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August 8, 2016

ATTORNEY DOCKET NO.: JHU3260-6
Parent Application No.: 14/645,282
Parent Filing Date: March 11, 2015
Parent Inventors: Atul Bedi and Rajani Ravi

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Presented for filing is a new **Continuation** patent application of:

INVENTORS : Atul Bedi and Rajani Ravi

TITLE : COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

The prior application is assigned of record to **THE JOHNS HOPKINS UNIVERSITY**, by virtue of an assignment recorded by the U.S. Patent and Trademark Office on October 16, 2012, at Reel 029136, Frame 0898.

Enclosed are the following papers, including all those required for a filing date under 37 C.F.R. § 1.53(b):

Pages of Cover Sheet	1
Pages of Specification	73
Pages of Claims	3
Pages of Abstract	1
Sheets of Drawing	75
Pages of Power of Attorney (from Parent) (with Statement under 37 CFR 3.73(c))	2
Pages of Declaration (from Parent)	2

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This request includes Three (3) sheets and the following:

- Application Data Sheet (9 pages);
- Information Disclosure Statement (2 pages);
- PTO Form SB/08 for the following unenclosed documents (2 pages);
    - Eight (8) U.S. Patents
    - Nine (9) U.S. Patent Application Publications
    - Three (3) Non Patent Literature Documents
- European Search Report from Related International Application No. EP 11 75 1481; and
- Sequence Listing in .txt format entitled "JHU3260-6_ST25.txt" (459 KB).

Under 35 USC § 120, this application is a **continuation** application of U.S. Application Serial No. 14/645,282 filed March 11, 2015, now pending; which is a continuation application of U.S. Application Serial No. 13/582,717 filed October 17, 2012, now issued as U.S. patent 8,993,524; which is a 35 USC § 371 National Stage application of International Application No. PCT/US2011/027317 filed March 4, 2011, now expired; which claims the benefit under 35 USC § 119(e) to U.S. Application Serial No. 61/435,671 filed January 24, 2011 and to U.S. Application Serial No. 61/311,255 filed March 5, 2010, both now expired. The disclosure of each of the prior applications is considered part of and is incorporated by reference in the disclosure of this application.

Applicants claim **SMALL ENTITY** status in the above-identified application. Pursuant to 37 CFR. § 1.27, a verified statement claiming small entity status is not required.

The filing fee is calculated as follows:

	Number Filed		Number Extra		Rate			Fee	
					Large Entity	Small Entity		Large Entity	Small Entity
Total Claims	**20**-20	=	**0**	X		$40	=		$ .00
Independent Claims	**1**-3	=	**0**	X		$210	=		$ .00
Multiple Dependent Claims Present: ____ Yes __x__ No						$390			N/A
Fee for Each Additional 66 Sheets in Excess of 133 Pages						$200			$ 200.00
					FILING FEE				$ 70.00
					SEARCH FEE				$ 300.00
					EXAM FEE				$ 360.00
					TOTAL FEE				**$ 930.00**

The fees for this filing total $930.00, which consist of $70.00 for the electronic filing fee, $300.00 for the search fee, $360.00 for the exam fee and $200.00 for the application size fee. The Commissioner is hereby authorized to charge any additional fees associated with the filing submitted herewith, or credit any overpayment, to Deposit Account 07-1896, referencing the Attorney Docket number shown above.

If this application is found to be INCOMPLETE, or if it appears that a telephone conference would helpfully advance prosecution, please telephone the undersigned at (858) 677-1456.

Please send all correspondence to: **USPTO Customer No. 100899**.

Respectfully submitted,

DLA Piper LLP (US)

August 8, 2016
Date

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Enclosures

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CONTINUATION
APPLICATION

for

UNITED STATES LETTERS PATENT

on

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

by

Atul Bedi

and

Rajani Ravi

Sheets of Drawings: Seventy Five (75)
Docket No.: JHU3260-6

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COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation application of U.S. Application Serial No. 14/645,282 filed March 11, 2015, now pending; which is a continuation application of U.S. Application Serial No. 13/582,717 filed October 17, 2012, now issued as U.S. patent 8,993,524; which is a 35 USC § 371 National Stage application of International Application No. PCT/US2011/027317 filed March 4, 2011, now expired; which claims the benefit under 35 USC § 119(e) to U.S. Application Serial No. 61/435,671 filed January 24, 2011 and to U.S. Application Serial No. 61/311,255 filed March 5, 2010, both now expired. The disclosure of each of the prior applications is considered part of and is incorporated by reference in the disclosure of this application.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

[0002] 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

[0003] 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

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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

[0004] 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-β)) 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.

[0005] 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-β); (ii) Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2); (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); (iv) Transforming growth factor-beta receptor (TGF-βR); (v) Programmed death-1 (PD-1); and (vi) Receptor activator of nuclear factor-κB (RANK).

[0006] 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.

[0007] 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-2α receptor; IL-2αR); (iii) cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); (iv) Interleukin-10 (IL-10); (v) Transforming growth factor-beta receptor (TGF-βR); (vi) Transforming growth factor-beta (TGF-β); (vii) Programmed Death-1 (PD-1); (viii) Programmed death-1 ligand (PD-L1 or PD-L2); (ix) Receptor activator of nuclear factor-κB (RANK); or (x) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL).

[0008] In one aspect, the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed death-1 ligand (PD-L1 or PD-L2); or (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL).

[0009] In one aspect, the immunomodulatory moiety includes a molecule that binds TGF-β. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Transforming growth factor-beta receptor TGF-βRII, TGF-βRIIb, or TGF-βRIII. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of TGF-β.

[0010] 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-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. 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.

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[0011] 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-βRII. In another aspect, the molecule includes PD-1 ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

[0012] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

[0013] 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).

[0014] 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-2α receptor; IL-2αR), 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.

[0015] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (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.

[0016] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Receptor activator of nuclear factor-κB ligand (RANKL).

[0017] 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-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (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.

[0018] 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.

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[0019] In one aspect, the targeting moiety specifically binds to Tumor Necrosis Factor-α (TNF-α), 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-α, 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.

[0020] 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.

[0021] In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), 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.

[0022] In one aspect, the targeting moiety includes transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), 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.

[0023] In one aspect, the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the immunomodulatory moiety activates the signaling function of transforming growth factor-β (TGF-β) receptor.

[0024] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from TGF-β. 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-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 51 or 52.

[0025] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25 (IL-2α receptor; IL-2αR), or CD4, and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 53, 54, 55, 56, 57 or 58.

[0026] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 59 or 60.

[0027] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, 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.

[0028] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

[0029] In one aspect, the targeting moiety includes includes a sequence from transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

[0030] In one aspect, the targeting moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

[0031] In various aspects, the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

[0032] 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.

[0033] 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.

[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 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.

[0035] 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+ 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.

[0036] 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.

[0037] 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.

[0038] 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.

[0039] 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

[0040] Figures 1A-1C show exemplary amino acid sequences of transforming growth factor beta receptor type II (TGF-β-RII) or TGF-β-RIIB or a fragment thereof, including (i) Transforming growth factor beta receptor type II (TGF-β-RII) (SEQ ID NO: 79); and (ii) Transforming growth factor beta receptor type IIB (TGF-β-RIIB) (SEQ ID NO: 80).

[0041] Also shown in Figures 1A-1C are exemplary truncated mutants of Transforming growth factor beta Receptor II (TGF-β-RII) or TGF-β-RIIB including the Extracellular domain (ECD) region that binds TGF-β, including (i) TGF-β R-II (ΔC terminus): TGFβ RII lacking the last 38 amino acids from the C-terminus (SEQ ID NO: 81) and TGF-β R-IIB (ΔC terminus): TGFβ RIIB lacking the last 38 aa from the C-terminus (SEQ ID NO: 82); (ii) TGF-βR-II (Δcyt): TGFβRII lacking the kinase domain & juxtamembrane region (SEQ ID NO: 83) and TGF-βR-IIB (Δcyt): TGFβRIIB lacking the kinase domain &

juxtamembrane region (SEQ ID NO: 84); (iii) TGF-β R-II containing the N-terminus region including the extracellular domain (SEQ ID NO: 85) and TGF-β R-IIB containing the N-terminus region including the extracellular domain (SEQ ID NO: 86); (iv) TGF-β R-II containing the extracellular domain that binds TGF-β (SEQ ID NO: 87) and TGF-β R-IIB containing the extracellular domain that binds TGF-β (SEQ ID NO: 88); and (v) TGF-β R-II containing the region of the extracellular domain that binds TGF-β (SEQ ID NO: 89).

[0042] In addition, Figures 1A-1C also show exemplary kinase-deficient mutants, deletion mutants, or point mutants of Transforming growth factor beta Receptor II (TGFβ-RII) or TGFβ-RIIB or a fragment thereof which binds TGF-β, including (i) Transforming growth factor beta Receptor II containing point mutations - amino acid sequence of TGF-β 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 (Δi) - TGF-β R-II (Δi2) contains a deletion of amino acids 498 to 508 and is inactive as a kinase (SEQ ID NO: 91).

[0043] Figure 2 shows exemplary fusion proteins including anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-HER2/neu heavy chain + TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 1) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

[0044] Figure 3 shows exemplary fusion proteins including anti-EGFR1 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-EGFR1 heavy chain + TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 2) and anti-EGFR1 light chain amino acid sequence (SEQ ID NO: 71).

[0045] Figure 4 shows exemplary fusion proteins including anti-CD20 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-CD20 heavy chain+ TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 3) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0046] Figure 5 shows exemplary fusion proteins including anti-VEGF antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-VEGF heavy chain+ TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 4) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

[0047] Figure 6 shows exemplary fusion proteins including anti-human CTLA-4 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including Anti-CTLA-4 heavy chain + TGFβ-RII Extracellular domain fusion amino acid sequence (SEQ ID NO: 5) and Anti-CTLA-4 light chain (SEQ ID NO: 74).

[0048] Figure 7 shows exemplary fusion proteins including IL-2, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including IL-2 + Fc + TGFβ-RII Extracellular domain (SEQ ID NO: 6) and TGFβ-RII Extracellular domain + Fc + IL-2 (SEQ ID NO: 7). 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.

[0049] Figures 8A-8B show exemplary fusion proteins including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-CD25 (Daclizumab) heavy chain and TGFβ-RII Extracellular domain (SEQ ID NO: 8) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 8A); and anti-CD25 (Basiliximab) heavy chain and TGFβ-RII Extracellular domain (SEQ ID NO: 9) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76).

[0050] Figure 9 shows exemplary fusion proteins including anti-CD4 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-CD4 heavy chain and TGFβ-RII Extracellular domain (SEQ ID NO: 10) and anti-CD4 light chain (SEQ ID NO: 77).

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[0051] Figure 10 shows exemplary fusion proteins including Programmed Death-1 (PD-1) Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ectodomain), including PD-1 ectodomain + Fc + TGFβRII ectodomain (SEQ ID NO: 11) and TGFβRII 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.

[0052] Figure 11 shows exemplary fusion proteins including Receptor activator of nuclear factor-kB (RANK) Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ectodomain), including RANK ectodomain + Fc + TGFβRII ectodomain (SEQ ID NO: 13) and TGFβRII 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.

[0053] 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).

[0054] 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).

[0055] 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).

[0056] Figure 15 shows exemplary fusion proteins including anti-CD20 antibody and PD-1 Ectodomain, including anti-CD20 heavy chain + PD-1 ectodomain fusion amino

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acid sequence (SEQ ID NO: 17) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0057] 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).

[0058] 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).

[0059] Figures 18A-18B show 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) (FIG. 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) (FIG. 18B).

[0060] 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.

[0061] 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).

[0062] 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.

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[0063] Figure 22 shows exemplary immunomodulatory moiety that binds Receptor activator of nuclear factor-kB (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).

[0064] 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).

[0065] 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).

[0066] 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).

[0067] 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).

[0068] 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).

[0069] Figures 28A-28B show 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) (FIG. 28A), and anti-CD25 (Basiliximab) heavy chain and RANK ectodomain (SEQ ID NO: 33) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 28B).

[0070] 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 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.

[0071] 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).

[0072] 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).

[0073] Figure 32 shows exemplary fusion proteins including anti-tumor necrosis factor (TNFα) antibody and PD-1 ligand, including anti-TNFα heavy chain + PD-1L (SEQ ID NO: 37) and anti-TNFα 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).

[0074] Figure 33 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand, including TNFR2 ECD + IgG Cγ1 + PD-L1 (SEQ ID NO: 38) and PD-L1 + IgG Cγ1 -TNFR2 ECD (SEQ ID NO: 39).

[0075] 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).

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[0076] Figures 35A-35B show 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) (FIG. 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) (FIG. 35B).

[0077] 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.

[0078] 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).

[0079] Figure 38 shows exemplary fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from PD-1 ligand (PD-L1) including Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + PD-L1 (SEQ ID NO: 46) and PD-1L1 + IgG Cγ1 + 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.

[0080] Figure 39 shows exemplary fusion proteins including a sequence of transforming growth factor-β (TGF-β), Immunoglobulin Fc (IgG Cγ1), and a sequence of PD-1 ligand (PD-L1) including TGFβ-1 + Fc + PD-L1 (SEQ ID NO: 48), and PD-1L1 + Fc + TGFβ-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.

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[0081] Figure 40 shows exemplary immunomodulatory moiety that binds Transforming growth factor-beta receptor (TGF-βR) including Transforming growth factor-beta (TGF-β1, TGF-β2, or TGF-β3 or a fragment thereof, TGF-β1 full sequence (SEQ ID NO: 102), and mature (active) TGF-β1 sequence (Ala 279 – Ser 390; 112 amino acids) (SEQ ID NO: 103).

[0082] Figure 41 shows exemplary fusion proteins including an antibody that binds TNF-α, and a sequence of transforming growth factor-β (TGF-β), including anti-TNFα heavy chain + TGF-β1 (SEQ ID NO: 50) and anti-TNFα 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).

[0083] Figure 42 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain (TNFR2 ECD), immunoglobulin Fc (IgG Cγ1), and a sequence from transforming growth factor-β (TGF-β) including TNFR2 ECD + IgG Cγ1 + TGF-β1 (SEQ ID NO: 51), and TGF-β1 + IgG Cγ1 + TNFR2 ECD (SEQ ID NO: 52).

[0084] Figure 43 shows exemplary fusion proteins including anti-CD20 antibody and a sequence from transforming growth factor-β (TGF-β), including anti-CD20 heavy chain + mature TGFβ1 sequence (SEQ ID NO: 53) and anti-CD20 light chain sequence (SEQ ID NO: 72).

[0085] Figures 44A-44B show exemplary fusion proteins including anti-CD25 antibody and a sequence from transforming growth factor-β (TGF-β), including anti-CD25 (Daclizumab) heavy chain and TGF-β1 (SEQ ID NO: 54) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 44A), and anti-CD25 (Basiliximab) heavy chain and TGF-β1 (SEQ ID NO: 55) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 44B).

[0086] Figure 45 shows exemplary fusion proteins including IL-2, Fc, and a sequence from transforming growth factor-β (TGF-β), including TGF-β1 + Fc + IL-2 (SEQ ID NO: 56) and IL-2 + Fc + TGF-β1 (SEQ ID NO: 57). 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.

[0087] Figure 46 shows exemplary fusion proteins including anti-CD4 antibody and a sequence from transforming growth factor-β (TGF-β), including anti-CD4 heavy chain and TGF-β (SEQ ID NO: 58) and anti-CD4 light chain (SEQ ID NO: 77).

[0088] Figure 47 shows exemplary Fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from transforming growth factor-β (TGF-β) including Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + TGF-β1 (SEQ ID NO: 59), and TGF-β1 + IgG Cγ1 + 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.

[0089] Figure 48 shows exemplary fusion proteins including an antibody that binds TNF-α, and a sequence of RANK ectodomain, including anti-TNFα heavy chain + RANK ectodomain (SEQ ID NO: 61) and anti-TNFα 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).

[0090] Figure 49 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain (TNFR2 ECD), immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including TNFR2 ECD + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 62), and RANK ectodomain + IgG Cγ1 + TNFR2 ECD (SEQ ID NO: 63).

[0091] Figure 50 shows exemplary Fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 64), and RANK ectodomain + IgG Cγ1 + 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.

[0092] Figure 51 shows exemplary fusion proteins including a sequence from transforming growth factor-β (TGF-β), immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including TGF-β + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 66), and RANK ectodomain + IgG Cγ1 + TGF-β (SEQ ID NO: 67).

[0093] Figure 52 shows exemplary fusion proteins including a sequence from PD-1 ligand (PD-L1), immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including PD-L1 + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 68), and RANK ectodomain + IgG Cγ1 + PD-L1 (SEQ ID NO: 69).

[0094] Figures 53A-53G show Regulatory T cells (Treg) accumulate in the tumor microenvironment and counteract the ability of chemotherapy to activate $CD8^+$ T cell-mediated antitumor immunity. (FIG. 53A) Surface exposure of calreticulin (CRT) in response to treatment of human (SW780) and murine (MB49) cancer cells with doxorubicin (10 μM) 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. (FIG. 53B) Priming of tumor-reactive immune responses by MB49 tumor cells treated with doxorubicin *ex vivo* or *in vivo*. $5x10^6$ MB49 cells that were pre-treated *ex vivo* with doxorubicin (10 μM) for 4h were injected into one flank of syngeneic immunocompetent C57BL/6 mice. Alternatively, C57BL/6 mice were injected with $5x10^5$ live MB49 tumor cells and then administered intratumoral doxorubicin (10 μg) at 10d following tumor inoculation. Tumor-reactive immune responses were determined by measuring production of IFN-γ 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. (FIG. 53C) Vaccination with doxorubicin-treated tumor cells induces $CD8^+$ T cell-mediated antitumor immunity that prevents tumor formation following re-challenge with live tumor cells. MB49 cells ($5x10^6$) that were pre-treated *in vitro* with doxorubicin (10 μM) 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-

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treatment with an anti-CD8 antibody (Clone GK2.43)(5 μg x 2 doses, iv) to deplete $CD8^+$ T cells. (FIG. 53D) Delayed administration of chemotherapy in mice with pre-established tumors decreases its immunogenicity and antitumor efficacy. C57BL/6 mice were injected with $5x10^5$ live syngeneic MB49 tumor cells and then administered intratumoral doxorubicin (10 μg) at d3, d7, or d10 foll^{owi}ng tumor inoculation. (FIG. 53E) Tumors foster the accumulation of $CD4^+CD25^+FoxP3^+$ cells (Tregs) in their microenvironment. Flow cytometric analyses of the percentage of $CD4^+CD25^+Fox^P3^+$ c^{e}lls (Tregs) among $CD4^+$ 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 $5x10^5$ live MB49 tumor cells. (FIG. 53F) Tregs infiltrating the tumor microenvironment suppress priming of tumor-reactive immune responses by doxorubicin-treated tumor cells. Naïve C57BL/6 mice were vaccinated with $5x10^6$ doxorubicin-killed MB49 cells with or without intravenous adoptive transfer of $5x10^6$ $CD4^+CD25^+$ cells isolated from tumors and DLN of tumor-bearing mice via immunomagnetic separation. Tumor-reactive immune responses were determined by measuring production of IFN-γ 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. (FIG. 53G) 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 $5x10^6$ doxorubicin-killed MB49 cells (left flank) with or without pre-treatment with either an anti-CD8 antibody (Clo^{ne} GK2.43)(5 μg x 2 doses, iv) to deplete $CD8^+$ T cells or adoptive transfer of $5x10^6$ $CD4^+CD25^+$ 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.

[0095] Figures 54A-54F show inhibition of TGF-β in the tumor microenvironment reduces 'adaptive' $FoxP3^+$ regulatory T cells and enhances the antitumor efficacy chemotherapy. (FIG. 54A) Tumor growth results in a progressive increase in the level of serum TGF-β. Levels of TGF-β in serum of mice at d0, d14, and d28 following inoculation of $5x10^5$ live MB49 tumor cells were evaluated utilizing ELISA.

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(FIG. 54B) Tumor cell-autonomous expression of TGF-β is the dominant source of elevated TGF-β 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-β/10^6 cells in supernatants was measured by ELISA. (FIG. 54C) TGFβRII:Fc sequesters TGF-β in supernatants of MB49 tumor cells in a concentration-dependent manner. MB49 tumor cells were cultured in the presence of graded concentrations of TGFβRII:Fc (0-400 ng/ml) for 24h followed by measurement of TGF-β (pg/ml/10^6 cells) in supernatants via ELISA. (FIG. 54D) TGF-β induces 'adaptive' FoxP3$^+$ regulatory T cells in the tumor microenvironment. At 5d following inoculation of MB49 tumor cells, mice were either left untreated (control) or treated with TGFβRII:Fc (1μg intratumoral; twice weekly) for 3 weeks followed by flow cytometric analyses of intracellular FoxP3 expression in CD4$^+$CD25$^+$ T cells infiltrating the tumors. (FIGS. 54E, 54F). Sequestration of intratumoral TGF-β with TGFβRII:Fc reduces CD4$^+$CD25$^+$FoxP3$^+$ 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 TGFβRII:Fc (1μg intratumoral). The percentage of CD4$^+$CD25$^+$FoxP3$^+$ cells (Tregs) among tumor cells was assessed by flow cytometry (FIG. 54E), and tumor volume was monitored to determine the effect of counteracting tumor-induced TGF-β-mediated immune tolerance on the *in vivo* antitumor efficacy of doxorubicin (FIG. 54F).

[0096] Figures 55A-55D show that anti-CD4 antibody-mediated depletion of CD4$^+$ regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive CD8$^+$ T cells and enhances the antitumor efficacy of chemotherapy. (FIG. 55A) *In vivo* depletion of tumor-infiltrating CD4$^+$CD25$^+$FoxP3$^+$ T cells by treatment of tumor-bearing mice with anti-CD4 antibody. C57BL/6 mice injected with 5 x10^5 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$^+$CD25$^+$FoxP3$^+$ T cells infiltrating tumors isolated from mice at d16 following tumor challenge were detected by flow cytometry. (FIG. 55B) Target-specific depletion of either CD4$^+$ T cells, CD4$^+$CD25$^+$FoxP3$^+$ T cells, or CD8$^+$ T cells by treatment of tumor-bearing mice with anti-CD4 antibody or anti-CD8 antibody.

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C57BL/6 mice injected s.c. with 5 x10^5 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 $CD4^+$ T cells or $CD8^+$ T cells among total mononuclear cells, and the percentage of $CD4^+CD25^+FoxP3^+$ T cells among total $CD4^+$ T cells. (FIG. 55C) Depletion of $CD4^+$ regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive $CD8^+$ T cells. C57BL/6 mice injected s.c. with 5 x10^5 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-γ expression in $CD8^+$ T cells from the tumor and draining lymph node in response to *in vitro* stimulation with MB49 cell lysates. (FIG. 55D) Depletion of $CD4^+$ regulatory T cells augments the *in vivo* antitumor efficacy of chemotherapy via activation of tumor-reactive $CD8^+$ T cells. C57BL/6 mice injected s.c. with 5 x10^5 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 $CD4^+$ T cells or $CD8^+$ T cells on the *in vivo* antitumor efficacy of doxorubicin.

[0097] Figures 56A-56F show anti-CD4 antibody-mediated depletion of $CD4^+$ regulatory T cells augments and sustains the antitumor effect of chemotherapy by enabling activation of adaptive antitumor immunity. (FIG. 56A) 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. (FIGS. 56B, 56C) Depletion of $CD4^+$ regulatory T cells enables cisplatin-

induced activation of tumor-reactive IFN-γ^+CD8^+ T cells and effector memory ($CD8^+CD62L^-$) T cells. C57BL/6 mice injected s.c. with 5 $x10^5$ 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-γ expression in $CD8^+$ T cells from the tumor and draining lymph node (DLN) in response to *in vitro* stimulation with MB49 cell lysates (FIG. 56B). The percentage of effector memory T_{EM} cells was determined by flow cytometric analyses of $CD8^+CD62L^-$ cells (FIG. 56C). (FIGS. 56D, 56E and 56F) Depletion of $CD4^+$ regulatory T cells augments the *in vivo* antitumor efficacy of chemotherapy via activation of tumor-reactive $CD8^+$ T cells. C57BL/6 mice injected s.c. with 5 $x10^5$ 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 $CD4^+$ T cells or $CD8^+$ 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.

