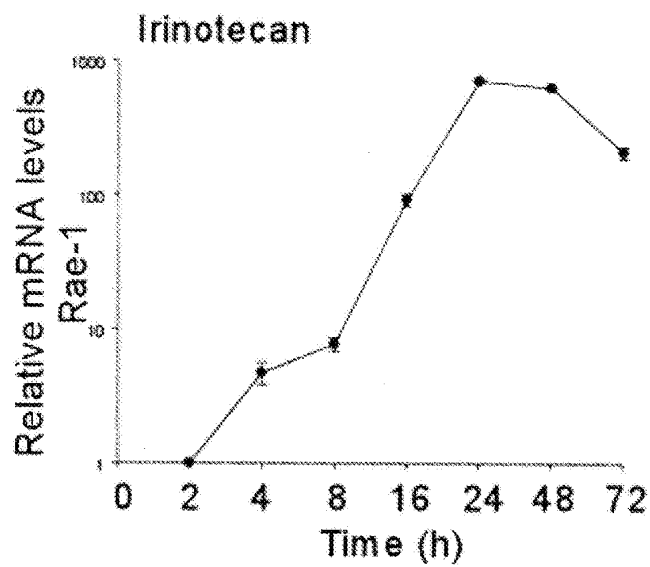
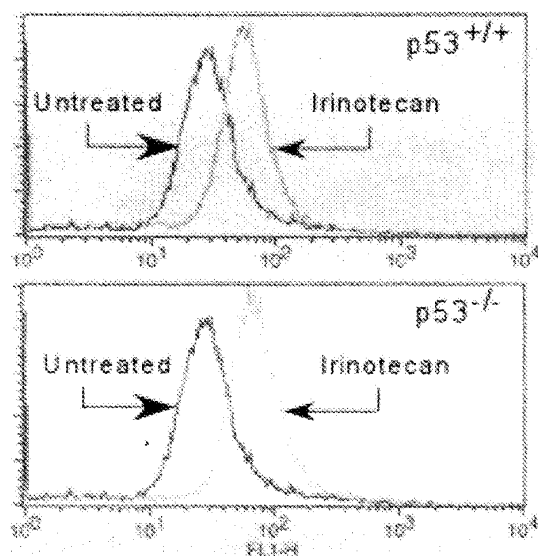


Fig. 57 E &amp; F

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G

Expression of MICA/B on HCT116 cells



H

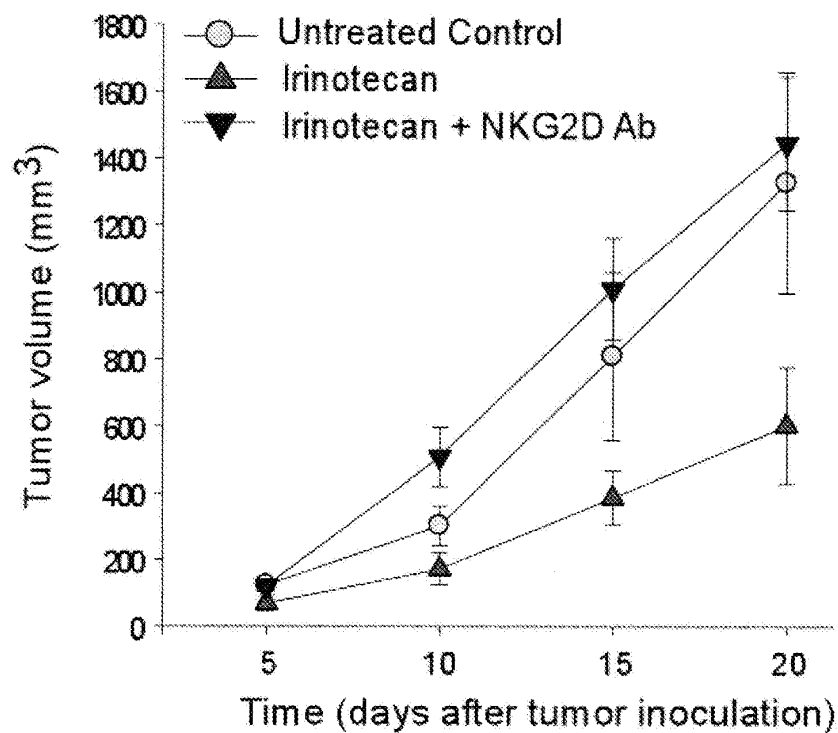


Fig. 57 G &amp; H