[0098] Figures 57A-57H show that 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. (FIG. 57A) Genotoxic chemotherapeutic agents induce expression of mouse NKG2D ligands (Rae-1) on cancer cells. Kinetics of the upregulation of Rae1 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-

CATCAGTGACAGTTACTTCTTCACCTTCTACACAGAGA-Tamra-3' (SEQ ID NO: 113)]. (FIG. 57B) 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 ($p53^{+/+}$) or p53-deficient ($p53^{-/-}$) 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 cytometryic analysis of tumor cells labeled with an anti-human MICA/B MAb (R&D Systems). (FIG. 57C) and (FIG. 57D) Induction of NKG2D ligands contributes to the antitumor effect of chemotherapy *in vivo*. Immunocompetent Balb/C mice injected s.c. with 2 $x10^5$ 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. (FIG. 57E) *In vivo* depletion of $CD4^+CD25^+FoxP3^+$ T cells by treatment of tumor-bearing mice with anti-CD4 antibody. Balb/C mice injected with 2 $x10^5$ 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. $CD4^+CD25^+FoxP3^+$ T cells in spleen and draining lymph node isolated from mice at d16 following tumor challenge were detected by flow cytometry. (FIG. 57F) Depletion of $CD4^+$ regulatory T cells facilitates irinotecan-induced activation of tumor-reactive IFN-γ^+CD8^+ T cells. Balb/C mice injected with 2 $x10^5$ 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-γ 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. (FIG. 57G) and (FIG. 57H) 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 $x10^5$ CT26 tumor cells s.c. were

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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

[0099] 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-adjuvant 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.

[0100] 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.

[0101] 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+ 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 cytoreduction 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.

[0102] 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.

[0103] 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 cytoreduction 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.

[0104] 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.

[0105] 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-β); (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 $CD4^+$ T cells (regulatory T cells; Treg cells) and myeloid-derived suppressor cells (MDSC). Tregs are a minority sub-population of $CD4^+$ T cells that constitutively express CD25 [the interleukin-2 (IL-2) receptor α-chain] and the forkhead box P3 (FOXP3) transcription factor. Tregs (CD4+CD25+FoxP3+ cells) maintain immune tolerance by restraining the activation, proliferation, and effector functions of a wide range of immune cells, including $CD4^+$ and $CD8^+$ 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-β; 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.

[0106] 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-β), 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-γ) in immune effector cells, and promoting the development and function of Tregs; (iv) Receptor activator of nuclear factor-κB ligand (RANKL), a ligand which engages Receptor activator of nuclear factor-κB (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.

[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 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).

[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 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.

[0109] 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-β); (ii) Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2); (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); (iv) Vascular endothelial growth factor (VEGF); (v) Transforming growth factor-beta receptor (TGF-βR); (vi) Programmed death-1 (PD-1); and (vii) Receptor activator of nuclear factor-κB (RANK).

[0110] 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.

[0111] 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-2α receptor; IL-2αR); (iii) cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); (iv) Interleukin-10 (IL-10); (v) Transforming growth factor-beta receptor (TGF-βR); (vi) Transforming growth factor-beta (TGF-β); (vii) Programmed Death-1 (PD-1); (viii) Programmed death-1 ligand (PD-L1 or PD-L2); (ix) Receptor activator of nuclear factor-κB (RANK); (x) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); (xi) LAG-3; or (xii) glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18).

[0112] In one aspect, the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed death-1 ligand (PD-L1 or PD-L2); (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); or (iv) vascular endothelial growth factor (VEGF).

[0113] In one aspect, the immunomodulatory moiety includes a molecule that binds TGF-β. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Transforming growth factor-beta receptor TGF-βRII, TGF-βRIIb, or TGF-βRIII. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes a TGF-β-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-β.

[0114] 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-2α

receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. 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.

[0115] 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-βRII. In another aspect, the molecule includes PD-1 ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

[0116] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

[0117] 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).

[0118] 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-2α receptor; IL-2αR), 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.

[0119] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (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.

[0120] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (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-κB ligand (RANKL).

[0121] 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-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of

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nuclear factor-κB (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.

[0122] 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.

[0123] 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.

[0124] 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

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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.

[0125] 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.

[0126] 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.

[0127] In one aspect, the targeting moiety specifically binds to Tumor Necrosis Factor-α (TNF-α), 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-α, 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.

[0128] 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.

[0129] In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), 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.

[0130] In one aspect, the targeting moiety includes transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), 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.

[0131] In one aspect, the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). 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-β (TGF-β) receptor.

[0132] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from TGF-β. 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-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 51 or 52.

[0133] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25 (IL-2α receptor; IL-2αR), or CD4, and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 53, 54, 55, 56, 57 or 58.

[0134] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 59 or 60.

[0135] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) or Osteoprotegerin (OPG). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, 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.

[0136] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) or Osteoprotegerin (OPG). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

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[0137] In one aspect, the targeting moiety includes includes a sequence from transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

[0138] In one aspect, the targeting moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

[0139] In various aspects, the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

[0140] 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.

[0141] 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.

[0142] 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

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another aspect, the cytotoxic anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

[0143] 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- β, 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.

[0144] 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- β, 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.

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[0145] In one aspect the chemotherapeutic agent is a topoisomerase-interacting agent, anthracycline, doxorubicin, mitoxantrone, camptothecin, camptothecin analogue, irinotecan, epipodophyilotoxin, etoposide, alkylating agent, cyclophosphamide, cisplatin, cisplatin analogue, oxaliplatin, antimetabolite, fluoropyrimidine analogue, 5-fluorouracil, gemcitabine, azacytidine, antimicrotubule agent, taxane, paclitaxel, or docetaxel.

[0146] 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+ T cell helper epitope from tetanus toxin. In one aspect, the CD4+ 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.

[0147] 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.

[0148] 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.

[0149] 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

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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.

[0150] 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.

[0151] 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.

[0152] 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.

[0153] 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).

[0154] 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.

[0155] A 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.

[0156] 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.

[0157] 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.

[0158] 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

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(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-α), TGF-α receptor; Transforming growth factor-beta (TGF-β), TGF-β receptor; Interleukin 13 receptor alpha2 chain (lL13Ralpha2), Interleukin-6 (IL-6), 1L-6 receptor, Interleukin-4, IL-4 receptor, Cytokine receptors, Class I (hematopoietin family) and Class II (interferon/lL-10 family) receptors, tumor necrosis factor (TNF) family, TNF-α, tumor necrosis factor (TNF) receptor superfamily (TNTRSF), 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-Lfucosyltraosferase (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 giucosaminyl transferase V, MGAT5), HERV-K-MEL, KK-LC, KM-HN-1, LAGE, LAGE-1, CTL-recognixed antigen on melanoma (CAMEL), MAGE-Al (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 (MUCl), MART-1/Melan-A (MLANA), gp100, gp100/Pme117

(S1LV), tyrosinase (TYR), TRP-1, HAGE, NA-88, NY-ESO-1, NY-ESO-l/LAGE-2, SAGE, Sp17, SSX-1,2,3,4, TRP2-1NT2, carcino-embryonic antigen (CEA), Kallikfein 4, mammaglobm-A, OA1, prostate specific antigen (PSA), prostate specific membrane antigen, TRP-l/gp75, TRP-2, adipophilin, interferon inducible protein absent in nielanorna 2 (AIM-2), BING-4, CPSF, cyclin D1, epithelial cell adhesion molecule (Ep-CAM), EpbA3, fibroblast growth factor-5 (FGF-5), glycoprotein 250 (gp250intestinal carboxyl esterase (iCE), alpha-feto protein (AFP), M-CSF, mdm-2, MUCI, p53 (TP53), PBF, FRAME, PSMA, RAGE-1, RNF43, RU2AS, SOX10, STEAP1, survivin (BIRCS), 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, FTHLI7, 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 enoJase, heat shock protein gp96, GM2, sargramostim, CTLA-4, 707 alanine proline (707-AP), adenocarcinoma antigen recognized by T cells 4 (ART-4), carcinoembryogenic 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 vims (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.

[0159] 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, CD40, or a combination thereof.

[0160] 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.

[0161] 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-2α receptor; IL-2αR); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF-βR); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); Programmed death-1 ligand (PD-L1 or PD-L2); Receptor activator of nuclear factor-κB (RANK); Receptor activator of nuclear factor-κB (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.

[0162] 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-α (TNF-α); 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.

[0163] 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-2α receptor; IL-2αR); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF-βR); Transforming growth factor-beta (TGF-β); 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-κB (RANK); Receptor activator of nuclear factor-κB (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-α (TNF-α); 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; 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.

[0164] Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as Zanulimumab (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-β antibody); metelimumab/CAT-192 (anti- TGF-β antibody); lerdelimumab/CAT-152 (anti- TGF-β antibody); ID11 (anti- TGF-β 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-α4 integrin); Vedolizumab (anti-α4 β7 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).

[0165] 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-βR); Transforming growth factor-beta (TGF-β); 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-κB (RANK); or Receptor activator of nuclear factor-κB (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-α (TNF-α); Interleukin-12 (IL-12); IL-12R; Interleukin-10 (IL-10); IL-10R. In another aspect, the

immunoodulatory 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.

[0166] 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-βRII, TGF-βRIIb, or TGF-βRIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor-κB (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.

[0167] In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: Transforming growth factor-beta (TGF-β); 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.

[0168] 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.

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[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 an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF-βRII, TGF-βRIIb, or TGF-βRIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor-κB (RANK); vascular endothelial growth factor receptor (VEGFR1 or VEGFR2); or IL-10R.

[0170] 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.

[0171] 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-βRII, TGF-βRIIb, or TGF-βRIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor-κB (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-2α receptor; IL-2αR); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); Programmed death-1 ligand (PD-L1 or PD-L2); Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); or Interleukin-4 receptor (IL-4R).

[0172] 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 differernt 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-βRII, TGF-βRIIb, or TGF-βRIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor-κB (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).

[0173] 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-β, 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-βRII, TGF-βRIIb, or TGF-βRIII), Programmed Death-1 (PD-1), Receptor activator of nuclear factor-κB (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.

[0174] 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-2α receptor; IL-2αR); or CD20; and the immunomodulatory moiety comprises one of the following ligands or active ligand fragments: Transforming growth factor-beta (TGF-β); PD-1 ligand 1 (PD-L1); PD-1 ligand 2 (PD-L2); or IL-10.

[0175] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds tumor necrosis factor-α (TNF-α), 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-β), 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).

[0176] 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-β), 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).

[0177] 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-α (TNF-α), 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-β), 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.

[0178] 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.*, IgGl, IgG2, IgG3, IgG4, IgAl, and IgA2) or subclass of immunoglobulin molecule.

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[0179] An antibody as disclosed herein includes an antibody fragment, such as, but not limited to, Fab, Fab' and F(ab')2, 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.

[0180] 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, CHI, CH2, and CH3 domains. Also includes 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.

[0181] 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.

[0182] 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.

[0183] 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)n 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.

[0184] For example, a hybridoma can produce the polypeptides corresponding to SEQ. ID. NO: 1-69.

[0185] 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')2, and Fv fragments; Fc fragments or Fc-fusion products; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragment(s).

[0186] 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 tor any other modified Fc (*e.g.* glycosylation or other engineered Fc).

[0187] 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 Clq 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).

[0188] Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different "classes." There arc 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, IgC3, IgG4, IgA, and IgA2. The heavy-chain constant domains (hat correspond to the different classes of antibodies

are called alpha (α), delta (δ), epsilon (ε), gamma (γ), and mu (μ) respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0189] 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.

[0190] 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.

[0191] 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.

[0192] 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.

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[0193] 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.

[0194] 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.

[0195] 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.

[0196] 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.

[0197] 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.

[0198] 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.

[0199] 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.

[0200] 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.

[0201] 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

[0202] Counteracting Tumor Immune Tolerance via Antibody-mediated Depletion of $CD4^+$ Regulatory T Cells Facilitates the Activation of Tumor-Reactive $CD8^+$ T Cells and Enhances the *In Vivo* Antitumor Efficacy of Cytotoxic Anticancer Agents.

[0203] Immunogenic death of tumor cells by chemotherapeutic agents can induce $CD8^+$ 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. 53A). 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-γ by draining lymph node (DLN) cells in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 53B). Vaccination with doxorubicin-killed MB49 cells generated a tumor-specific immune response since no corresponding increase in IFN-γ 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 $CD8^+$ T cells with an anti-CD8 antibody before challenge with live tumor cells (FIG. 53C). These observations indicate that *ex vivo* treatment with chemotherapeutic agents can induce an immunogenic death of tumor cells that generates $CD8^+$ T cell-mediated adaptive antitumor immunity.

[0204] Tumor-induced immune tolerance inhibits activation of $CD8^+$ T cells in response to chemotherapy. To examine whether *in vivo* treatment with chemotherapeutic agents can activate $CD8^+$ 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-γ secretion by DLN cells in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 53B). 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. 53D). 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.

[0205] Regulatory T cells (Treg) accumulate in the tumor microenvironment and counteract the ability of chemotherapy to activate $CD8^+$ T cell mediated antitumor immunity. To investigate whether $FoxP3^+$ Tregs are involved in enforcing immune tolerance in the tumor microenvironment, we examined the percentage of $CD4^+CD25^+FoxP3^+$ cells (Tregs) among $CD4^+$ 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 $CD4^+$ T cells in the spleen and DLN at d14 following tumor inoculation, a majority of tumor-infiltrating $CD4^+$ T cells at this time were $CD4^+CD25^+FoxP3^+$ cells (FIG. 53E). To investigate whether Tregs infiltrating the tumor microenvironment can suppress the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death, $CD4^+CD25^+$ 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 $CD4^+CD25^+$ cells into naïve mice inhibited the ability of subsequent *in vivo* vaccination with doxorubicin-treated MB49 tumor cells to increase production of IFN-γ by draining lymph node (DLN) cells in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 53F). Consistent with the ability of tumor-infiltrating $CD4^+CD25^+$ 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. 53G). These results indicate that the tumor microenvironment fosters the accumulation of $FoxP3^+$ Tregs which counteract the activation of $CD8^+$ T cell mediated antitumor immunity in response to chemotherapy-induced tumor cell death.

[0206] Inhibition of TGF-β in the tumor microenvironment reduces tumor-infiltrating $FoxP3^+$ regulatory T cells and enhances the antitumor efficacy chemotherapy. TGF-β induces FoxP3 expression in naïve peripheral $CD4^+CD25^-FoxP3^-$ T cells and facilitates their conversion into 'adaptive' $FoxP3^+$ Tregs that share the immunosuppressive ability of

natural FoxP3$^+$ Tregs generated in the thymus. Since human cancers frequently become refractory to the growth-inhibitory effect of TGF-β and acquire an ability to increase expression and secretion of TGF-β, 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-β 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-β (FIG. 54A). To assess the precise source of TGF-β in tumor-bearing mice, the total amount of TGF-β 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-β/10^6 cells showed that tumor cells were the dominant source of the increased level of TGF-β in tumor-bearing mice (FIG. 54B). In addition to tumor cell-autonomous expression of TGF-β, T cells from tumor-bearing mice also expressed higher levels of TGF-β compared to their counterparts from tumor-free mice (FIG. 54B). To determine whether the elevation of TGF-β 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 TGFβRII and the Fc portion of the murine IgG1 heavy chain (TGFβRII:Fc). This fusion protein interferes with the binding of TGF-β to endogenous TGFβRII and functions as a stable TGF-β antagonist. ELISA assays confirmed the ability of TGFβRII:Fc to sequester TGF-β in supernatants of MB49 tumor cells in a concentration-dependent manner (FIG. 54C). At 5d following inoculation of MB49 tumor cells, mice were either left untreated or treated with TGFβRII:Fc (1μg intratumoral; twice weekly) for 3 weeks followed by flow cytometric analyses of intracellular FoxP3 expression in CD4$^+$CD25$^+$ T cells infiltrating the tumors. *In vivo* treatment of tumors with TGFβRII:Fc resulted in a significant decline in FoxP3 expression in tumor-infiltrating CD4$^+$ T cells (FIG. 54D) and a dramatic reduction of CD4$^+$CD25$^+$FoxP3$^+$ Tregs in tumor tissue (FIG. 54E). To determine whether inhibition of TGF-β 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 TGFβRII:Fc (1μg intratumoral). In contrast to treatment

with either doxorubicin or TGFβRII: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-β in the tumor microenvironment induces 'adaptive' $FoxP3^+$ Tregs and that counteracting tumor-induced TGF-β-mediated immune tolerance enhances the antitumor efficacy of chemotherapy.

[0207] Anti-CD4 antibody-mediated depletion of $CD4^+$ regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive $CD8^+$ T cells and enhances the antitumor efficacy of chemotherapy. To determine whether depletion of $CD4^+$ regulatory T cells can improve the antitumor efficacy of chemotherapy by enhancing the activity of $CD8^+$ T cells in the tumor microenvironment, immunocompetent mice bearing syngeneic tumors were administered an anti-CD4 antibody (Clone GK1.5) to deplete $CD4^+$ T cells or an anti-CD8 antibody (Clone GK2.43) to deplete $CD8^+$ 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 $CD4^+$ T cells or $CD8^+$ T cells, respectively (FIG. 55A). Mice treated with anti-CD4 antibody showed loss of $CD4^+CD25^+FoxP3^+$ T cells in the peripheral blood as well as among tumor-infiltrating cells (FIGS. 55A, 55B). To determine whether antibody-mediated depletion of $CD4^+CD25^+FoxP3^+$ cells facilitates chemotherapy-induced activation of tumor-reactive $CD8^+$ T cells in the tumor microenvironment, we evaluated the expression of IFN-γ in $CD8^+$ 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 $CD8^+$ T cells from untreated mice did not express IFN-γ in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 55C). Whereas IFN-γ^+CD8^+ T cells became evident in mice treated with doxorubicin alone, antibody-mediated depletion of $CD4^+$ T cells further enhanced the percentage of tumor-reactive $CD8^+$ T cells that expressed IFN-γ in doxorubicin-treated animals (FIG. 55C). To directly evaluate whether the activation of tumor-reactive $CD8^+$ T cells determines the *in vivo* antitumor efficacy of chemotherapy, we examined the effect of antibody-mediated depletion of $CD8^+$ T cells or $CD4^+$ 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 $CD8^+$ T cells completely impaired the *in vivo* antitumor efficacy of doxorubicin, depletion of $CD4^+$ T cells enhanced the response to doxorubicin and resulted in tumor regression (FIG. 55D).

[0208] Anti-CD4 antibody-mediated depletion of $CD4^+$ 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. 56A). Whereas tumor-reactive IFN-γ^+CD8^+ T cells were evident in tumors of MB49 tumor-bearing mice treated with doxorubicin (FIG. 55C), treatment with cisplatin was unable to induce a corresponding elevation of IFN-γ expression in $CD8^+$ T cells in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 56B). 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 $CD4^+$ T cells enhanced the percentage of tumor-reactive IFN-γ^+CD8^+ T cells as well as $CD8^+CD62L^-$ T cells in cisplatin-treated animals (FIGS. 56B, 56C). Treatment of MB49 tumor-bearing mice with cisplatin partially inhibited tumor growth but failed to arrest tumor progression. Whereas depletion of $CD8^+$ T cells completely negated the *in vivo* antitumor effect of cisplatin, depletion of $CD4^+$ T cells enhanced the response to cisplatin and arrested tumor growth (FIG. 56D). 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. 56E). In contrast, mice depleted of $CD4^+$ 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.

[0209] Chemotherapy-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD^{4+} regulatory T cells to stimulate CD^{8+} T cell-mediated tumor regression. NKG2D (NK group 2, member D) is a lectin-like type II transmembrane stimulatory receptor used by NK cells, $\gamma\delta$-TC^{R+} T cells and $\alpha\beta$-TC^{R+} 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 telengiectasia, mutated) or ATR (ATM- and Rad3-related) protein kinase**s.** 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. 57A). 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. 57A). 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. 53B). Isogenic HCT116 cells that differ only in their p53 status demonstrated that p53 is not required for irinotecan-induced upregulation of MICA/B (FIG. 53B). 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^{8+} T cells, we investigated whether DNA damage-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD^{4+} regulatory T cells to stimulate CD^{8+} T cell-mediated tumor regression. Balb/C mice bearing CT26 tumors were administered an anti-CD4 antibody (Clone GK1.5) to deplete CD^{4+} T cells and/or an anti-CD8 antibody (Clone GK2.43) to deplete CD^{8+} T cells and then treated with irinotecan. Flow cytometric analyses confirmed the loss of $CD^{4+}CD2^{5+}FoxP^{3+}$ T cells in the spleen and draining lymph node of mice treated with anti-CD4 antibody (FIG. 57D). Antibody-mediated depletion of CD^{4+} T cells enhanced the percentage of tumor-reactive IFN-$\gamma^{+}CD^{8+}$ T cells in irinotecan-treated animals (FIG. 57E). Whereas treatment of CT26 tumor-bearing mice with irinotecan only slowed tumor growth, depletion of CD^{4+} T cells enhanced the response to irinotecan and arrested tumor growth (FIG. 57F). The ability of CD^{4+} T cell depletion to augment the antitumor efficacy of irinotecan was mediated by CD^{8+} T cells since antibody-mediated depletion of CD^{8+} T cells completely negated the *in vivo* antitumor effect of chemotherapy in CD^{4+} T cell-depleted mice (FIG. 57F).

[0210] These data provide the following insights: (i) activation of tumor-reactive $CD8^{+}$ 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 $CD8^{+}$ T cells in the tumor microenvironment; and (iii) Counteracting tumor-induced immune tolerance via antibody-mediated depletion of $CD4^{+}$ 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+ regulatory T cells in the tumor microenvironment can increase the activation of CD8+ 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

[0211] Exemplary Targeted Immunomodulatory Antibodies and Fusion Proteins

[0212] 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.

[0213] 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-β). SEQ ID NO: 1 provides a fusion protein including anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 2). SEQ ID NO: 2 provides a fusion protein including anti-EGFR1 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 3). SEQ ID NO: 3 provides a fusion protein including anti-CD20 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 4). SEQ ID NO: 4 provides a fusion protein including anti-VEGF antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 5). SEQ ID NO: 5 provides a fusion protein including anti-human CTLA-4 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 6). SEQ ID NO: 6 provides a fusion protein including IL-2, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 7). SEQ ID NO: 7 provides a fusion protein including Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), Fc, and IL-2 (FIG. 7). SEQ ID NO: 8 provides a fusion protein including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 8A). SEQ ID NO: 9 provides a fusion protein including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular

domain (ECD) (FIG. 8B). SEQ ID NO: 10 provides a fusion protein including anti-CD4 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 9). SEQ ID NO: 11 provides a fusion protein including PD-1 Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ectodomain) (PD-1 ectodomain + Fc + TGFβRII ectodomain; FIG. 10). SEQ ID NO: 12 provides a fusion protein including Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ectodomain), Fc, and PD-1 Ectodomain (TGFβRII ectodomain + Fc + PD-1 ectodomain; FIG. 10). SEQ ID NO: 13 provides a fusion protein including RANK Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ectodomain) (RANK ectodomain + Fc + TGFβRII ectodomain; FIG. 11). SEQ ID NO: 14 provides a fusion protein including Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ectodomain), Fc, and RANK Ectodomain (TGFβRII ectodomain + Fc + RANK ectodomain; FIG. 11).

[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 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 (FIG. 13). SEQ ID NO: 16 provides a fusion protein including anti-EGFR1 antibody and PD-1 Ectodomain (FIG. 14). SEQ ID NO: 17 provides a fusion protein including anti-CD20 antibody and PD-1 Ectodomain (FIG. 15). SEQ ID NO: 18 provides a fusion protein including anti-VEGF antibody and PD-1 Ectodomain (FIG. 16). SEQ ID NO: 19 provides a fusion protein including anti-human CTLA-4 antibody and PD-1 Ectodomain (FIG. 17). SEQ ID NO: 20 provides a fusion protein including anti-CD25 antibody and PD-1 Ectodomain (FIG. 18A). SEQ ID NO: 21 provides a fusion protein including anti-CD25 antibody and PD-1 Ectodomain (FIG. 18B). SEQ ID NO: 22 provides a fusion protein including IL-2, Fc, and PD-1 ectodomain (IL-2 + Fc + PD-1 ectodomain; FIG. 19). SEQ ID NO: 23 provides a fusion protein including PD-1 ectodomain, Fc, and IL-2 (PD-1 ectodomain + Fc + IL-2; FIG. 19).

SEQ ID NO: 24 provides a fusion protein including anti-CD4 antibody and PD-1 Ectodomain (FIG. 20). SEQ ID NO: 25 provides a fusion protein including RANK Ectodomain, Fc, and PD-1 ectodomain (RANK ectodomain + Fc + PD-1 ectodomain; FIG. 21). SEQ ID NO: 26 provides a fusion protein including PD-1 ectodomain, Fc, and RANK Ectodomain (PD-1 ectodomain + Fc + RANK ectodomain; FIG. 21).

[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 is a molecule that specifically binds to Receptor activator of NF-kB ligand (RANKL). SEQ ID NO: 27 provides a fusion protein including anti-HER2/neu antibody and RANK Ectodomain (FIG. 23). SEQ ID NO: 28 provides a fusion protein including anti-EGFR1 antibody and RANK Ectodomain (FIG. 24). SEQ ID NO: 29 provides a fusion protein including anti-CD20 antibody and RANK Ectodomain (FIG. 25). SEQ ID NO: 30 provides a fusion protein including anti-VEGF antibody and RANK Ectodomain (FIG. 26). SEQ ID NO: 31 provides a fusion protein including anti-human CTLA-4 antibody and RANK Ectodomain (FIG. 27). SEQ ID NO: 32 provides a fusion protein including anti-CD25 antibody and RANK Ectodomain (FIG. 28A). SEQ ID NO: 33 provides a fusion protein including anti-CD25 antibody and RANK Ectodomain (FIG. 28B). SEQ ID NO: 34 provides a fusion protein including IL-2, Fc, and RANK ectodomain (IL-2 + Fc + RANK ectodomain; FIG. 29). SEQ ID NO: 35 provides a fusion protein including RANK ectodomain, Fc, and IL-2 (RANK ectodomain + Fc + IL-2; FIG. 29). SEQ ID NO: 36 provides a fusion protein including anti-CD4 antibody and RANK Ectodomain (FIG. 30).

[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 Programmed death-1 (PD-1). SEQ ID NO: 37 provides a fusion protein including anti-tumor necrosis factor (TNFα) antibody and PD-1 ligand 1 (FIG. 32). SEQ ID NO: 38 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand: (TNFR2 ECD + IgG Cγ1 + PD-L1; FIG. 33). SEQ ID NO: 39 provides a fusion protein including PD-1 ligand, Fc, and TNFR2

Extracellular ligand binding domain: (PD-L1 + IgG Cγ1 -TNFR2 ECD; FIG. 33). SEQ ID NO: 40 provides a fusion protein including anti-CD20 antibody and PD-1 ligand 1 (PD-L1) (FIG. 34). SEQ ID NO: 41 provides a fusion protein including anti-CD25 antibody and PD-1 ligand 1 (PD-L1) (FIG. 35A). SEQ ID NO: 42 provides a fusion protein including anti-CD25 antibody and PD-1 ligand 1 (PD-L1) (FIG. 35B). SEQ ID NO: 43 provides a fusion protein including PD-1 ligand 1 (PD-L1), Fc, and IL-2 (PD-L1 – Fc – IL2; FIG. 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; FIG. 36). SEQ ID NO: 45 provides a fusion protein including anti-CD4 antibody and PD-1 ligand 1 (PD-L1) (FIG. 37). SEQ ID NO: 46 provides a fusion protein including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from PD-1 ligand (PD-L1) (Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + PD-L1; FIG. 38). SEQ ID NO: 47 provides a fusion protein including the extracellular domain of PD-1 ligand (PD-L1), immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4: (PD-L1 + IgG Cγ1 + CTLA-4 ECD; FIG. 38). SEQ ID NO: 48 provides a fusion protein including Transforming growth factor-β (TGF-β), immunoglobulin Fc (IgG Cγ1), and a sequence from PD-1 ligand 1 (PD-L1) (TGFβ-1 + Fc + PD-L1; FIG. 39). SEQ ID NO: 49 provides a fusion protein including a sequence from PD-1 ligand 1 (PD-L1), immunoglobulin Fc (IgG Cγ1), and Transforming growth factor beta (TGF-□β) (PD-L1 + Fc + TGFβ-1; FIG. 39).

[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 includes a molecule that specifically binds to Transforming growth factor-beta receptor (TGF-βR). SEQ ID NO: 50 provides a fusion protein including an antibody that binds TNF-α, and a sequence from Transforming growth factor-β (TGF-β) (FIG. 41). SEQ ID NO: 51 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and a sequence from Transforming growth factor-β (TGF-β) (TNFR2 ECD + IgG Cγ1 + TGF-β; FIG. 42). SEQ ID NO: 52 provides a fusion protein including a sequence from Transforming growth factor-β (TGF-β), Fc, and TNFR2 Extracellular ligand binding

domain: (TGF-β + IgG Cγ1 + TNFR2 ECD; FIG. 42). SEQ ID NO: 53 provides a fusion protein including anti-CD20 antibody and a sequence from Transforming growth factor-β (TGF-β) (FIG. 43). SEQ ID NO: 54 provides a fusion protein including anti-CD25 antibody and a sequence from transforming growth factor-β (TGF-β) (FIG. 44A). SEQ ID NO: 55 provides a fusion protein including anti-CD25 antibody and a sequence from transforming growth factor-β (TGF-β) (FIG. 44B). SEQ ID NO: 56 provides a fusion protein including a sequence from Transforming growth factor-β (TGF-β), Fc, and IL-2 (TGF-β + Fc + IL-2; FIG. 45). SEQ ID NO: 57 provides a fusion protein including IL-2, Fc, and Transforming growth factor-β (TGF-β)(IL-2 + Fc + TGF-β; FIG. 45). SEQ ID NO: 58 provides a fusion protein including anti-CD4 antibody and a sequence from transforming growth factor-β (TGF-β) (FIG. 46). SEQ ID NO: 59 provides a fusion protein including the extracellular domain of CTLA-4, immunoglobulin Fc (IgG Cγ1), and a sequence from a sequence from transforming growth factor-β (TGF-β) (Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + TGF-β1; FIG. 47). SEQ ID NO: 60 provides a fusion protein including a sequence from Transforming growth factor-β (TGF-β), immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4: (TGF-β1 + IgG Cγ1 + CTLA-4 ECD) (FIG. 47).

[0218] 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-kB ligand (RANKL). SEQ ID NO: 61 provides a fusion protein including an antibody that binds TNF-α, and a sequence from RANK Ectodomain (FIG. 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 Cγ1 + RANK Ectodomain; FIG. 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 Cγ1 + TNFR2 ECD; FIG. 49). SEQ ID NO: 64 provides a fusion protein including the extracellular domain of CTLA-4, immunoglobulin Fc (IgG Cγ1), and a sequence from a sequence from RANK Ectodomain (Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + RANK

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Ectodomain; FIG. 50). SEQ ID NO: 65 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4: (RANK Ectodomain + IgG Cγ1 + CTLA-4 ECD) (FIG. 50). SEQ ID NO: 66 provides a fusion protein including a sequence from transforming growth factor-β (TGF-β), immunoglobulin Fc region (IgG Cγ1), and an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) (TGF-β + IgG Cγ1 + RANK Ectodomain; FIG. 51). SEQ ID NO: 67 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG Cγ1), and a sequence from transforming growth factor-β (TGF-β): (RANK Ectodomain + IgG Cγ1 + TGF-β) (FIG. 51). SEQ ID NO: 68 provides a fusion protein including a sequence from Programmed death-1 ligand 1 (PD-L1), immunoglobulin Fc region (IgG Cγ1), and an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) (PD-L1 + IgG Cγ1 + RANK Ectodomain; FIG. 52). SEQ ID NO: 69 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG Cγ1), and a sequence from Programmed death-1 ligand 1 (PD-L1): (RANK Ectodomain + IgG Cγ1 + PD-L1) (FIG. 52).

[0219] 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. An isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:

(a) the targeting moiety specifically binds Programmed Death-1 ligand (PD-1 ligand); and

(b) the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor (TGFβR).

2. The molecule of claim 1, wherein the targeting moiety comprises an antibody, antibody fragment, antigen-binding domain of an immunoglobulin, single chain variable fragment (scFv), Fc-containing polypeptide, or fusion protein that specifically binds PD-1 ligand or a fragment thereof.

3. The molecule of claim 2, wherein the targeting moiety specifically binds PD-1 ligand 1 (PD-L1) or PD-1 ligand 2 (PD-L2).

4. The molecule of claim 3, wherein the molecule comprises the amino acid sequence corresponding to the heavy chain or light chain of an antibody that specifically binds PD-L1.

5. The molecule of claim 1, wherein the targeting moiety comprises an amino acid sequence of the extracellular domain of PD-1.

6. The molecule of claim 5, wherein the targeting moiety comprises the amino acid sequence corresponding to SEQ ID NO: 93 or a binding fragment thereof.

7. The molecule of claim 1, wherein the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor selected from the group consisting of SEQ ID NO: 79-91.

8. The molecule of claim 1, wherein the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor II (TGFβRII) or a binding fragment thereof.

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9. The molecule of claim 7, wherein the immunomodulatory moiety comprises the amino acid sequence corresponding to SEQ ID NO: 87 or a binding fragment thereof.

10. The molecule of claim 1, wherein the molecule comprises the amino acid sequence set forth in SEQ ID NOs: 87 and SEQ ID NO:93 or a binding fragment thereof.

11. The isolated molecule of claim 1, wherein the targeting moiety is fused to the immunomodulatory moiety via a linker.

12. The isolated molecule of claim 11, further comprising a linker at the CH3 region of the Fc.

13. The isolated molecule of claim 11, wherein the linker is (GGGGS)n and wherein n is 1, 2, 3, 4, 5, 6, 7 or 8.

14. The isolated molecule of claim 13, wherein the linker comprises the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO:104).

15. The molecule of claim 10, wherein the molecule comprises the amino acid sequence set forth in SEQ ID NOs: 11 or SEQ ID NO:12 or a binding fragment thereof.

16. A method of treating a neoplastic disease or cancer comprising:
administration to a subject in need thereof a molecule of claim 1.

17. The method of claim 16, further comprising administering an additional cancer therapy.

18. The method of claim 17, wherein the cancer therapy is selected from the group consisting of a chemotherapeutic molecule, immunotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent, ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, and radiofrequency ablation.

19. The method of claim 16, further comprising administration in combination with a vaccine.

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20. The method of claim 16, wherein the subject is human.

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

ABSTRACT OF THE DISCLOSURE

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 antobodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ablity of cancer cells is recognized as immune tolerance. Such immunosuppressive mechanisms include immunosuppressive cytokines (for example, Transforming growth factor beta (TGF-β)) and regulatory T cells and/or immunosuppressive myeloid dendritic cells (DCs). By conteracting 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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1. Amino acid sequences of transforming growth factor beta receptor type II (TGF-β-RII) or TGF-β-RIIB or a fragment thereof:

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

MGRGLLRGLW PLHIVLWTRI AS*TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST*
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
*CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDL LLVIFQ*VTGI SLLPPLGVAI
SVIIIFYCYR VNRQQKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNTE
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSGQVG
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 (TGFβ RII)]
[Underlined = TGFβ RII Extracellular domain (ECD) region that binds TGF-β)]

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

MGRGLLRGLW PLHIVLWTRI AS*TIPPHVQK S**DVEMEAQKD EIICPSCNRT AHPLRHI***NND*
MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS ICEKPQEVCV AVWRKNDENI
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT
*SNPDLLLVIF Q*VTGISLLPP LGVAISVIII FYCYRVNRQQ KLSSTWETGK TRKLMEFSEH
CAIILEDDRS DISSTCANNI NHNTELLPIE LDTLVGKGRF AEVYKAKLKQ NTSEQFETVA VKI FP
YEEYASWKTE KDIFSDINLK HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH
VISWEDLRKL GSSLARGIAH LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL
SLRLDPTLSV DDLANSGQVG 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 (TGFβ RIIB)]
[Underlined = TGFβ RIIB Extracellular domain (ECD) region that binds TGF-β)]

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

(i) TGF-β R-II (ΔC terminus): TGFβ RII lacking the last 38 amino acids from the C-terminus (SEQ ID NO: 81):

MGRGLLRGLW PLHIVLWTRI AS*TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST*
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
*CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDL LLVIFQ*VTGI SLLPPLGVAI
SVIIIFYCYR VNRQQKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNTE
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSGQVG
TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC WDHDPEARL

FIG. 1A

TGF-β R-IIB (ΔC terminus): TGFβ RIIB lacking the last 38 aa from the C-terminus (SEQ ID NO: 82):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK S**DVEMEAQKD EIICPSCNRT AHPLRHI**NND
MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS ICEKPQEVCV AVWRKNDENI
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT
SNPDLLLVIF QVTGISLLPP LGVAISVIII FYCYRVNRQQ KLSSTWETGK TRKLMEFSEH
CAIILEDDRS DISSTCANNI NHNTELLPIE LDTLVGKGRF AEVYKAKLKQ NTSEQFETVA VKI FP
YEEYASWKTE KDIFSDINLK HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH
VISWEDLRKL GSSLARGIAH LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL
SLRLDPTLSV DDLANSGQVG TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR
CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC
WDHDPEARL

(ii) TGF-βR-II (Δcyt): TGFβRII lacking the kinase domain & juxtamembrane region (SEQ ID NO: 83):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDL LLVIFQVTGI SLLPPLGVAI
SVIIIFYCYR VNRQQKLSS

TGF-βR-IIB (Δcyt): TGFβRIIB lacking the kinase domain & juxtamembrane region (SEQ ID NO: 84):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK S**DVEMEAQKD EIICPSCNRT AHPLRHI**NND
MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS ICEKPQEVCV AVWRKNDENI
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT
SNPDLLLVIF QVTGISLLPP LGVAISVIII FYCYRVNRQQ KLSS

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

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDL LLVIFQ

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

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK S**DVEMEAQKD EIICPSCNRT AHPLRHI**NND
MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS ICEKPQEVCV AVWRKNDENI
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT
SNPDLLLVIF Q

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

TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP
QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS
DECNDNIIFS EEYNTSNPD

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

TIPPHVQK S**DVEMEAQKD EIICPSCNRT AHPLRHI**NND MIVTDNNGAV KFPQLCKFCD
VRFSTCDNQK SCMSNCSITS ICEKPQEVCV AVWRKNDENI TLETVCHDPK LPYHDFILED
AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT SNPD

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

PQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD
FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD

FIG. 1B

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

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

Amino acid sequence of TGF-β 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 QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDL LLVIFQVTGI SLLPPLGVAI
SVIIIFYCYR VNRQQKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNTE
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVRIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSGQVG
TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
LEHLDRLSGR SCSEEKIPED GSLNTTK

(ii) Transforming growth factor beta Receptor II containing deletions in the amino acid sequence (deletion mutants): Transforming growth factor beta Receptor II (Δi)

TGF-β R-II (Δi2) 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 QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDL LLVIFQVTGI SLLPPLGVAI
SVIIIFYCYR VNRQQKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNTE
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSGQVG
TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
HPCVESMKD**A** **S**GIQMVCETL TECWDHDPEA RLTAQCVAER FSELEHLDRL SGRSCSEEKI
PEDGSLNTTK

4. Amino acid sequences of transforming growth factor beta receptor type III (TGF-β-RIII) or or a fragment thereof.

5. Hybrid or fusion proteins containing amino acid sequences of transforming growth factor beta receptor type II (TGF-β-RII) and TGF-β-RIII.

6. Hybrid or fusion proteins containing amino acid sequences of transforming growth factor beta receptor type IIB (TGF-β-RIIB) and TGF-β-RIII.

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

GGGGSGGGGSGGGGS

FIG. 1C

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

Anti-HER2/neu heavy chain + TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 1):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKG
RFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST**
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD

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

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSR
SGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-HER2 antibody variable region
Plain: Anti-HER2 antibody constant region
Italic: *Linker*
Bold: **TGFβRII ectodomain**

FIG. 2

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

Anti-EGFR1 heavy chain + TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 2):

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPFTSR
LSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVSAASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST**
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD

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

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSG
SGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-EGFR antibody variable region

Plain: Anti-EGFR antibody constant region

Italic: *Linker*

Bold: **TGFβRII ectodomain**

FIG. 3

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

Anti-CD20 heavy chain+ TGFβ-RII ECD fusion amino acid sequence
(SEQ ID NO: 3):

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

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Underlined: Anti-CD20 antibody variable region
Plain: Anti-CD20 antibody constant region
Italic: *Linker*
Bold: **TGFβRII ectodomain**

FIG. 4

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

Anti-VEGF heavy chain+ TGFβ-RII ECD fusion amino acid sequence
(SEQ ID NO: 4):

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFKR
RFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS
LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST**
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD

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

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSG
SGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-VEGF antibody variable region
Plain: Anti-VEGF antibody constant region
Italic: *Linker*
Bold: **TGFβRII ectodomain**

FIG. 5

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

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

QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYTMHWVRQA PGKGLEWVTF ISYDGNNKYY
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGQ 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 NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
GNVFSCSVMH EALHNHYTQK SLSLSPGK *GGGGSGGGGSGGGGS* **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 DSTYSLSSTL
TLSKADYEKH KVYACEVTHQ GLSSPVTKSF NRGEC

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

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

Italic: *Linker*

Bold: **TGFβRII ectodomain**

FIG. 6

Fusion proteins comprising IL-2, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD).

IL-2 + Fc + TGFβ-RII Extracellular domain (SEQ ID NO: 6):

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**EEM**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS* **TIPPHVQK SVNNDMIVTD**
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV
CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFSEEYNTSNPD

TGFβ-RII Extracellular domain + Fc + IL-2 (SEQ ID NO: 7):

TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP
QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS
DECNDNIIFS EEYNTSNPD *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**E**E**M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T

Bold: **TGFβRII ectodomain**
Italic: *Linker (Optional; other linker sequences)*
Plain: Fc
Underlined: IL-2 fragment
(Note: Can replace linker *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 in Fc)

FIG. 7

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

Anti-CD25 (Daclizumab) heavy chain and TGFβ-RII Extracellular domain (SEQ ID NO: 8):

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 KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK *GGGGSGGGGSGGGGS* **TIPPHVQK SVNNDMIVTD**
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK 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 TYSLSSTLTL
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

Plain: Anti-CD25 antibody

Italic: *Linker*

Bold: **TGFβRII ectodomain**

FIG. 8A

Anti-CD25 (Basiliximab) heavy chain and TGFβ-RII Extracellular domain (SEQ ID NO: 9):

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPG QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDYGY YFDFWGQGTT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
FSCSVMHEAL HNHYTQKSLS LSPGK *GGGGSGGGGSGGGGS* **TIPPHVQK SVNNDMIVTD**
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV
CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD

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

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

Plain: Anti-CD25 antibody

Italic: *Linker*

Bold: **TGFβRII ectodomain**

FIG. 8B

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

Anti-CD4 heavy chain and TGFβ-RII Extracellular domain (SEQ ID NO: 10):

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSISIDTSKNLFSLKLRSVTAADTAVYYCASNILKYLHWLLYWGQGVLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRF**
STCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMK
EKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

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

Italic: *Linker*

Bold: **TGFβRII ectodomain**

FIG. 9

Fusion proteins comprising PD-1 Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ectodomain).

PD-1 ectodomain + Fc + TGFβRII ectodomain (SEQ ID NO: 11):

PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVW
RKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYN
TSNPD

TGFβRII ectodomain + Fc + PD-1 ectodomain (SEQ ID NO: 12):

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVW
RKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYN
TSNPD *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV

Underlined: PD-1 ectodomain

Italic: *Linkers*

Plain: IgG1 Fc

Bold: **TGFβRII ectodomain**

(Note: The first linker sequence *GGGGSGGGGSGGGGS* 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 (TGFβ-RII) ectodomain, Fc, and Receptor activator of nuclear factor–κB (RANK) Ectodomain:

TGFβRII ectodomain – Fc - RANK ectodomain (SEQ. ID. NO: 13):

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVW
RKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYN
TSNPD*GGGGSGGGGSGGGGS*THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS* QI
APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

RANK ectodomain – Fc - TGFβRII ectodomain (SEQ. ID. NO: 14):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
*GGGGSGGGGSGGGGS*THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGS**TIPPHVQKS**
VNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITL
ETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

Bold: TGFβRII 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 MQIPQAPWPV VWAVLQLGWR *PGWFLDSPDR PWNPPTFSPA 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 PWNPPTFSPA 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 PWNPPTFSPA 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
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **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):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSR
SGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
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):

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPFTSR
LSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVSAASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **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):

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSG
SGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASV
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
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV**

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
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
RFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS
LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS**
ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT
YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV

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

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSG
SGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
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 γ1-chain)-PD-1 ectodomain fusion protein, disulfide with human κ-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 WLGPFDYWGQ 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 NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
GNVFSCSVMH EALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS* **PGWFLDSPDR**
PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG
QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA ELRVTERRAE
VPTAHPSPSP 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 DSTYSLSSTL
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
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 KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK *GGGGSGGGGSGGGGS*
PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNT
SESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRAR
RNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLV

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*
Bold: **PD-1 ectodomain**

FIG. 18A

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

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPG QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDYGY YFDFWGQGTT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
FSCSVMHEAL HNHYTQKSLS LSPGK *GGGGSGGGGSGGGGS*
PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNT
SESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRAR
RNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLV

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

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

Plain: Anti-CD25 antibody

Italic: *Linker*

Bold: **PD-1 ectodomain**

FIG. 18B

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

IL-2 + Fc + PD-1 ectodomain (SEQ ID NO: 22):

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**E**E**M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV

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

PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**E**E**M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T

Underlined: IL-2 fragment
Italic: *Linker*
Plain: Fc
Bold: **human PD-1 ectodomain**
(Note: Can replace optional 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. 19

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
NRVSISIDTSKNLFSLKLRSVTAADTAVYYCASNILKYLHWLLYWGQGVLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

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

Italic: *Linker*

Bold: **PD-1 ectodomain**

FIG. 20

Fusion proteins comprising PD-1 Ectodomain, Fc, and Receptor activator of nuclear factor–κB (RANK) Extracellular domain (ectodomain):

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

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVP TAHPSPSPRPAGQFQTLV

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

PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVP TAHPSPSPRPAGQFQTLV*GGGGSGGGGSGGGGS***
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS* QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Bold: PD-1 ectodomain

Italics: Linker (optional: may delete linker 1 or replace with IEGRDMD (SEQ. ID. NO: 106) or EPKSCDK (SEQ. ID. NO: 105))

Plain: IgG1 Fc

Underlined: RANK ectodomain

FIG. 21

Moieties that bind Receptor activator of nuclear factor–κB (RANK) ligand

(i) Full-length RANK (TNFRSF11A) or fragment thereof (SEQ. ID. NO: 95):

1 MAPRARRRRP LFALLLLCAL LARLQVAL**QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
61 TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW
121 SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
181 RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPGLIILLLF ASVALVAAII FGVCYRKKGK
241 ALTANLWHWI NEACGRLSGD KESSGDSCVS THTANFGQQG ACEGVLLLTL EEKTFPEDMC
301 YPDQGGVCQG TCVGGGPYAQ GEDARMLSLV SKTEIEEDSF RQMPTEDEYM DRPSQPTDQL
361 LFLTEPGSKS TPPFSEPLEV GENDSLSQCF TGTQSTVGSE SCNCTEPLCR TDWTPMSSEN
421 YLQKEVDSGH CPHWAASPSP NWADVCTGCR NPPGEDCEPL VGSPKRGPLP QCAYGMGLPP
481 EEEASRTEAR DQPEDGADGR LPSSARAGAG SGSSPGGQSP ASGNVTGNSN STFISSGQVM
541 NFKGDIIVVY 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 CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW 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 LARLQVAL**QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
61 TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VC*DTGK*ALVA VVAGNSTTPR RCACTAGY*HW*
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–κB (RANK) ligand (RANKL)

(v) Full-length Osteoprotegerin (OPG, TNFRSF11B) or fragment thereof (SEQ. ID. NO: 98):

1 MNKLLCCALV FLDISIKWTT **QETFPPKYLH YDEETSHQLL CDKCPPGTYL KQHCTAKWKT**
61 VCAPCPDHYY TDSWHTSDEC LYCSPVCKEL QYVKQECNRT HNRVCECKEG RYLEIEFCLK
121 HRSCPPGFGV VQAGTPERNT VCKRCPDGFF SNETSSKAPC RKHTNCSVFG LLLTQKGNAT
181 HDNICSGNSE STQKCGIDVT LCEEAFFRFA VPTKFTPNWL SVLVDNLPGT KVNAESVERI
241 KRQHSSQEQT FQLLKLWKHQ NKAQDIVKKI IQDIDLCENS VQRHIGHANL TFEQLRSLME
301 SLPGKKVGAE DIEKTIKACK PSDQILKLLS LWRIKNGDQD TLKGLMHALK HSKTYHFPKT
361 VTQSLKKTIR FLHSFTMYKL YQKLFLEMIG NQVQSVKISC L

Bold: RANK ligand-binding domain

FIG. 22

Fusion proteins comprising Anti-HER2/neu antibody and Receptor activator of nuclear factor–κB (RANK) Extracellular domain (ECD).

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

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISA
DTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK
KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW
SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFT
LTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Underlined: Anti-HER2 antibody variable region
Plain: Anti-HER2 antibody constant region
Italics: Linker
Bold: RANK ectodomain

FIG. 23

Fusion proteins comprising Anti-EGFR1 antibody and Receptor activator of nuclear factor–κB (RANK) Extracellular domain (ECD).

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

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSINKD
NSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVSAASTKGPSVFPLAPSSKSTSGGTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKR
VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW
SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFT
LSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Underlined: Anti-EGFR antibody variable region
Plain: Anti-EGFR antibody constant region
Italics: Linker
Bold: RANK ectodomain

FIG. 24

Fusion proteins comprising Anti-CD20 antibody and Receptor activator of nuclear factor–κB (RANK) Extracellular domain (ECD).

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

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKGKATLTA
DKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
TTTSDSVCLP CGPDEYLDSW 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):

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGSGTSYSL
TISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Underlined: Anti-CD20 antibody variable region

Plain: Anti-CD20 antibody constant region

Italics: Linker

Bold: RANK ectodomain

FIG. 25

Fusion proteins comprising anti-VEGF antibody and Receptor activator of nuclear factor–κB (RANK) Extracellular domain (ECD).

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

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADF
KRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP**
CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN
TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS
DAVCSSSLPA RKPPNEPHVY LPG

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

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL
SSPVTKSFNRGEC

Underlined: Anti-VEGF antibody variable region
Plain: Anti-VEGF antibody constant region
Italics: Linker
Bold: RANK ectodomain

FIG. 26

Fusion proteins comprising anti-human CTLA-4 antibody and Receptor activator of nuclear factor–κB (RANK) Extracellular domain (ECD).

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

QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYTMHWVRQA PGKGLEWVTF ISYDGNNKYY
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGQ 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 NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
GNVFSCSVMH EALHNHYTQK SLSLSPGK *GGGGSGGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC**
EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR
RCACTAGYHW 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 DSTYSLSSTL
TLSKADYEKH KVYACEVTHQ GLSSPVTKSF NRGEC

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–κB (RANK) Extracellular domain (ECD).

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

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 KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK *GGGGSGGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC**
EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR
RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR
PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Anti-CD25 - 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
Italics: Linker
Bold: RANK ectodomain

FIG. 28A

Fusion proteins comprising anti-CD25 antibody and Receptor activator of nuclear factor–κB (RANK) Extracellular domain (ECD).

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

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPG QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDYGY YFDFWGQGTT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
FSCSVMHEAL HNHYTQKSLS LSPGK *GGGGSGGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC**
EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR
RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR
PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

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

Plain: Anti-CD25 antibody
Italics: Linker
Bold: RANK ectodomain

FIG. 28B

Fusion proteins comprising IL-2, Fc, and Receptor activator of nuclear factor–κB (RANK) Extracellular domain (ECD).

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

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT*GGGGSGGGGSG*
*GGGS*THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGK *GGGGSGGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
TTTSDSVCLP CGPDEYLDSW 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 CGPDEYLDSW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
*GGGGSGGGGSGGGGS*THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGS*APTSSSTKKTQLQLEHLLLDLQMILNGINNYKN
PKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMC
EYADETATIVEFLNRWITFCQSIISTLT

Bold: RANK ectodomain

Italics: Linker (Optional; any linker sequence)

Plain: Fc

Underlined: IL2 fragment that binds CD25 (IL-2Rα)

(Can replace linker 1 with EPKSCDK (SEQ. ID. NO: 105) or IEGRDMD (SEQ. ID. NO: 106))
(Can replace underlined aa in Fc: E with D and M with L)

FIG. 29

Fusion protein comprising anti-CD4 antibody and Receptor activator of nuclear factor–κB (RANK) Extracellular domain (ECD)

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

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSISIDTSKNLFSLKLRSVTAADTAVYYCASNILKYLHWLLYWGQGVLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP**
CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN
TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS
DAVCSSSLPA RKPPNEPHVY LPG

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

Underlined: Anti-CD4 antibody variable region

Plain: Anti-CD4 antibody constant region

Italic: *Linker*

Bold: **PD-1 ectodomain**

FIG. 30

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

MRIFAVFIFM TYWHLLNAFT VTVPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV NAPYNKINQR ILVVDPVTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL FNVTSTLRIN TTTNEIFYCT FRRLDPEENH TAELVIPELP LAHPPNERTH LVILGAILLC LGVALTFIFR LRKGRMMDVK 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 ILVVDPVTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL FNVTSTLRIN TTTNEIFYCT FRRLDPEENH TAELVI

(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 TYWHLLNAFT VTVPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV NAPYNKINQR ILVVDPVTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL FNVTSTLRIN TTTNEIFYCT FRRLDPEENH TAELVIPELP LAHPPNERTH LVILGAILLC LGVALTFIFR LRKGRMMDVK KCGIQDTNSK KQSDTHLEET

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

Underlined Bold: **Ligand Binding domain**

FIG. 31

Fusion proteins comprising anti-tumor necrosis factor (TNFα) antibody and PD-1 ligand 1 or PD-1 ligand 2

Anti-TNFα heavy chain + PD-L1 (SEQ ID NO: 37):

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSVEG
RFTISRDNAKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVD**KKAE**PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

Anti-TNFα light chain (SEQ ID NO: 78):

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLPGKAPKLLIYAASTLQSGVPSRFSGSG
SGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: anti-TNFα antibody variable region

Plain: anti-TNFα antibody constant region

Italic: *Linker*

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

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

FIG. 32

Fusion proteins comprising TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand: (TNFR2 ECD + IgG Cγ1 + PD-L1)

TNFR2 ECD + IgG Cγ1 + PD-L1 (SEQ ID NO: 38):

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGD *EPKSCDK*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

Fusion proteins comprising PD-1 ligand, Fc, and TNFR2 Extracellular ligand binding domain: (PD-L1 + IgG Cγ1 + TNFR2 ECD)

PD-L1 + IgG Cγ1 + TNFR2 ECD (SEQ ID NO: 39):

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQ
FVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
PYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFR
LRKGRMMDVKKCGIQDTNSKKQSDTHLEET *GGGGSGGGGSGGGGS EPKSCDK*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGD

Underlined: TNFR2 ligand-binding domain
Plain: human IgG1 heavy chain constant region (IgG Cγ1 domain = H, CH2, CH3)
Italic: *Linker (Linker 1 is optional and may be replaced with another linker such as EPKSCDK* (SEQ. ID. NO: 105) *or GGGGSGGGGSGGGGS* (SEQ. ID. NO: 104))
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
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
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

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
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 KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK *GGGGSGGGGSGGGGS*
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECK
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

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

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPG QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDYGY YFDFWGQGTT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
FSCSVMHEAL HNHYTQKSLS LSPGK *GGGGSGGGGSGGGGS*
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECK
FPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNA
ALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHEL
TCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYC
TFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

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

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

Plain: anti-CD25 antibody

Italic: *Linker*

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

FIG. 35B

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):

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEE
DLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKV
NAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTT
TNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFR
LRKGRMMDVKKCGIQDTNSKKQSDTHLEET *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR**E**E**M**TKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK *GGGGSGGGGSGGGGS*
APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

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

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
GGGGSGGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR**E**E**M**TKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK *GGGGSGGGGSGGGGS*
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEED
LKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD
PVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEE
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 *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. 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
NRVSISIDTSKNLFSLKLRSVTAADTAVYYCASNILKYLHWLLYWGQGVLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
**RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET**

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
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 Cγ1), and a sequence from PD-1 ligand (PD-L1)

Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + PD-L1 (SEQ ID NO: 46):

*MGVLLTQRTLLSLVLALLFPSMASM*AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQA
DSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGI
GNGTQIYVIDPEPCPDSD *QEPKSCDK*THTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
**RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET**

Fusion proteins comprising the extracellular domain of PD-1 ligand (PD-L1), Immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4.

PD-L1 + IgG Cγ1 + CTLA-4 ECD (SEQ ID NO: 47):

**MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQ
FVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
PYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFR
LRKGRMMDVKKCGIQDTNSKKQSDTHLEET** *GGGGSGGGGSGGGGS*
*QEPKSCDK*THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSI
CTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD

Underlined Italic: *Oncostatin M Signal Peptide (-25 to -1)*
Underlined: CTLA-4 extracellular domain (1-125)
Plain: human IgG1 heavy chain constant region (IgG Cγ1 domain = H, CH2, CH3)
Italic: *Linker (optional)*
Bold: **human PD-1 ligand 1 (PD-1L) or extracellular binding domain of PD-1L (underlined)**
(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

Fusion proteins comprising a sequence of transforming growth factor-β (TGF-β), Immunoglobulin Fc (IgG Cβ1), and a sequence of PD-1 ligand (PD-L1)

TGFβ-1 + Fc + PD-L1 (SEQ ID NO: 48):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**E**E**M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
**RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET**

Fusion protein comprising a sequence of PD-1 ligand (PD-L1), Immunoglobulin Fc (IgG Cγ1), and a sequence of Transforming growth factor beta (TGF- β)

PD-L1 + Fc + TGFβ-1 (SEQ ID NO: 49):

**MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQ
FVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
PYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKG
RMMDVKKCGIQDTNSKKQSDTHLEET** *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**E**E**M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Underlined: TGFβ-1
Italic: *Linker (optional)*
Plain: Fc
Bold: **human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)**
(Note: Can replace linker *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. 39

(i) Transforming growth factor-beta (TGF-β1, TGF-β2, or TGF-β3 or a fragment thereof:

TGF-β1 full sequence (SEQ ID NO: 102):

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

Underlined: Mature (active) TGF-β1 sequence (Ala 279 – Ser 390; 112 aa).

(ii) Mature (active) TGF-β1 sequence (Ala 279 – Ser 390; 112 aa)
(SEQ ID NO: 103):

AL DTNYCFSSTE KNCCVRQLYI DFRKDLGWKW IHEPKGYHAN FCLGPCPYIW
SLDTQYSKVL ALYNQHNPGA SAAPCCVPQA LEPLPIVYYV GRKPKVEQLS NMIVRSCKCS

FIG. 40

Fusion proteins comprising an antibody that binds TNF-α, and a sequence of transforming growth factor-β (TGF-β).

Anti-TNFα heavy chain + TGF-β1 (SEQ ID NO: 50):

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSVEG
RFTISRDNAKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVD**KKAE**PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Anti-TNFα light chain (SEQ ID NO: 78):

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLPGKAPKLLIYAASTLQSGVPSRFSGSG
SGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: anti-TNFα antibody variable region

Plain: anti-TNFα antibody constant region

Italic: *Linker*

Bold: **Mature (active) human TGF-β1**

(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-β (TGF-β)

TNFR2 ECD + IgG Cγ1 + TGF-β1 (SEQ ID NO: 51):

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGD *EPKSCDK*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**E**E**M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

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

TGF-β1 + IgG Cγ1 + TNFR2 ECD (SEQ ID NO: 52):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSR**E**E**M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGD

Underlined: TNFR2 ligand-binding domain

Plain: human IgG1 heavy chain constant region (IgG Cγ1 domain = H, CH2, CH3)

Italic: *Linker*

Bold: **Mature (active) human TGF-β1**

(Note: Can replace linker 1 with *GGGGSGGGGSGGGGS* 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-β (TGF-β)

Anti-CD20 heavy chain + mature TGFβ1 sequence (SEQ ID NO: 53):

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKG
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Underlined: anti-CD20 antibody variable region

Plain: anti-CD20 antibody constant region

Italic: *Linker*

Bold: **Mature (active) human TGF-β1**

FIG. 43

Fusion proteins comprising anti-CD25 antibody and a sequence from transforming growth factor-β (TGF-β).

Anti-CD25 (Daclizumab) heavy chain and TGF-β1 (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 KTTPPVLDSD 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 TYSLSSTLTL
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

Plain: anti-CD25 antibody

Italic: *Linker*

Bold: **Mature (active) human TGF-β1**

FIG. 44A

Anti-CD25 (Basiliximab) heavy chain and TGF-β1 (SEQ ID NO: 55):

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPG QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDYGY YFDFWGQGTT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
FSCSVMHEAL HNHYTQKSLS LSPGK *GGGGSGGGGSGGGGS*
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHAN
FCLGPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQ
LSNMIVRSCKCS

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

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

Plain: anti-CD25 antibody

Italic: *Linker*

Bold: **Mature (active) human TGF-β1**

FIG. 44B

Fusion proteins comprising IL-2, Fc, and a sequence from transforming growth factor-β (TGF-β).

TGF-β1 + Fc + IL-2 (SEQ ID NO: 56):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**E**E**M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T

IL-2 + Fc + TGF-β1 (SEQ ID NO: 57):

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**E**E**M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Underlined: IL-2 fragment
Italic: *Linker (optional)*
Plain: Fc
Bold: **Mature (active) human TGF-β1**
(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

Fusion protein comprising anti-CD4 antibody and a sequence from transforming growth factor-β (TGF-β).

Anti-CD4 heavy chain and TGF-β1 (SEQ ID NO: 58)

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSISIDTSKNLFSLKLRSVTAADTAVYYCASNILKYLHWLLYWGQGVLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

Underlined: Anti-CD4 antibody variable region

Plain: Anti-CD4 antibody constant region

Italic: *Linker*

Bold: **Mature (active) human TGF-β1**

FIG. 46

Fusion proteins comprising the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from a sequence from transforming growth factor-β (TGF-β)

Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + TGF-β1 (SEQ ID NO: 59):

*MGVLLTQRTLLSLVLALLFPSMASM*AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQA
DSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGI
GNGTQIYVIDPEPCPDSD*QEPKSCDK* THTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
**ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS**

Fusion proteins comprising a sequence from transforming growth factor-β (TGF-β), Immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4

TGF-β1 + IgG Cγ1 + CTLA-4 ECD (SEQ ID NO: 60):

**ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS** *EPKSCDK*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSI
CTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD

Underlined Italic: *Oncostatin M Signal Peptide (-25 to -1)*
Underlined: CTLA-4 extracellular domain (1-125)
Plain: human IgG1 heavy chain constant region (IgG Cγ1 domain = H, CH2, CH3)
Italic: *Linker (optional)*
Bold: **Mature (active) human TGF-β1**
(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

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

Anti-TNFα heavy chain + RANK ectodomain (SEQ ID NO: 61):

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSVEG
RFTISRDNAKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVD**KKAE**PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG**

Anti-TNFα light chain (SEQ ID NO: 78):

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLPGKAPKLLIYAASTLQSGVPSRFSGSG
SGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: anti-TNFα antibody variable region
Plain: anti-TNFα 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

Fusion proteins comprising TNFR2 Extracellular ligand binding domain, Fc, and a RANKL-binding sequence of receptor activator of nuclear factor-kB (RANK)

TNFR2 ECD + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 62):

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGD *EPKSCDK*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**E**E**M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS* **QI APPCTSEKHY**
EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA
VVAGNSTTPR RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS
DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Fusion proteins comprising a RANKL-binding sequence of receptor activator of nuclear factor-kB (RANK), Fc, and TNFR2 Extracellular ligand binding domain:

RANK ectodomain + IgG Cγ1 + TNFR2 ECD (SEQ ID NO: 63):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW
NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN TECAPGLGAQ
HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA
RKPPNEPHVY LPG
GGGGSGGGGSGGGGS THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSR**E**E**M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGD

Underlined: TNFR2 ligand-binding domain

Plain: human IgG1 heavy chain constant region (IgG Cγ1 domain = H, CH2, CH3)

Italic: *Linker*

Bold: **RANK ectodomain**

(Note: Can replace linker 1 with *GGGGSGGGGSGGGGS* 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

Fusion proteins comprising the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a RANKL-binding sequence of receptor activator of nuclear factor-kB (RANK)

Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 64):

*MGVLLTQRTLLSLVLALLFPSMASM*AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQA
DSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGI
GNGTQIYVIDPEPCPDSD*QEPKSCDK* THTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG**

Fusion proteins comprising a RANKL-binding sequence of receptor activator of nuclear factor-kB (RANK), Immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4

RANK ectodomain + IgG Cγ1 + CTLA-4 ECD (SEQ ID NO: 65):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG *EPKSCDK*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSI
CTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD

Underlined Italic: *Oncostatin M Signal Peptide (-25 to -1)*
Underlined: CTLA-4 extracellular domain (1-125)
Plain: human IgG1 heavy chain constant region (IgG Cγ1 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

Fusion proteins comprising a RANKL-binding sequence of receptor activator of nuclear factor-kB (RANK), Fc, and a sequence from transforming growth factor-β (TGF-β).

TGF-β1 + Fc + RANK ectodomain (SEQ ID NO: 66):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**E**E**M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS* **QI APPCTSEKHY**
EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA
VVAGNSTTPR RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS
DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

RANK ectodomain + Fc + TGF-β1 (SEQ ID NO: 67):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW
NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN TECAPGLGAQ
HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA
RKPPNEPHVY LPG *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**E**E**M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Underlined: Mature (active) human TGF-β1
Italic: *Linker (optional)*
Plain: Fc
Bold: **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. 51

Fusion proteins comprising a RANKL-binding sequence of receptor activator of nuclear factor-kB (RANK), Fc, and a sequence from PD-1 ligand 1

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

RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEED
LKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD
PVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEE
T *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR**E**E**M**TKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK *GGGGSGGGGSGGGGS* QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP
CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN
TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS
DAVCSSSLPA RKPPNEPHVY LPG

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

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
GGGGSGGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR**E**E**M**TKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK *GGGGSGGGGSGGGGS*
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEED
LKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD
PVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEE
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

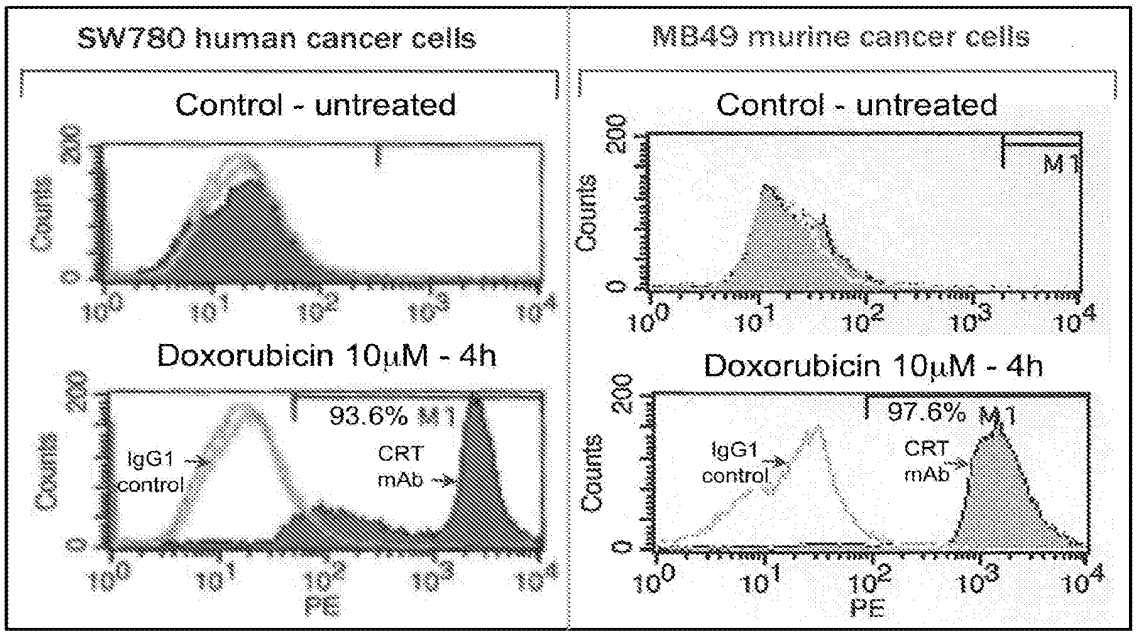


FIG. 53A

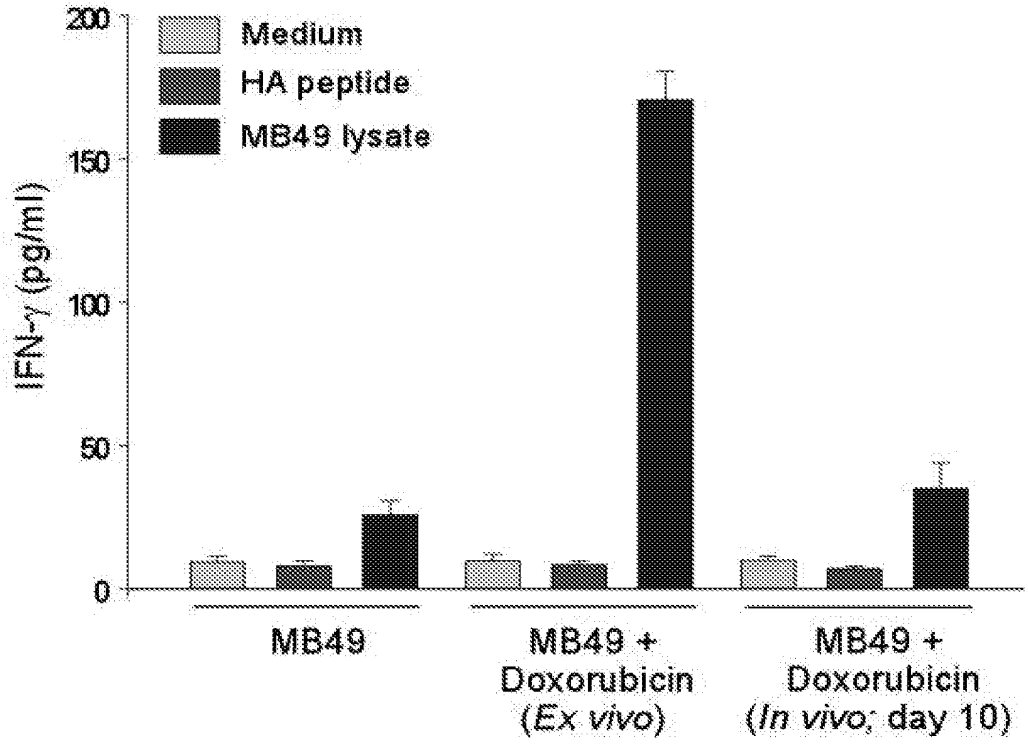


FIG. 53B

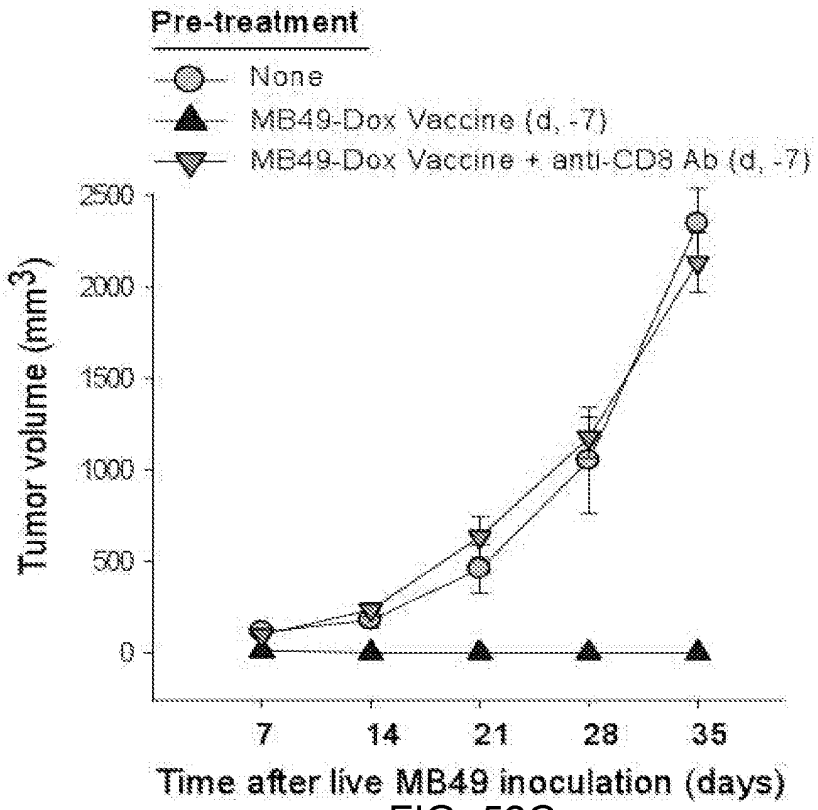


FIG. 53C

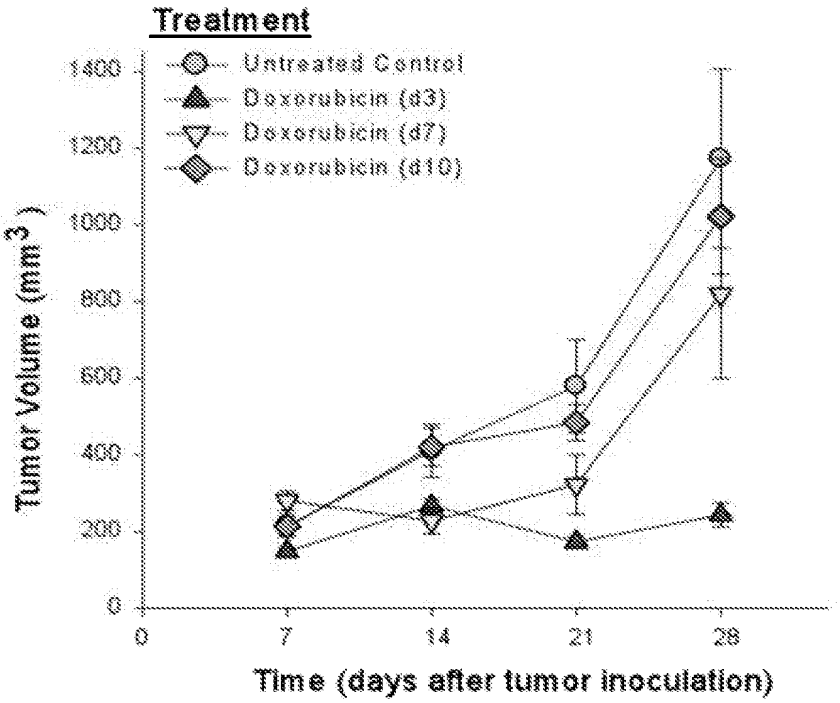


FIG. 53D

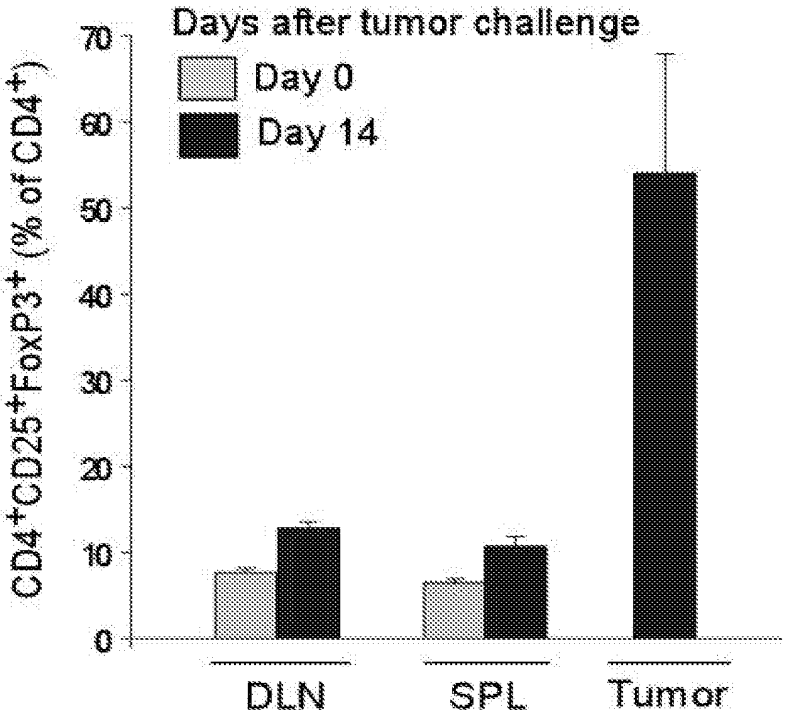


FIG. 53E

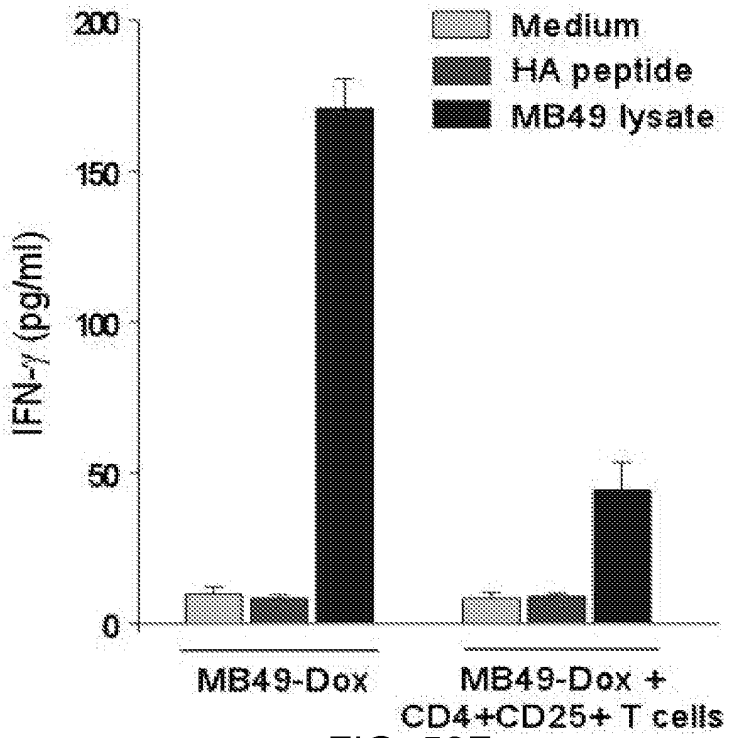


FIG. 53F

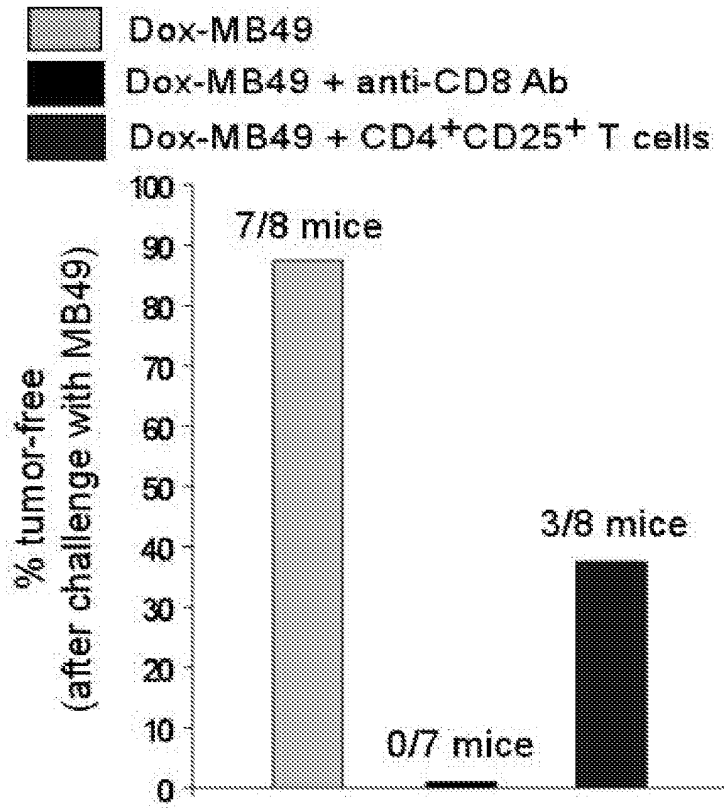

Dox-MB49
Dox-MB49 + anti-CD8 Ab
Dox-MB49 + CD4+CD25+ T cells
% tumor-free
(after challenge with MB49)
100
90
80
70
60
50
40
30
20
10
0
7/8 mice
0/7 mice
3/8 mice

FIG. 53G

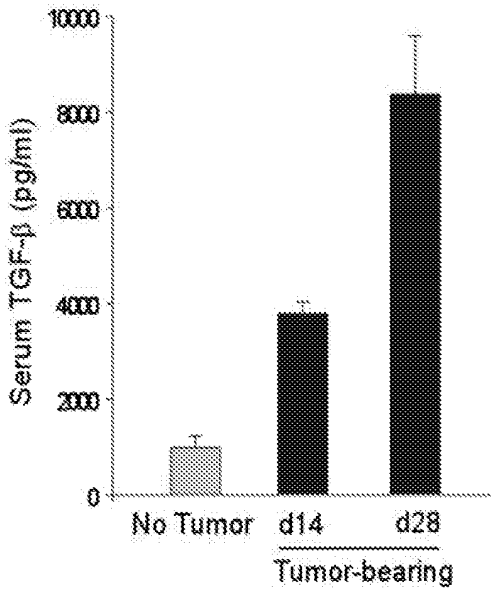


FIG. 54A

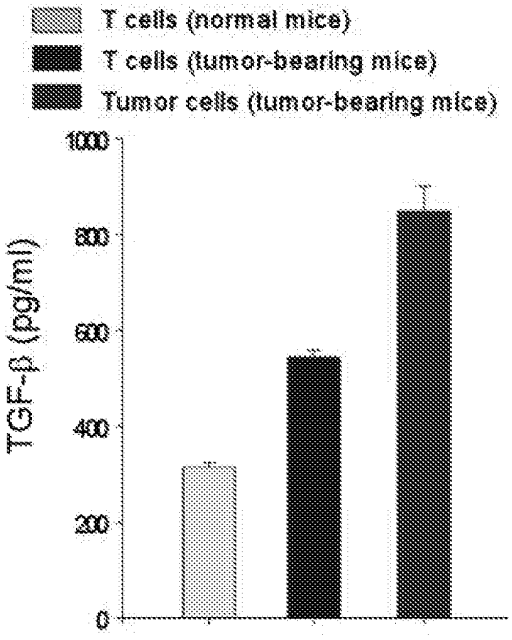


FIG. 54B

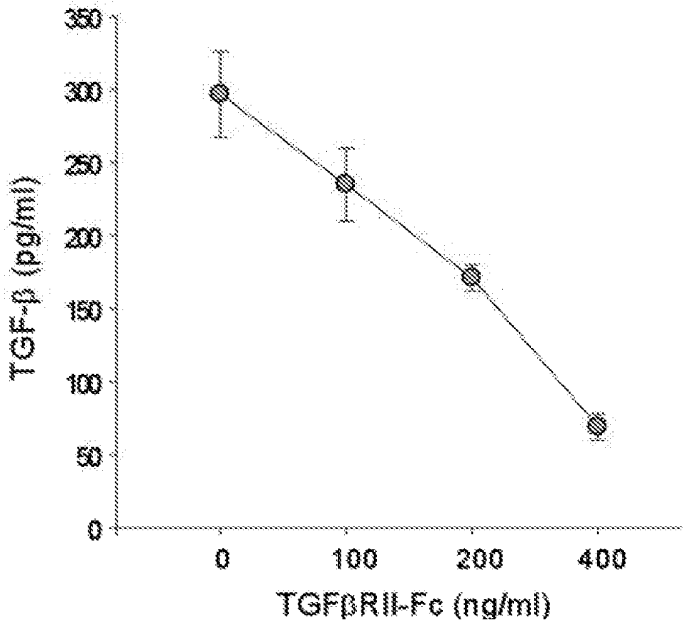


FIG. 54C

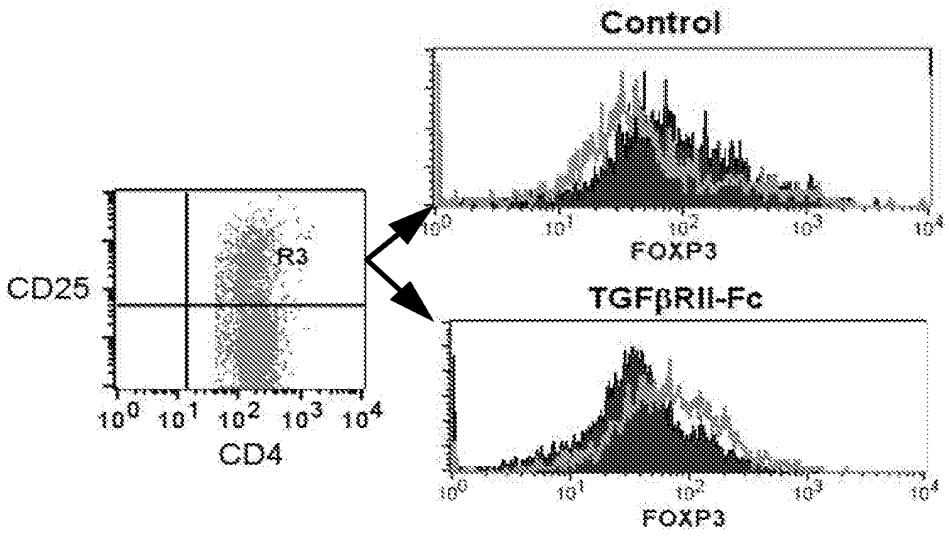


FIG. 54D

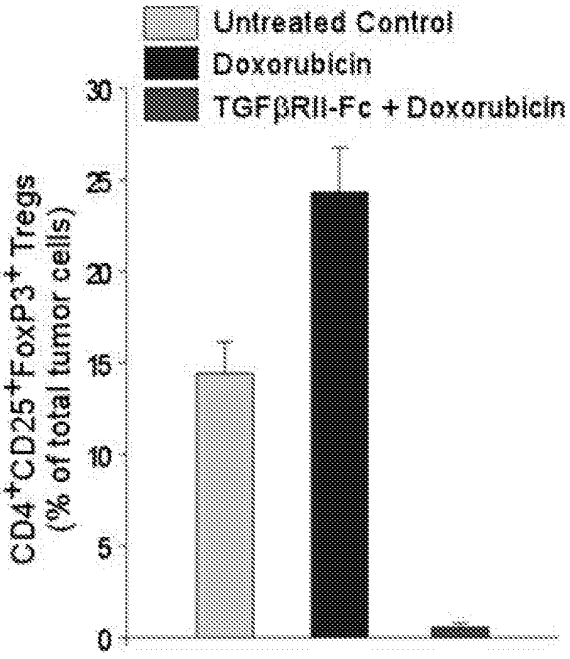


FIG. 54E

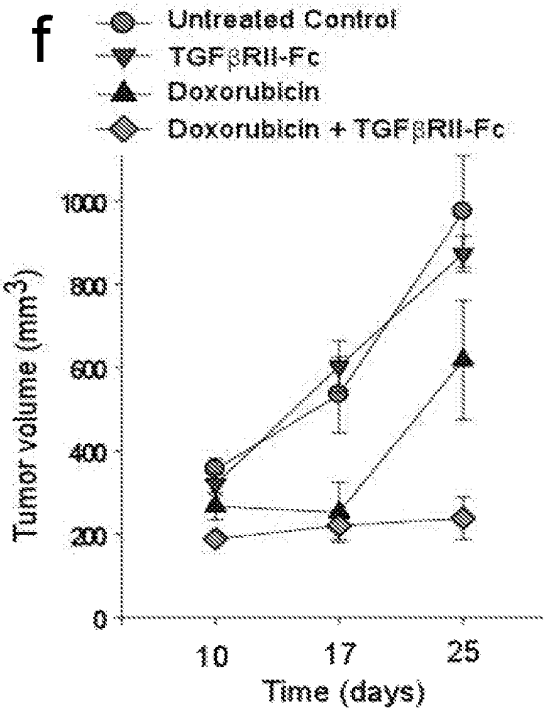


FIG. 54F

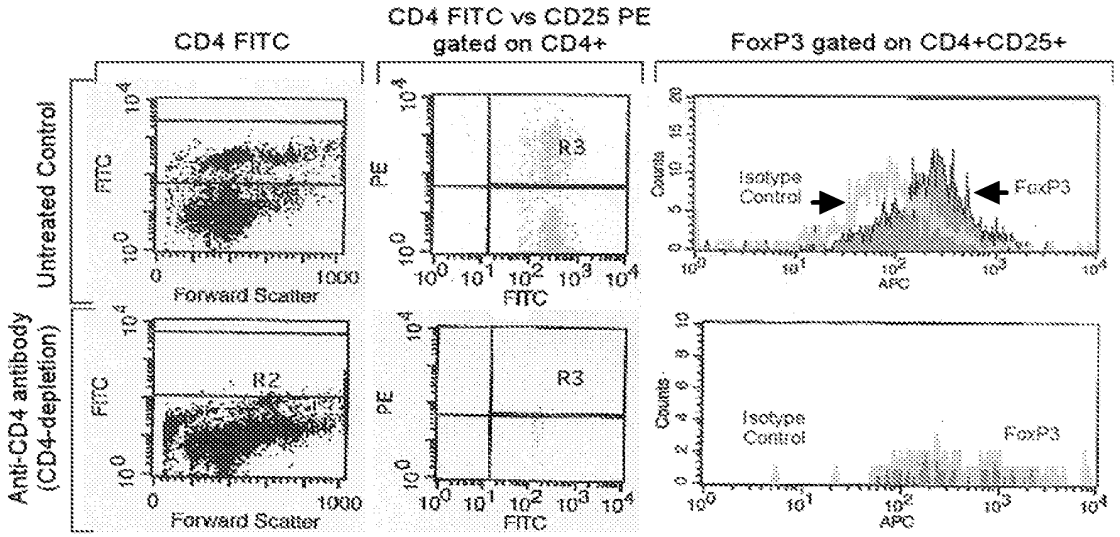


FIG. 55A

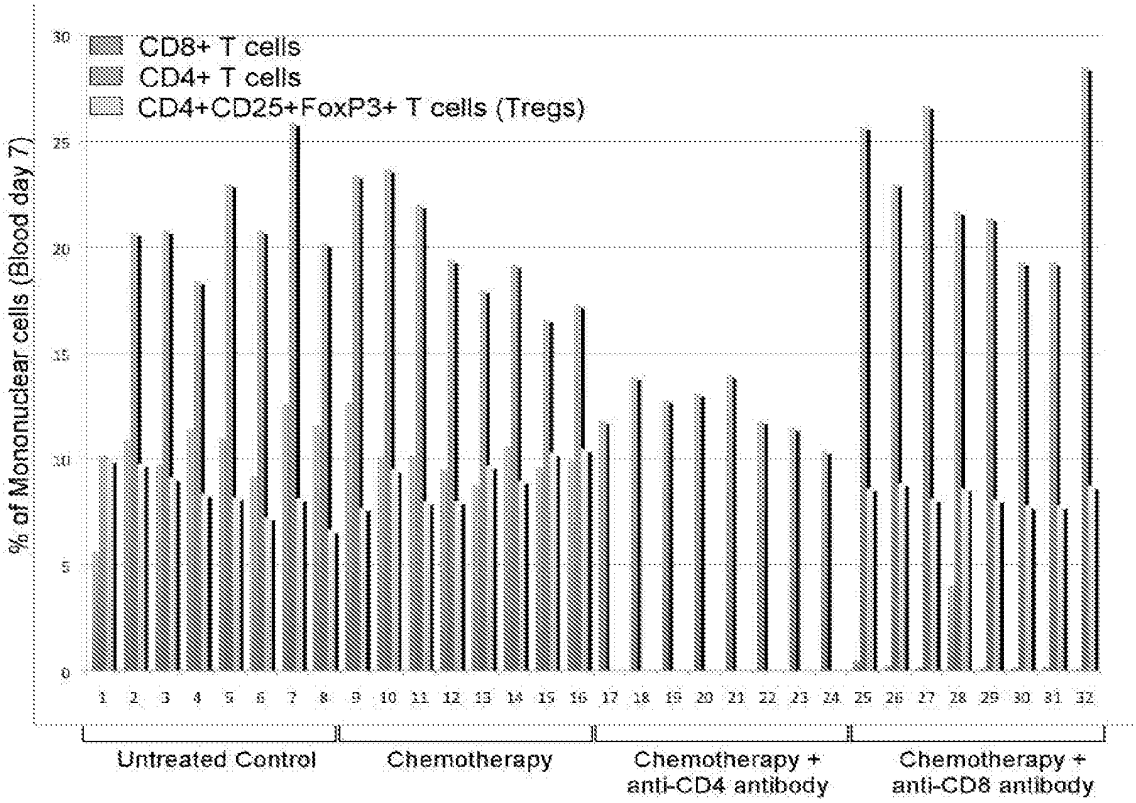


FIG. 55B

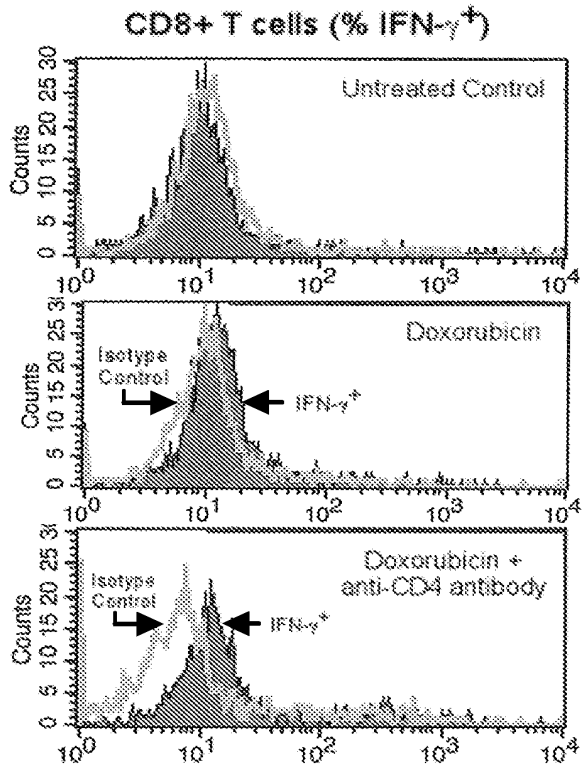


FIG. 55C

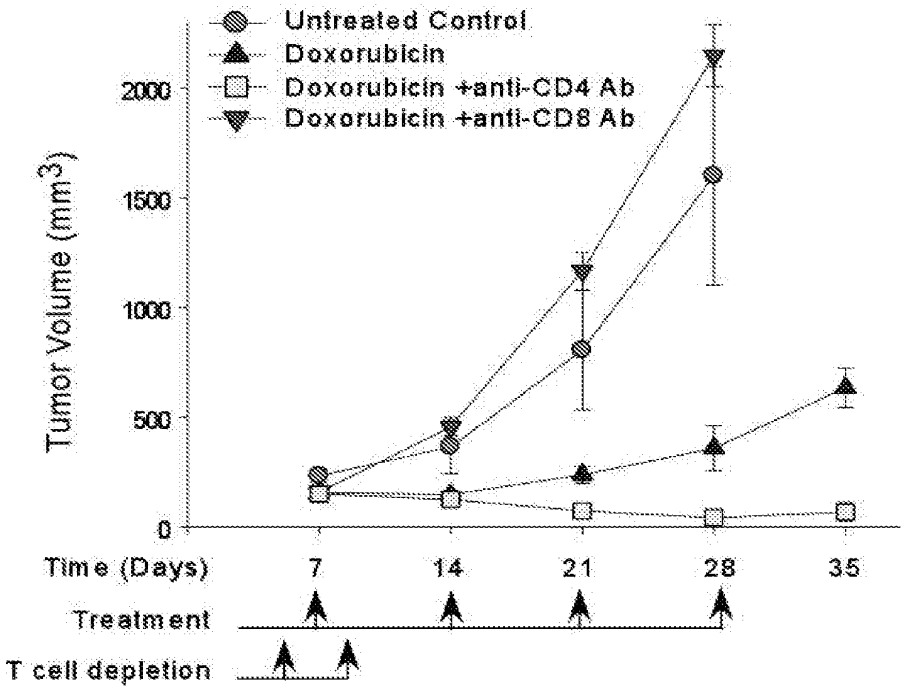


FIG. 55D

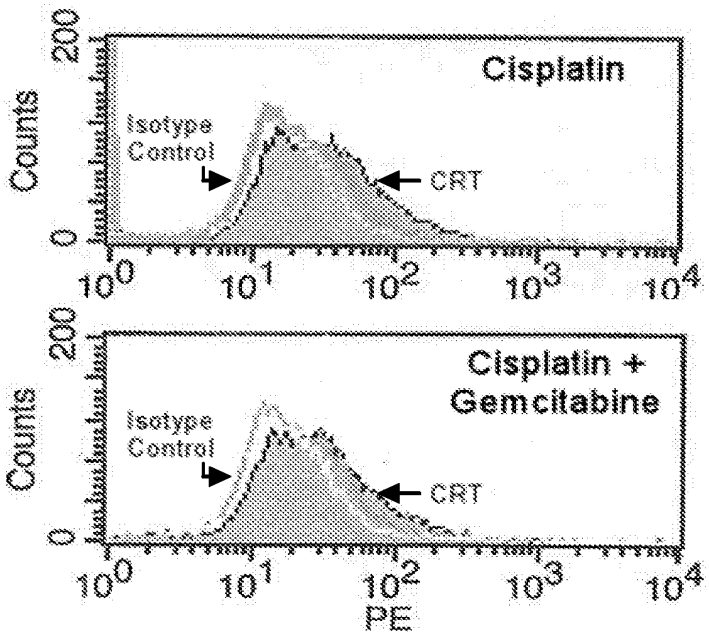


FIG. 56A

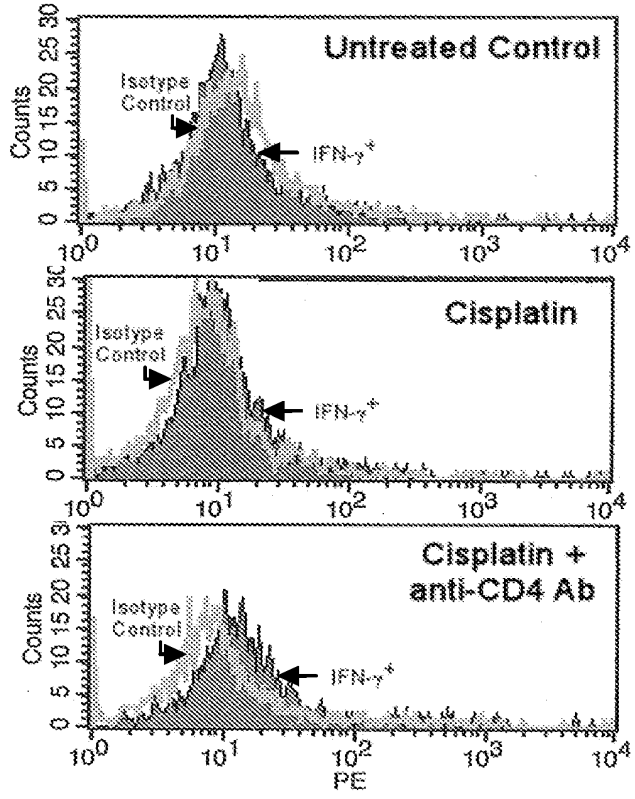


FIG. 56B

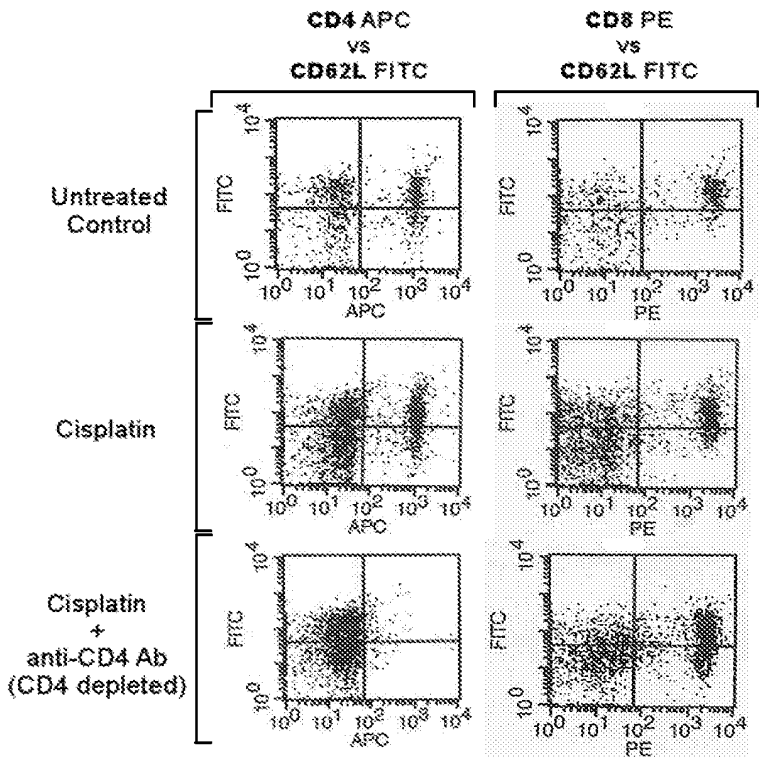


FIG. 56C

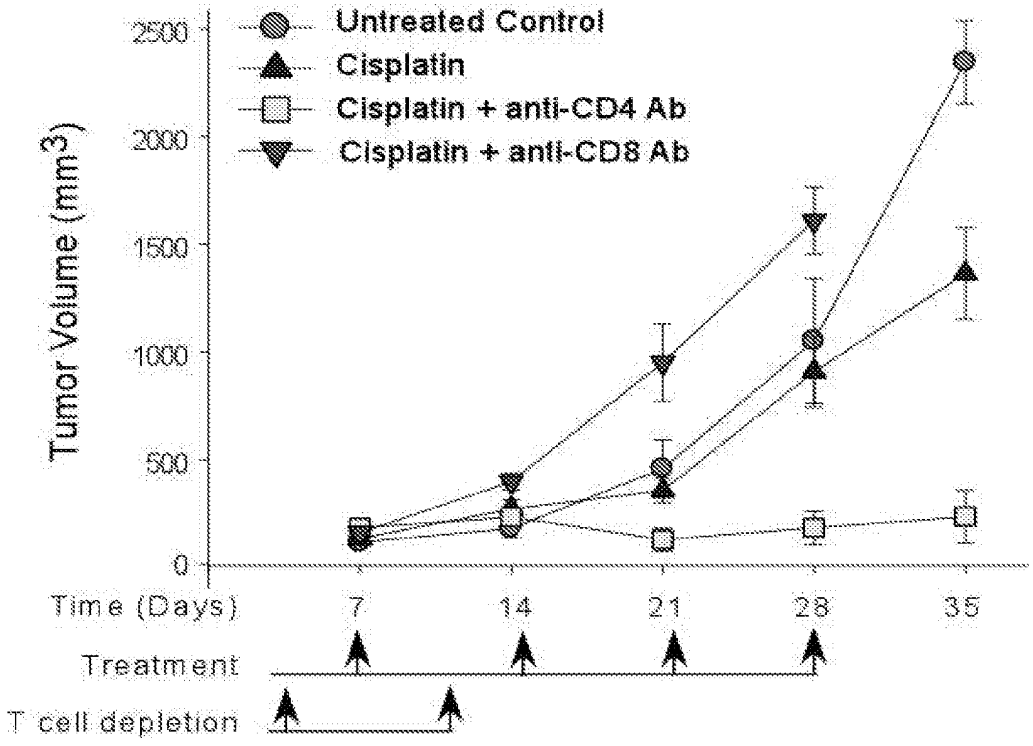


FIG. 56D

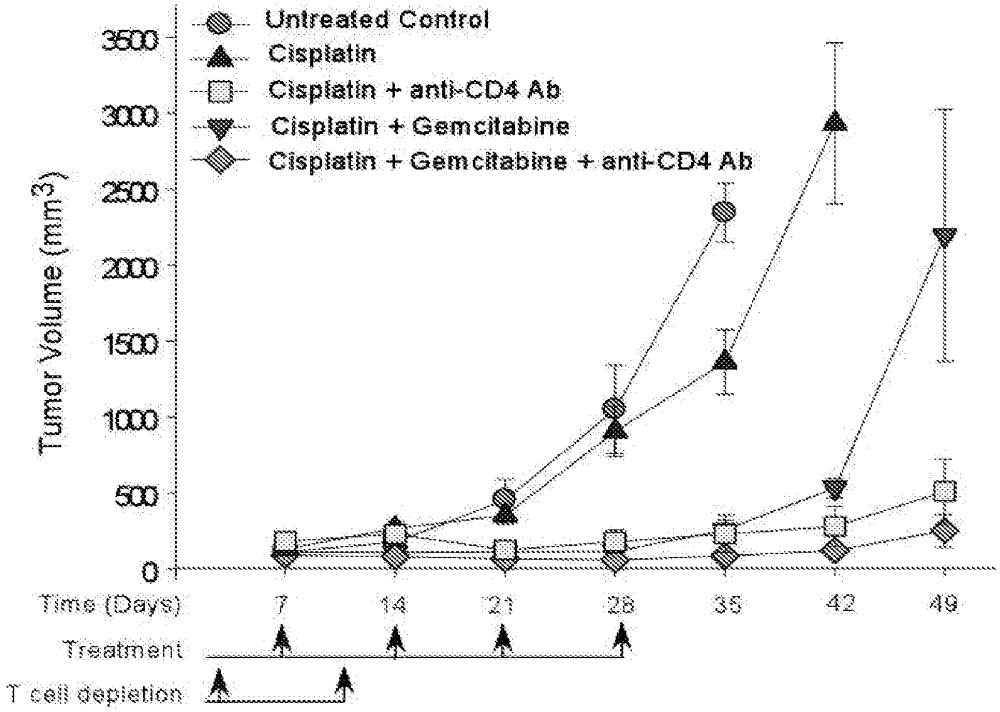


FIG. 56E

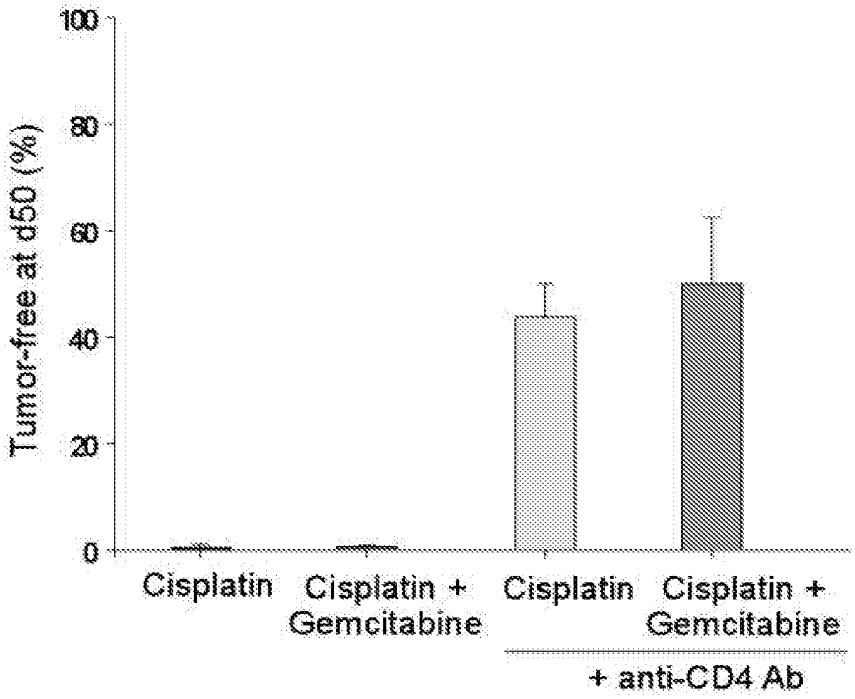


FIG. 56F

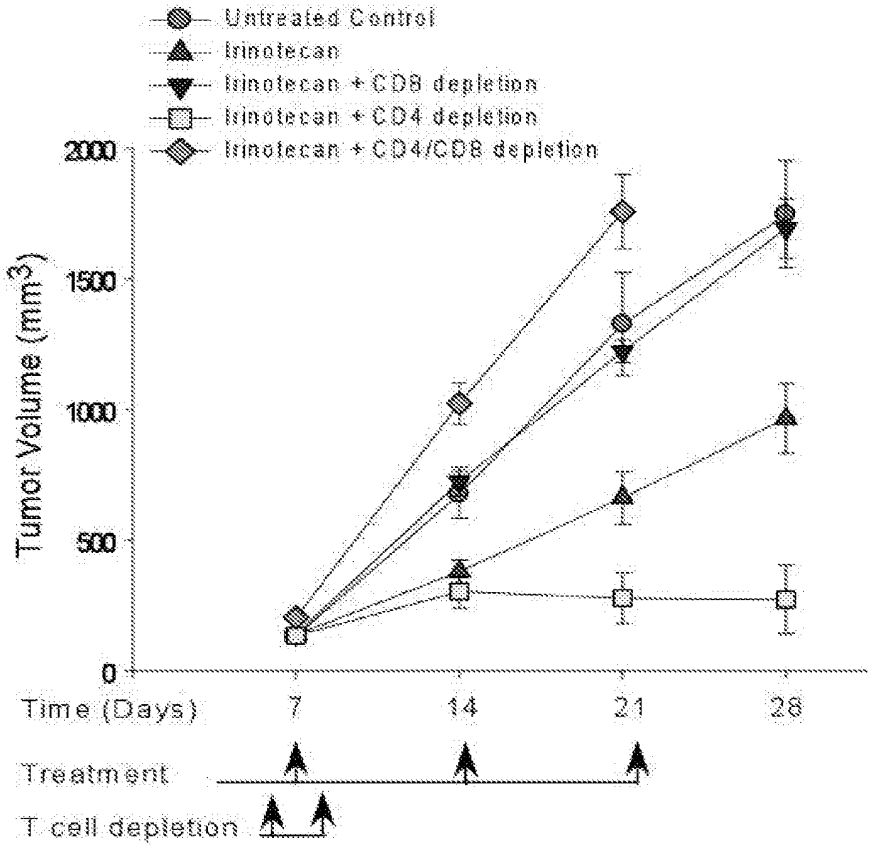


FIG. 57A

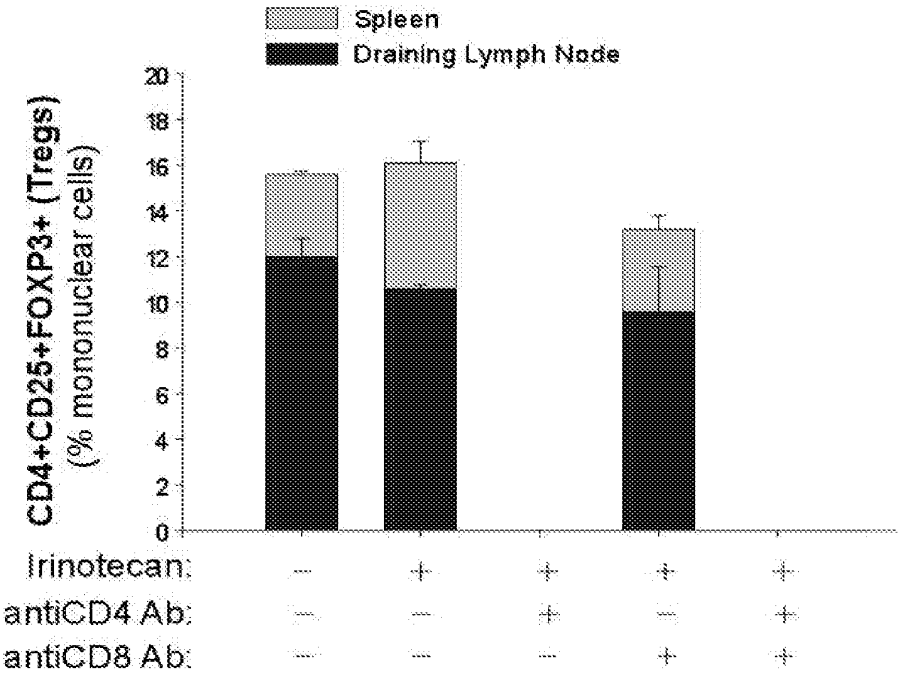


FIG. 57B

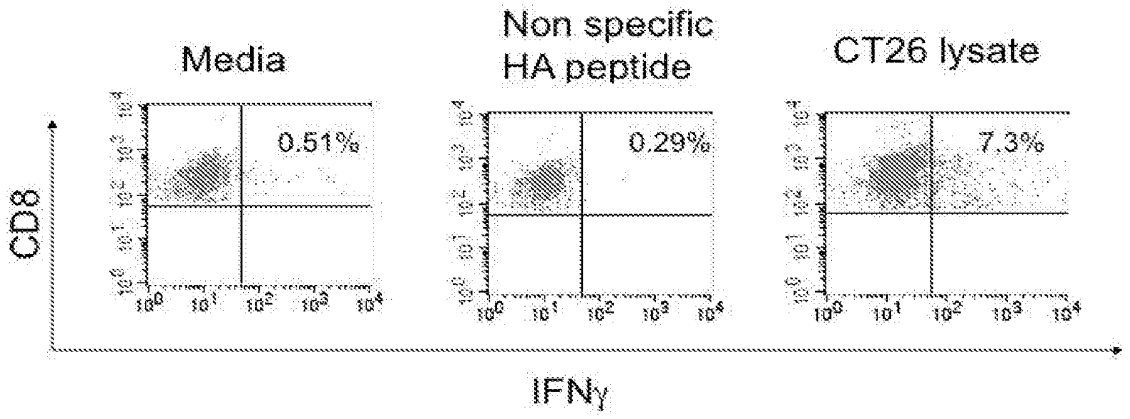


FIG. 57C

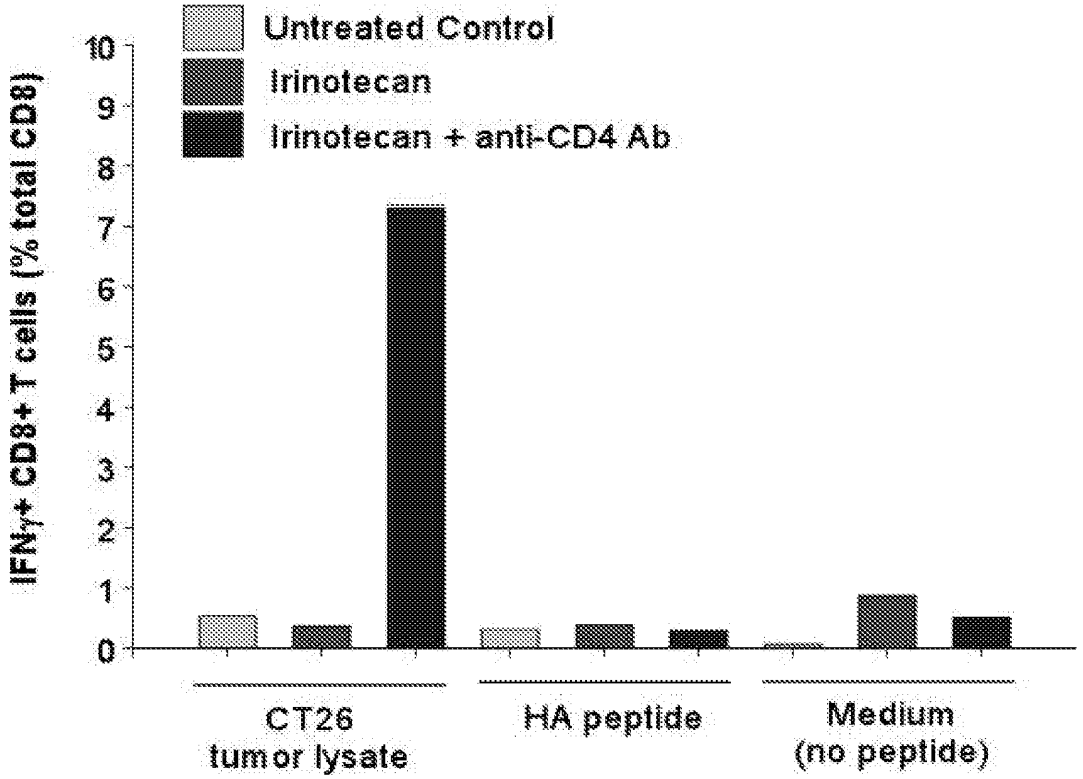


FIG. 57D

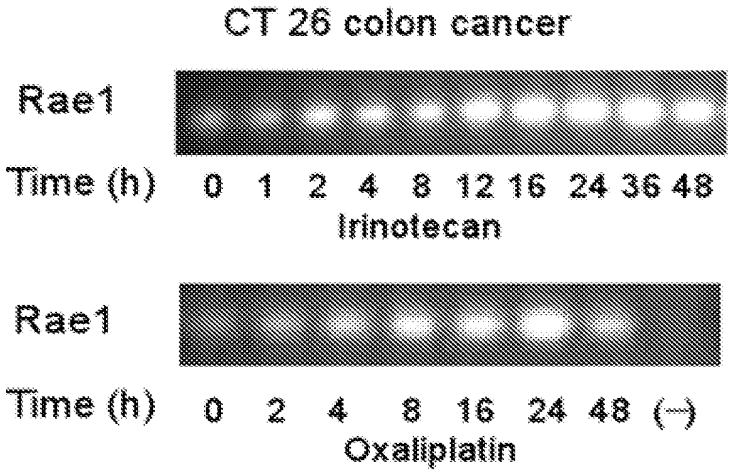


FIG. 57E

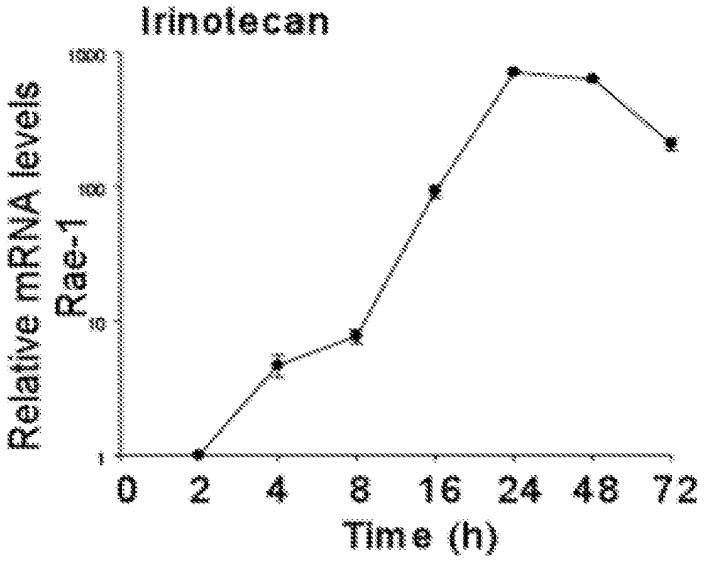


FIG. 57F

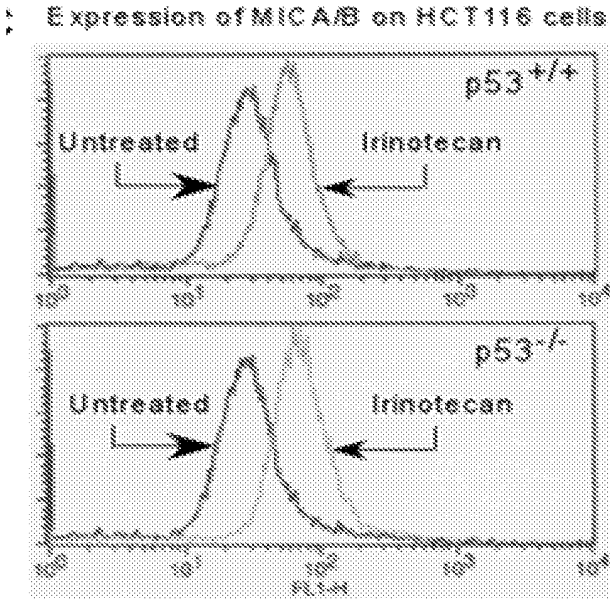


FIG. 57G

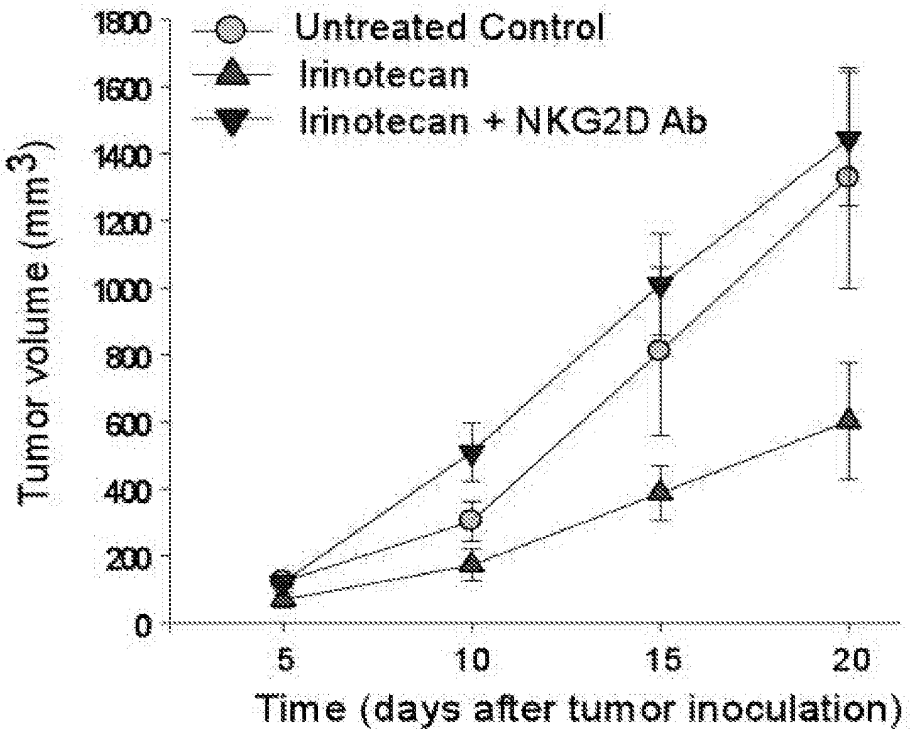


FIG. 57H

PATENT
ATTORNEY DOCKET NO.: JHU3260-6

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	Not Yet Assigned
Parent Serial No.:	14/645,282	Examiner:	Not Yet Assigned
Parent Filing Date:	March 11, 2015		
Serial No.:	Not Yet Assigned		
Filed:	August 8, 2016		
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Sir:

In accordance with Applicant's duty of disclosure pursuant with 37 C.F.R. §§ 1.56 and 1.97, attached is a list of references identified on the attached Form PTO/SB/08 for the Examiner's review and consideration.

The listed documents are of record in prior U.S. Application Serial No. 14/645,282 filed March 11, 2015, now pending; and U.S. Application Serial No. 13/582,717 filed October 17, 2012, now issued as U.S. patent 8,993,524, upon which Applicant relies on for an earlier effective filing date under 35 U.S.C. § 120. Consequently, copies of those references are not provided pursuant to 37 C.F. R. § 1.98(d); however, Applicant will provide copies of those references at the request of the Examiner.

Identification of the references listed on the attached Form PTO/SB/08 is not to be construed as an admission of Applicant or Attorneys for Applicant that such references are available as prior art against the subject application.

It is respectfully requested that these references be considered in the examination of this application and their consideration be made of written record in the application file.

This Information Disclosure Statement is being submitted within three months of the filing date of a national application other than a continued prosecution application under 37 C.F.R. §§ 1.53(d) and 1.97. Consequently, no fee is deemed necessary in connection with the filing of this Information Disclosure Statement. However, if any fee is required, authorization is hereby given to charge any fees associated with the filings submitted herewith, or credit any overpayment to Deposit Account No. 07-1896.

Respectfully submitted,

Date: August 8, 2016

Lisa A. Haile, J.D., Ph.D.
Registration No.: 38,347
Telephone: (858) 677-1456
Facsimile: (858) 638-5040

USPTO Customer No. 100899
DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

PTO/SB/08a (07-09)
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Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	
Filing Date	August 8, 2016
First Named Inventor	Atul Bedi
Art Unit	
Examiner Name	
Attorney Docket Number	JHU3260-6

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	AA	US-7,317,091 B2	01-08-2008	Lazar et al.	
	AB	US-7,527,791 B2	05-05-2009	Adams et al.	
	AC	US-7,795,389 B2	09-14-2010	Sun and Hinck	
	AD	US-7,803,378 B2	09-28-2010	Tranchand-Bunel, Denis	
	AE	US-7,820,165 B2	10-26-2010	McKenna et al.	
	AF	US-8,101,720 B2	01-24-2012	Lazar et al.	
	AG	US-8,399,618 B2	03-19-2013	Lazar et al.	
	AH	US-8,815,247 B2	08-26-2014	Govindappa et al.	
	AI	US-2005/0054832 A1	03-10-2005	Lazar et al.	
	AJ	US-2006/0135459 A1	06-22-2006	Epstein and Khawli	
	AK	US-2006/0263368 A1	11-23-2006	Rosenblum and Ellington	
	AL	US-2007/0212337 A1	09-13-2007	Bedi et al.	
	AM	US-2007/0244042 A1	10-18-2007	Sun and Hinck	
	AN	US-2008/0075717 A1	03-27-2008	Tranchand-Bunel, Denis	
	AO	US-2009/0053240 A1	02-26-2009	Lazar and Karki	
	AP	US-2009/0226435 A1	09-10-2009	Khare, Sanjay	
	AQ	US-2011/0052585 A1	03-03-2011	Scaria and White	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] "Number[4] "Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]

Examiner Signature		Date Considered	

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(Use as many sheets as necessary)

Sheet 2 of 2

Complete if Known	
Application Number	
Filing Date	August 8, 2016
First Named Inventor	Atul Bedi
Art Unit	
Examiner Name	
Attorney Docket Number	JHU3260-6

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	AR	KWIATKOWSKA et al., "Expression of Soluble Recombinant TGF-β Type II Receptor Fused with the Fc Portion of Human IgG1 (sTβRII-Fc) in NSO Cells," *Acta Biochim. Pol.* (2006), 53(2):361-369.	
	AS	ZHANG et al., "Design and Optimization of a Linker for Fusion Protein Construction," *Prog. Natural Sci.* (2009), 19:1197-1200, Elsevier.	
	AT	ZHANG et al., "Expression of a Soluble TGF-β Receptor by Tumor Cells Enhances Dendritic Cell/Tumor Fusion Vaccine Efficacy," *J. Immunol.* (2008), 181:3690-3697, The American Association of Immunologists, Inc.	
	AU	Supplementary European Search Report from Related International Application No. EP 11 75 1481.	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.
[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.
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331323-001150

PATENT
ATTORNEY DOCKET NO.: JHU3260-5

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	1647
Application No.:	14/645,282	Examiner:	S.H. Shafer
Filed:	March 11, 2015	Conf. No.:	1031
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

POWER OF ATTORNEY BY ASSIGNEE

As the below-named assignee of the above-identified application ("Application"), **THE JOHNS HOPKINS UNIVERSITY**, through its duly-delegated representative, hereby appoints the practitioners of **USPTO CUSTOMER NO. 100899** at DLA Piper LLP (US) to represent and transact all business for the undersigned as attorneys or agents before the United States Patent and Trademark Office (USPTO) in connection with the above-identified patent application according to USPTO assignment records.

Statement under 37 C.F.R. §3.73(c):

THE JOHNS HOPKINS UNIVERSITY is the assignee of the entire right, title and interest in the above-identified application by virtue of an assignment recorded in the U.S. Patent and Trademark Office at Reel 029136, Frame 0898.

Direct all telephone calls to:

LISA A. HAILE, J.D., Ph.D.
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

Address all correspondence to: **USPTO CUSTOMER NO. 100899**

The Johns Hopkins University
c/o DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

THE JOHNS HOPKINS UNIVERSITY

By : Jill E. Uhl
(Signature)

Name : Jill E. Uhl
(Typed or Printed Name)

Title : Intellectual Property Director, The Johns Hopkins University

Date : 31 MAR 2015

PTO/AIA/01 (06-12)
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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

As the below named inventor, I hereby declare that:

This declaration is directed to:

- ☐ The attached application, or
- ☑ United States application or PCT international application number 14/645,282 filed on March 11, 2015.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Atul BEDI Date (Optional): _____

Signature: Atul Bedi

Note: An application data sheet (PTO/AIA/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/SB/AIA01 form for each additional inventor.

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

As the below named inventor, I hereby declare that:

This declaration is directed to:

- ☐ The attached application, or
- ☑ United States application or PCT International application number 14/645,282 filed on March 11, 2015.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

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LEGAL NAME OF INVENTOR

Inventor: Rajani RAVI Date (Optional): _____

Signature: Rajani Ravi

Note: An application data sheet (PTO/AIA/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/SB/AIA01 form for each additional inventor.

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Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS
First Named Inventor/Applicant Name:	Atul Bedi
Filer:	Lisa A. Haile/Aldon Griffis
Attorney Docket Number:	JHU3260-6

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility filing Fee (Electronic filing)	4011	1	70	70
Utility Search Fee	2111	1	300	300
Utility Examination Fee	2311	1	360	360
Pages:				
Utility Appl Size fee per 50 sheets >100	2081	1	200	200
Claims:				
Miscellaneous-Filing:				
Petition:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**930**

Electronic Acknowledgement Receipt

EFS ID:	26580150
Application Number:	15231309
International Application Number:	
Confirmation Number:	3446
Title of Invention:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Lisa A. Haile/Aldon Griffis
Filer Authorized By:	Lisa A. Haile
Attorney Docket Number:	JHU3260-6
Receipt Date:	08-AUG-2016
Filing Date:	
Time Stamp:	17:17:07
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$930
RAM confirmation Number	080916INTEFSW00004501071896
Deposit Account	071896
Authorized User	Aldon Griffis
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37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	JHU3260-6ADS_EFS.pdf	1823569 06febd6b359fb9b0b7bdd6fe0200ed1e9bd335bc	no	9
Warnings:					
Information:					
2		JHU3260-6ContinuationTxEFS.PDF	35484 bd07c19524246b122ea0e8dfabb511a8bc599c99	yes	4

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Transmittal of New Application	1	3
	Miscellaneous Incoming Letter	4	4

Warnings:					
Information:					
3		JHU3260-6ContinuationEFS.pdf	3060961 eccb1073514dbc917e9e7b1b42945ee6f9b00b07	yes	152

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Specification	1	73
	Claims	74	76
	Abstract	77	77
	Drawings-only black and white line drawings	78	152

Warnings:					
Information:					

4		JHU3260-6IDS_EFS.PDF	56139 67eca2bb854ff5cc6388605281230e95710806b7	yes	4
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Transmittal Letter		1	2	
	Information Disclosure Statement (IDS) Form (SB08)		3	4	
Warnings:					
Information:					
5	Power of Attorney	JHU3260-6ParentPowerAttyEFS.pdf	53472 658f3c0e99e41d01a1390688238ee21e434649fb	no	2
Warnings:					
Information:					
6	Oath or Declaration filed	JHU3260-6ParentInventorDeclsEFS.pdf	56548 71722f650861d9c73acc4dae46f7f950bbcc0c37	no	2
Warnings:					
Information:					
7	Sequence Listing (Text File)	JHU3260-6_ST25.txt	469806	no	-
Warnings:					
Information:					
8	Fee Worksheet (SB06)	fee-info.pdf	37159 52bbb75dd1f4379f2b6815a832f5c211a1879195	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	5593138		

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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	JHU3260-6
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Atul		Bedi	

Residence Information (Select One) ● US Residency ○ Non US Residency ○ Active US Military Service

City	State/Province	Country of Residence
Timonium	MD	US

Mailing Address of Inventor:

Address 1	2211 Dalewood Road
Address 2	
City	Timonium
State/Province	MD
Postal Code	21093
Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Rajani		Ravi	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	State/Province	Country of Residence
Ruxton	MD	US

Mailing Address of Inventor:

Address 1	7810 Ballston Road
Address 2	
City	Ruxton
State/Province	MD
Postal Code	21204
Country	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

PTO/AIA/14 (11-15)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	JHU3260-6
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	100899		
Email Address		Add Email	Remove Email

Application Information:

Title of the Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		
Attorney Docket Number	JHU3260-6	**Small Entity Status Claimed**	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	75	**Suggested Figure for Publication (if any)**	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	● Customer Number	US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	100899		

PTO/AIA/14 (11-15)
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	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	14/645282	2015-03-11
Prior Application Status	Patented		Remove

Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
14/645282	Continuation of	13/582717	2012-10-17	8993524	2015-03-31

Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
13/582717	a 371 of international	PCT/US2011/027317	2011-03-04
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2011/027317	Claims benefit of provisional	61/435671	2011-01-24
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2011/027317	Claims benefit of provisional	61/311255	2010-03-05

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	JHU3260-6
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

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Approved for use through 04/30/2017. OMB 0651-0032
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	JHU3260-6
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	JHU3260-6
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 — Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

● Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
Person to whom the inventor is obligated to assign.	Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	The Johns Hopkins University		
Mailing Address Information For Applicant:			
Address 1	3400 N. Charles Street		
Address 2			
City	Baltimore	**State/Province**	MD
Country	US	Postal Code	21218
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

Remove

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Lisa A. Haile/			Date (YYYY-MM-DD)	2016-08-08
First Name	Lisa	Last Name	Haile	Registration Number	38,347

Additional Signature may be generated within this form by selecting the Add button. Add

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	JHU3260-6
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

SCORE Placeholder Sheet for IFW Content

Application Number: 15231309 Document Date: 08/08/2016

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- Drawings – Other than Black and White Line Drawings

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

To access the documents in the SCORE database, refer to instructions below.

At the time of document entry (noted above):

- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://Score.uspto.gov/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: September 30, 2013

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Sequence Listing was accepted.
If you need help call the Patent Electronic Business Center at (866)
217-9197 (toll free).
Reviewer: Saleem, Syed (ASRC)
Timestamp: [year=2016; month=8; day=15; hr=7; min=25; sec=43; ms=185; ]
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Validated By CRFValidator v 1.0.5

Application No: 15231309 Version No: 1.0

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SEQUENCE LISTING

<110> THE JOHNS HOPKINS UNIVERSITY
BEDI, Atul
RAVI, Rajani

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

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

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

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

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335
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Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
    450                 455                 460

Thr Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val
465                 470                 475                 480

Thr Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe Cys
                485                 490                 495

Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn
            500                 505                 510

Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala
        515                 520                 525

Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His
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Asp Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala Ser
545                 550                 555                 560
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Pro Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe
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Glu Glu Tyr Asn Thr Ser Asn Pro Asp
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Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu Glu Trp Ile
        35                  40                  45

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

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

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

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

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

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
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Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
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145                 150                 155                 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
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Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
            180                 185                 190

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

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Ala Glu Pro Lys Ser Cys
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Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
225                 230                 235                 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
                245                 250                 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
            260                 265                 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
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    290                 295                 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305                 310                 315                 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
                325                 330                 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
            340                 345                 350

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
        355                 360                 365

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
    370                 375                 380
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Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
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Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
                405                 410                 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
            420                 425                 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
        435                 440                 445

Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
    450                 455                 460

Gly Ser Thr Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met
465                 470                 475                 480

Ile Val Thr Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys
                485                 490                 495

Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met
            500                 505                 510

Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys
        515                 520                 525

Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val
    530                 535                 540

Cys His Asp Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala
545                 550                 555                 560

Ala Ser Pro Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr
                565                 570                 575

Phe Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile
            580                 585                 590

Phe Ser Glu Glu Tyr Asn Thr Ser Asn Pro Asp
        595                 600
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<210> 4
<211> 605
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<213> Homo sapiens

<400> 4

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            20                  25                  30

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

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

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

Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

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

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

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

Asn

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www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/231,309	08/08/2016	1645	930	JHU3260-6	20	1

CONFIRMATION NO. 3446

100899
The John Hopkins University
c/o DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

FILING RECEIPT



Date Mailed: 08/17/2016

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Atul Bedi, Timonium, MD;
Rajani Ravi, Ruxton, MD;

Applicant(s)

The Johns Hopkins University, Baltimore, MD;

Power of Attorney: The patent practitioners associated with Customer Number 100899

Domestic Priority data as claimed by applicant

This application is a CON of 14/645,282 03/11/2015
which is a CON of 13/582,717 10/17/2012 PAT 8993524
which is a 371 of PCT/US2011/027317 03/04/2011
which claims benefit of 61/435,671 01/24/2011
and claims benefit of 61/311,255 03/05/2010

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 08/16/2016
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/231,309**
Projected Publication Date: 11/24/2016
Non-Publication Request: No
Early Publication Request: No
**** SMALL ENTITY ****
Title

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Preliminary Class

424

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

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This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 15/231,309

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	70		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	300		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	360		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	20 minus 20 =	*	x 40 =	0.00	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*	x 210 =	0.00			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			200			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	930		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/231,309	08/08/2016	Atul Bedi	JHU3260-6

CONFIRMATION NO. 3446

100899
The John Hopkins University
c/o DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

PUBLICATION NOTICE



Title:COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Publication No.US-2016-0340430-A1
Publication Date:11/24/2016

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently http://pair.uspto.gov/. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/231,309	08/08/2016	Atul Bedi	JHU3260-6	3446

100899 7590 12/21/2016

The John Hopkins University
c/o DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

EXAMINER	
SHAFER, SHULAMITH H	

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
12/21/2016	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

gtdocket@dlapiper.com

Office Action Summary	Application No. 15/231,309	Applicant(s) BEDI ET AL.	
	Examiner SHULAMITH H. SHAFER	Art Unit 1647	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 2 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) 1-20 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☐ Claim(s) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☒ Claim(s) 1-20 are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

Election/Restrictions

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-15, drawn to drawn to a molecule, comprising a targeting moiety fused with an immunomodulatory moiety, classified in 530, subclass 387.3.
II. Claims 16-20, drawn to a method of treating a neoplastic disease or cancer comprising administration of a targeting moiety fused with an immunomodulatory moiety, classified in 424, subclass 130.1.

The inventions are distinct, each from the other because of the following reasons:

Inventions I and II are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product. See MPEP § 806.05(h). In the instant case, the neoplastic diseases may be treated with standard chemo- and/or radiation therapy.

Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above and there would be a serious search and examination burden if restriction were not required because one or more of the following reasons apply:

(a) the inventions have acquired a separate status in the art in view of their different classification;

(b) the inventions have acquired a separate status in the art due to their recognized divergent subject matter;

(c) the inventions require a different field of search (for example, searching different classes/subclasses or electronic resources, or employing different search queries);

(d) the prior art applicable to one invention would not likely be applicable to another invention;

(e) the inventions are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a invention to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected invention**.

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected invention.

If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention.

Species Election

This application contains claims directed to the following patentably distinct species: Sequences. The species are independent or distinct because each sequence comprises a sequence of unique and distinct amino acid residues. In addition, these species are not obvious variants of each other based on the current record.

If Group I is elected, Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, chosen from SEQ ID NOs:79-91, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, Claim 1 is generic.

There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply: each sequence comprises a sequence of unique and distinct amino acid residues. A search for SEQ ID NO:79, for example, would not reveal art reading on SEQ ID NO:90.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected species or grouping of patentably indistinct species**, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species or grouping of patentably indistinct species.

Should applicant traverse on the ground that the species, or groupings of patentably indistinct species from which election is required, are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing them to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103 or pre-AIA 35 U.S.C. 103(a) of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

The examiner has required restriction between product or apparatus claims and process claims. Where applicant elects claims directed to the product/apparatus, and all product/apparatus claims are subsequently found allowable, withdrawn process claims that include all the limitations of the allowable product/apparatus claims should be considered for rejoinder. All claims directed to a nonelected process invention must include all the limitations of an allowable product/apparatus claim for that process invention to be rejoined.

In the event of rejoinder, the requirement for restriction between the product/apparatus claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product/apparatus are found allowable, an otherwise proper restriction requirement between product/apparatus claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product/apparatus claim will not be rejoined. See MPEP § 821.04. Additionally, in order for rejoinder to occur, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product/apparatus claims. **Failure to do so may result in no rejoinder.** Further, note

that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SHULAMITH H. SHAFER whose telephone number is (571)272-3332. The examiner can normally be reached on Monday through Friday, 8 AM to 5 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joanne Hama can be reached on 571-272-2911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

SHULAMITH H. SHAFER
Primary Examiner
Art Unit 1647

/SHULAMITH H. SHAFER/
Primary Examiner, Art Unit 1647

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Bedi and Ravi	Art Unit:	1647
Application No.:	15/231,309	Examiner:	Shafer, Shulamith H.
Filing Date:	August 8, 2016	Conf. No.	3446
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

Mail Stop AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO RESTRICTION REQUIREMENT

Dear Sirs:

In response to the Requirement for Restriction mailed December 21, 2016 in the above-identified patent application, Applicant submits the following amendments and remarks.

Applicants Arguments and Remarks begin on page **2** of this paper.

REMARKS

Restriction Requirement

As set forth in the Office Action issued December 21, 2016 ("the Office Action") Applicants are required under 35 U.S.C. 121 to restrict the application to one of the following groups:

Group I, Claims 1-15, drawn to drawn to a molecule, comprising a targeting moiety fused with an immunomodulatory moiety, classified in 530, subclass 387.3.

Group II, Claims 16-20, drawn to a method of treating a neoplastic disease or cancer comprising administration of a targeting moiety fused with an immunomodulatory moiety, classified in 424, subclass 130.1.

In response to the restriction requirement, Applicant elects the invention of Group I, encompassing claims 1-15, without traverse.

Additionally, Applicant selects species SEQ ID NO: 87 as in claim 9.

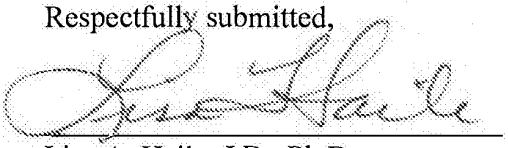
Applicants reserve the right to pursue prosecution of non-elected subject matter and/or species upon an indication of allowance of any generic claims or in a later filed application claiming the benefit of priority of the above-identified application.

CONCLUSION

Applicant provides that the claims are ready for examination. The Examiner is invited to contact Applicant's undersigned representative if there are any questions relating to this application.

No fee is currently believed due in conjunction with this submission. However, the Commissioner is authorized to charge any fees that may be due in connection with the filing of this paper, or to credit any overpayment thereof, to Deposit Account No. 07-1896 referencing the above-identified attorney docket number.

Respectfully submitted,



Date: January 23, 2017

Lisa A. Haile, J.D., Ph.D.
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4365 Executive Drive, Suite 1100
San Diego, California 92121-2133
USPTO Customer No.: 100899

Electronic Acknowledgement Receipt

EFS ID:	28142620
Application Number:	15231309
International Application Number:	
Confirmation Number:	3446
Title of Invention:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Lisa A. Haile/Shannon Warner
Filer Authorized By:	Lisa A. Haile
Attorney Docket Number:	JHU3260-6
Receipt Date:	23-JAN-2017
Filing Date:	08-AUG-2016
Time Stamp:	19:11:21
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Response to Election / Restriction Filed	JHU3260_6_Response_RR.pdf	53327 573705d528a870b7430a2cecae6b4387f4ee14c8	no	3
Warnings:					

Information:	
Total Files Size (in bytes):	53327

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/231,309	08/08/2016	Atul Bedi	JHU3260-6	3446

100899 7590 02/22/2017

The John Hopkins University
c/o DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

EXAMINER	
SHAFER, SHULAMITH H	

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
02/22/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

gtdocket@dlapiper.com

Office Action Summary	Application No. 15/231,309	Applicant(s) BEDI ET AL.	
	Examiner SHULAMITH H. SHAFER	Art Unit 1647	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *1/23/17*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-20* is/are pending in the application.

5a) Of the above claim(s) *16-20* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-14* is/are rejected.

8)☒ Claim(s) *15* is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *8/8/16*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

Detailed Action

The present application is being examined under the pre-AIA first to invent provisions.

Restriction Requirement:

Applicants' election, without traverse, of Group I, drawn to an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds PD-1 ligand, in response of 23 January 2017, is acknowledged.

In response to requirement for species election, applicants have elected SEQ ID NO:87.

Claims 1-20 are pending in the instant application. Claims 16-20 have been withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim.

Claims 1-15 are under consideration to the extent they read on the elected invention.

Information Disclosure Statement:

The Information Disclosure statements (IDS) submitted on the 8 August 2016 has been considered. The signed copy is attached.

Rejections

35 U.S.C. § 112:

The following is a quotation of 35 U.S.C. 112(b):

> (b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 12 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 12 recites the limitation "further comprising a linker at the CH3 region of the Fc". There is insufficient antecedent basis for this limitation in the claim as the claim depends from claims 11 and 1 which do not recite a “CH3 region of the Fc”.

35 U.S.C. § 112:

The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

> (a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of the first paragraph of pre-AIA 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 2, in part (with respect to fusion protein), 7, 8, and 11-14 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim (s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Applicant is directed to the Written Description Training Materials, Revision 1, March 25, 2008.

The claims are drawn to an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein: (a) the targeting moiety specifically binds Programmed Death-1 ligand (PD-1 ligand); and (b) the immunomodulatory moiety

comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor (TGFβR).

Vas-Cath Inc. V. Mahurkar, 19 USPQ2d 1111, states that Applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention, for purposes of the written description inquiry, is whatever is now claimed (see page 1117). A review of the language of the claim indicates that these claims are drawn to a genus, i.e., a targeting moiety specifically binds Programmed Death-1 ligand (PD-1 ligand).

To provide adequate written description and evidence of possession of a claimed genus, the specification must provide sufficient distinguishing characteristics of the genus. The factors to be considered include disclosure of complete or partial structure, physical and/or chemical properties, functional characteristics, structure/function correlation, methods of making the claimed product, or any combination thereof.

A description of a genus may be achieved by means of a recitation of a representative number of species falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus. *Regents of the University of California v. Eli Lilly & Co.*, 119 F3d 1559, 1569, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). In *Regents of the University of California v. Eli Lilly* (43 USPQ2d 1398-1412), the court held that a generic statement which defines a genus of nucleic acids by only their functional activity does not provide an adequate written description of the genus. The court indicated that, while applicants are not required to disclose every species encompassed by a genus, the description of the genus is achieved by the recitation of a representative number of species falling within the scope of the claimed genus. At section B(1), the court states, "An adequate written description of a DNA ... requires a precise definition, such as by structure, formula, chemical name, or physical properties, not a mere wish or plan for obtaining the claimed chemical invention."

A description of a genus may be achieved by means of a recitation of a representative number of species falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a

substantial portion of the genus. *Regents of the University of California v. Eli Lilly & Co.*, 119 F3d 1559, 1569, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). In *Regents of the University of California v. Eli Lilly* (43 USPQ2d 1398-1412), the court held that a generic statement which defines a genus of nucleic acids by only their functional activity does not provide an adequate written description of the genus. The court indicated that, while applicants are not required to disclose every species encompassed by a genus, the description of the genus is achieved by the recitation of a representative number of species falling within the scope of the claimed genus. At section B(1), the court states, "An adequate written description of a DNA ... requires a precise definition, such as by structure, formula, chemical name, or physical properties, not a mere wish or plan for obtaining the claimed chemical invention."

The specification, at paragraph 0163 of PGPUB 2016/0340430, the PGPUB of the instant application, discloses "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. With respect to targeting moieties that specifically bind PD-1 ligands, the disclosure teaches that 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).

The specification discloses the reduction to practice of two species within the claimed genus: a molecule of SEQ ID NO: 11 which provides a fusion protein including PD-1 Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGFβRII) Extracellular domain (ectodomain) (PD-1 ectodomain+Fc+ TGFβRII ectodomain) and a molecule of SEQ ID NO: 12 which provides a fusion protein including Transforming growth factor-beta receptor II (TGFβRII) Extracellular domain (ectodomain), Fc, and PD-1 Ectodomain (TGFβRII ectodomain+Fc+PD-1 ectodomain) (paragraph 0220 and Figure 10). The technology for producing antibodies to PD-1 is well known in the art;

thus, the Examiner considers the Applicants to be in possession of a targeting moiety wherein the targeting moiety is an antibody or antigen binding fragment thereof.

There are no structural formulas disclosed of any other targeting moieties that specifically bind PD-1 ligand.

The specification does not place any structure, or chemical limitations on “targeting moieties that specifically bind PD-1 ligand”.

"A patentee will not be deemed to have invented species sufficient to constitute the genus by virtue of having disclosed a single species when ... the evidence indicates ordinary artisans could not predict the operability in the invention of any species other than the one disclosed." In re Curtis, 354 F.3d 1347, 1358, 69 USPQ2d 1274, 1282 (Fed. Cir. 2004).

For inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus. See, e.g., Eli Lilly.

Further, it is not sufficient to define the genus solely by its principal biological property, because an alleged conception having no more specificity than that is simply a wish to know the identity of any material with that biological property. Per the Enzo court's example, (Enzo Biochem, Inc. v. Gen-Probe Inc., 63 USPQ2d 1609 (CA FC 2002) at 1616) of a description of an anti-inflammatory steroid, i.e., a steroid (a generic structural term) couched "in terms of its function of lessening inflammation of tissues" which, the court stated, "fails to distinguish any steroid from others having the same activity or function" and the expression "an antibiotic penicillin" fails to distinguish a particular penicillin molecule from others possessing the same activity and which therefore, fails to satisfy the written description requirement. Similarly, the function of the variant as claimed does not distinguish a particular variant from others having the same activity or function and as such, fails to satisfy the written-description requirement. Applicant has not disclosed any relevant, identifying characteristics, such as structure or other physical and/or chemical properties, sufficient to show possession of the claimed genus. Mere idea or function is insufficient for written description; isolation and characterization at a minimum are required. A description of what a material does,

rather than what it is, usually does not suffice. (Eli Lilly, 119 F.3d at 1568, 43 USPQ2d at 1406).

No common structural attributes identify the members of the genus. The general knowledge and level of skill in the art do not supplement the omitted description, because specific, not general guidance is needed.

In the absence of sufficient recitation of distinguishing characteristics, the specification does not provide adequate written description of the claimed genus, which is "a targeting moiety specifically binds Programmed Death-1 ligand (PD-1 ligand)". One of skill in the art would not recognize from the disclosure that the applicant was in possession of the genus. The specification does not clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed (see *Vas-Cath* at page 1116).

Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C. 112 is severable from its enablement provision (see page 1115).

Note

It is noted that PD-1 ligand 1 is also known as B7-H1 and PD-1 ligand 2 is also known as B7-DC.

35 U.S.C. § 103:

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-5, 7-9 and 11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Langermann et al. (US 8,114,845, the '845 patent, filed 25 August 2009) and Sun et al. (US 7,795,389, cited on IDS, reference AC, the '389 patent).

The cited references establish the following fact pattern:

The '845 patent teaches compositions containing antagonists of PD-1 receptors; these antagonists bind to and lock a ligand of PD-1 [column 13, lines 43-46]. PD-1 receptor ligands are identified as B7-H1 (PD-1 ligand 1) and B7-DC (PD-1 ligand 2) [column 2 lines 57-58]. The antagonists may comprise fusion proteins wherein the first polypeptide possesses PD-1 antagonist activity in that it binds to and blocks a ligand of PD-1. The first polypeptide is linked to the second polypeptide portion by an oligopeptide, where said linking amino acids do not substantially decrease the PD-1 antagonist activity of the fusion protein [column 17, lines 18-37]. Among the fusion partners are the hinge and Fc domain of an IgG antibody [column 7, lines 65-67]. The reference teaches that the ligand binding proteins include soluble fragments of the PD-1 receptor protein which can bind to the PD-1 ligands and prevent binding to the endogenous receptor; such fragments also include ECD portion of the PD-1 protein [column 22, lines 1-12]. In disclosing fusion proteins that bind to a PD-1 ligand, the reference teaches that said antagonist fusion protein may comprise at least a portion of an antibody [column 26, lines 47-50]. The reference teaches some specific anti-B7-H1

antibodies which would be useful in the disclosed fusion proteins [column 32, lines 38-48].

Thus, the '845 patent teaches a fusion protein comprising a targeting moiety that specifically binds PD-1 ligand, wherein the moiety specifically binds PD-L1 or PD-L2. The targeting moiety may comprise and antibody or the amino acid sequence of the extracellular domain of PD-1. The fusion protein may also comprise a linker. The fusion proteins increase T cell responses and may be utilized in the treatment of various diseases, including cancer [column 6, lines 22-29].

The '845 patent does not teach an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety wherein the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor II (TGF- βRII), wherein the immunomodulatory moiety comprises the amino acid sequence corresponding to SEQ ID NO:87.

The '389 patent teaches TGF-beta antagonists comprising soluble cell surface type II TGF-beta receptors and fusion proteins comprising said soluble cell surface type II TGF-beta receptors (column 3, lines 26-28, 31-33). These soluble type II receptors comprise the extracellular domain of the TGFβ-RII (see claim 1, for example). Said antagonists may be used to treat cancers (abstract). The reference teaches a sequence, SEQ ID NO:8, which comprises an amino acid sequence which is 100% identical to SEQ ID NO:87 of the instantly claimed invention (see results in SCORE and alignment below).

```
Query Match             100.0%;  Score 764;  DB 3;  Length 538;
  Best Local Similarity   100.0%;
  Matches  137;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps
0;

Qy          1 TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE 60
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         23 TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE 82

Qy         61 VCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
120
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         83 VCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
142

Qy        121 CNDNIIFSEEYNTSNPD 137
```

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                ||||||||||||||||
Db        143 CNDNIIFSEEYNTSNPD 159
```

In view of the facts set forth above, it would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made to combine the prior art teachings of the '845 patent and '389 patent to construct the isolated molecule comprising a targeting moiety which specifically binds to PD-1 ligand (as disclosed by the '845 patent) and an immunomodulatory moiety comprising an amino acid sequence of the extracellular domain of TGFβ-RII of SEQ ID NO:87, as taught by the '389 patent.

One would be motivated to make these modifications and have a reasonable expectation of success as both references disclose fusion proteins comprising said polypeptides as therapeutic agents to treat cancers.

"It is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." *In re Kerkhoven*, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980) (MPEP 2144.06)

One of ordinary skill would be motivated to combine the two therapeutic proteins as a fusion protein, as one would recognize that administration of one therapeutic protein composition would be advantageous as a treatment protocol.

Claims 6 and 10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Langermann et al. (the '845 patent) and Sun et al. (the '389 patent) as applied to claims 1 and 5, further in view of Langermann (USPGPUB 2013/0017199, priority claimed to SN 61/263,983, filed on 24 November 2009).

The teachings of the '845 patent and the '389 patent are set forth in detail above. The references, singularly or in combination, do not teach a molecule wherein the targeting moiety comprises the amino acid sequence corresponding to SEQ ID NO: 93 or a binding fragment thereof (claim 6), wherein the molecule comprises the amino acid sequence set forth in SEQ ID NOs: 87 and SEQ ID NO:93 or a binding fragment thereof (Claim 10).

Langermann (2013) teaches a sequence, SEQ ID NO:45, which is 100% identical to SEQ ID NO:93 of the instant application (See alignment below and results in SCORE). The sequence is identified as the extracellular domain of PD-1 [paragraph 0225].

```
SEQ ID NO 45
;  LENGTH: 150
;  TYPE: PRT
;  ORGANISM: Homo sapien
US-13-511-879-45

  Query Match             100.0%;  Score 804;  DB 10;  Length 150;
  Best Local Similarity   100.0%;
  Matches  150;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps
0;

Qy          1 PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLA 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLA 60

Qy         61 AFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRA
120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRA
120

Qy        121 ELRVTERRAEVPTAHPSPSPRPAGQFQTLV 150
              ||||||||||||||||||||||||||||||
Db        121 ELRVTERRAEVPTAHPSPSPRPAGQFQTLV 150
```

Therefore, it would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made to modify the prior art teachings of the '845 patent and the '389 patent which teach a fusion protein comprising a targeting moiety fused with an immunomodulatory moiety wherein the immunomodulatory moiety comprises SEQ ID NO:87 and substitute the specific sequence of the extracellular domain of PD-1 of SEQ ID NO:45 (which is 100% identical to instantly claimed SEQ ID NO:93), as taught by Langermann (2013) to construct a molecule comprising the amino acid sequence set forth in SEQ ID No:87 and SEQ ID NO:93.

One would be motivated to make these modifications and have a reasonable expectation of success the '845 patent and the '389 patent teach a fusion protein comprising a targeting moiety comprising the ECD of PD-1 and the immunomodulatory

domain of SEQ ID NO:87, and Langermann (2013) teaches a specific sequence of the ECD of PD-1.

Claim 12 is rejected under 35 U.S.C. 103(a) as being unpatentable over the '845 patent and the '389 patent as applied to claim 11 in view of Scaria et al. (US 2011/0052585, priority claimed to SN 60/974,892, filed 25 Sept. 2007, cited on IDS, reference AQ).

The teachings of the '845 patent and the '389 patent are set forth in detail above. The references, singularly or in combination, do not teach a molecule comprising a linker at the CH3 region of the Fc (Claim 12).

Scaria et al teaches fusion proteins comprising a cytokine cell surface receptor and Fc (paragraph 0017). The Fc component comprises the CH3 domain of an IgG (paragraph 0021). The fusion construct may comprise a soluble cytokine receptor fragment linked to the CH3 domain of the Fc via a linker (paragraph 0034 and 0035).

Therefore, it would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made to combine the prior art teachings of '845 patent and the '389 patent. to construct the isolated molecule comprising a targeting moiety which specifically binds to PD-1 ligand (as disclosed by the '845 patent) and an immunomodulatory moiety comprising an amino acid sequence of the extracellular domain of TGFβ-RII of SEQ ID NO:87, as taught by the '389 patent and include a linker at the CH3 region of the Fc, as taught by Scaria et al. One would be motivated to make these modifications and have a reasonable expectation of success as utilization of Fc fusion proteins is taught by the '845 patent and Scaria et al teaches fusion proteins comprising a linker at the CH3 domain of the Fc.

Claims 13 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over the '845 patent and the '389 patent as applied to claim 11 in view of Krauss et al (USPGPUB 2006/0193849).

The teachings of the '845 patent and the '389 patent are set forth in detail above. The references, singularly or in combination, do not teach a molecule wherein the linker is $(GGGGS)_3$.

Krauss et al. teach fusion proteins comprising a linker of $(GGGGS)_3$. The reference teaches that such a linker allows for constraint free pairing of two protein domains [paragraph 0207].

Therefore, it would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made to combine the prior art teachings of '845 patent and the '389 patent to construct the isolated molecule comprising a targeting moiety which specifically binds to PD-1 ligand (as disclosed by the '845 patent) and an immunomodulatory moiety comprising an amino acid sequence of the extracellular domain of TGFβ-RII of SEQ ID NO:87, as taught by the '389 patent and include a linker of $(GGGGS)_3$ as taught by Krauss et al. One would be motivated to make these modifications and have a reasonable expectation of success as Krauss et al. teaches construction of fusion proteins and teaches that such a linker allows for constraint free pairing of two protein domains.

Conclusion:

Claims 1-14 are rejected. Claim 15 is objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SHULAMITH H. SHAFER whose telephone number is (571)272-3332. The examiner can normally be reached on Monday through Friday, 8 AM to 5 PM.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an

interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joanne Hama can be reached on 571-272-2911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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SHULAMITH H. SHAFER
Primary Examiner
Art Unit 1647

/SHULAMITH H. SHAFER/
Primary Examiner, Art Unit 1647

Notice of References Cited	Application/Control No. 15/231,309	Applicant(s)/Patent Under Reexamination BEDI ET AL.	
	Examiner SHULAMITH H. SHAFER	Art Unit 1647	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-8,114,845 B2	02-2012	Langermann; Solomon	A61K31/675	514/21.2
*	B	US-2013/0017199 A1	01-2013	Langermann; Solomon	A61K38/17	424/134.1
*	C	US-2006/0193849 A1	08-2006	Krauss; Juergen	A61K47/4843	424/133.1
	D	US-				
	E	US-				
	F	US-				
	G	US-				
	H	US-				
	I	US-				
	J	US-				
	K	US-				
	L	US-				
	M	US-				

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15231309	BEDI ET AL.
	Examiner	Art Unit
	SHULAMITH H SHAFER	1647

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Inventor name search, PALM, EAST and STN, search history included	2/14/17	SHS
EAST search, search history included	2/14/17	SHS
STN search, search history included	2/14/17	SHS
Review prosecution history of parent applications, SN 13/582,717 and 14/645,282, now US 8,993,524 and 9,441,044	2/14/17	SHS
SEQ searches, see results in SCORE	2/13/17	SHS

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

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(FILE 'HOME' ENTERED AT 14:43:26 ON 14 FEB 2017)

FILE 'MEDLINE, AGRICOLA, BIOSIS, CAPLUS, EMBASE, SCISEARCH' ENTERED AT 14:43:41 ON 14 FEB 2017

L1 180 SEA ABB=ON PLU=ON BEDI ATUL/AU OR RAVI RAJANI/AU

L2 45 SEA ABB=ON PLU=ON L1 AND IMMUNE

L3 21 DUP REM L2 (24 DUPLICATES REMOVED)

DIS L3 1-21 TI SO

L4 7248 SEA ABB=ON PLU=ON "PROGRAMMED DEATH-1 LIGAND" OR "PD-1 LIGAND" OR CD279

L5 97 SEA ABB=ON PLU=ON L4 (P) (CONJUGATE OR FUSION OR HYBRID OR CHIMER?)

L6 57 DUP REM L5 (40 DUPLICATES REMOVED)

L7 23 SEA ABB=ON PLU=ON L4 (S) (CONJUGATE OR FUSION OR HYBRID OR CHIMER?)

L8 19 DUP REM L7 (4 DUPLICATES REMOVED)

DIS L8 1-19 TI SO AU

DIS L6 1-57 TI SO AU

L9 35755 SEA ABB=ON PLU=ON "TRANSFORMING GROWTH FACTOR-BETA RECEPTOR" OR "TGF BETA RECEPTOR"

L10 432 SEA ABB=ON PLU=ON L9 (S) (HYBRID OR CONJUGATE OR CHIMER? OR FUSION)

L11 1 SEA ABB=ON PLU=ON L10 AND L4

DIS L11 IBIB ABS

L12 67 SEA ABB=ON PLU=ON L10 AND (IMMUNE OR IMMUNOMODULATORY)

L13 54 DUP REM L12 (13 DUPLICATES REMOVED)

DIS L13 1-54 TI SO AU

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	
Filing Date	August 8, 2016
First Named Inventor	Atul Bedi
Art Unit	
Examiner Name	
Attorney Docket Number	JHU3260-6

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	AA	US-7,317,091 B2	01-08-2008	Lazar et al.	
	AB	US-7,527,791 B2	05-05-2009	Adams et al.	
	AC	US-7,795,389 B2	09-14-2010	Sun and Hinck	
	AD	US-7,803,378 B2	09-28-2010	Tranchand-Bunel, Denis	
	AE	US-7,820,165 B2	10-26-2010	McKenna et al.	
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	AI	US-2005/0054832 A1	03-10-2005	Lazar et al.	
	AJ	US-2006/0135459 A1	06-22-2006	Epstein and Khawli	
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	AL	US-2007/0212337 A1	09-13-2007	Bedi et al.	
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	AN	US-2008/0075717 A1	03-27-2008	Tranchand-Bunel, Denis	
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	AP	US-2009/0226435 A1	09-10-2009	Khare, Sanjay	
	AQ	US-2011/0052585 A1	03-03-2011	Scaria and White	

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Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] "Number[4] "Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]

Examiner Signature		Date Considered	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 2 of 2

Complete if Known	
Application Number	
Filing Date	August 8, 2016
First Named Inventor	Atul Bedi
Art Unit	
Examiner Name	
Attorney Docket Number	JHU3260-6

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	AR	KWIATKOWSKA et al., "Expression of Soluble Recombinant TGF-β Type II Receptor Fused with the Fc Portion of Human IgG1 (sTβRII-Fc) in NSO Cells," *Acta Biochim. Pol.* (2006), 53(2):361-369.	
	AS	ZHANG et al., "Design and Optimization of a Linker for Fusion Protein Construction," *Prog. Natural Sci.* (2009), 19:1197-1200, Elsevier.	
	AT	ZHANG et al., "Expression of a Soluble TGF-β Receptor by Tumor Cells Enhances Dendritic Cell/Tumor Fusion Vaccine Efficacy," *J. Immunol.* (2008), 181:3690-3697, The American Association of Immunologists, Inc.	
	AU	Supplementary European Search Report from Related International Application No. EP 11 75 1481.	

Examiner Signature	/SHULAMITH H SHAFER/	Date Considered	02/13/2017

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.
[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.
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331323-001150

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	891	"programmed death-1 ligand" or "PD-1 ligand" or CD279	US-PGPUB; USPAT; USOCR; FPRS; JPO; DERWENT	OR	OFF	2017/02/14 13:28
L2	156	l1 same (fusion or chimer? or hybrid or conjugate)	US-PGPUB; USPAT; USOCR; FPRS; JPO; DERWENT	OR	OFF	2017/02/14 13:29
L3	11	l2 and ("transforming growth factor beta receptor" or TGFbetaR)	US-PGPUB; USPAT; USOCR; FPRS; JPO; DERWENT	OR	OFF	2017/02/14 13:30
L4	65	l1 with (fusion or chimer? or hybrid or conjugate)	US-PGPUB; USPAT; USOCR; FPRS; JPO; DERWENT	OR	OFF	2017/02/14 14:12
L5	1461	"transforming growth factor beta receptor" or TGFbetaR	US-PGPUB; USPAT; USOCR; FPRS; JPO; DERWENT	OR	OFF	2017/02/14 14:19
L6	165	l5 same (fusion or conjugate or hybrid or chimer?)	US-PGPUB; USPAT; USOCR; FPRS; JPO; DERWENT	OR	OFF	2017/02/14 14:19
L7	147	l6 and (PD-1 ligand)	US-PGPUB; USPAT; USOCR; FPRS; JPO; DERWENT	OR	OFF	2017/02/14 14:20
L8	48	l6 same(PD-1 ligand)	US-PGPUB; USPAT; USOCR; FPRS; JPO; DERWENT	OR	OFF	2017/02/14 14:20
L9	97	l5 with (fusion or conjugate or hybrid or chimer?)	US-PGPUB; USPAT; USOCR; FPRS; JPO; DERWENT	OR	OFF	2017/02/14 14:32
L10	180	Langermann adj solomon	US-PGPUB; USPAT; USOCR; FPRS; JPO; DERWENT	OR	OFF	2017/02/14 15:09
L11	31	l10 and PD-L1	US-PGPUB; USPAT; USOCR; FPRS; JPO; DERWENT	OR	OFF	2017/02/14 15:10
S1	1	("8993524").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2017/02/13 17:12
S2	1	("9441044").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2017/02/13 17:13
S3	1	("20160340430").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2017/02/13 17:14
S4	1	("8993524").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2017/02/14 11:53
S5	1	("9441044").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2017/02/14 11:54

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CONFIRMATION NO. 3446

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
15/231,309	08/08/2016 RULE	530	1647	JHU3260-6

APPLICANTS
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INVENTORS
Atul Bedi, Timonium, MD;
Rajani Ravi, Ruxton, MD;

**** CONTINUING DATA ***************************
This application is a CON of 14/645,282 03/11/2015 PAT 9441044
which is a CON of 13/582,717 10/17/2012 PAT 8993524
which is a 371 of PCT/US2011/027317 03/04/2011
which claims benefit of 61/435,671 01/24/2011
and claims benefit of 61/311,255 03/05/2010

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED **** ** SMALL ENTITY **
08/16/2016

Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☐ No Verified and Acknowledged /SHULAMITH H SHAFER/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		MD	75	20	1

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UNITED STATES

TITLE
COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Bedi and Ravi	Art Unit:	1647
Application No.:	15/231,309	Examiner:	Shafer, Shulamith H.
Filing Date:	August 8, 2016	Conf. No.	3446
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

Mail Stop AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO NON-FINAL OFFICE ACTION

Dear Sirs:

In response to the non-final Office Action mailed February 22, 2017 in the above-identified patent application, Applicant submits the following amendments and remarks.

Amendments to the Claims begin with the Listing of the claims on page **2** of this paper.

Applicants Arguments and Remarks begin on page **5** of this paper.

AMENDMENTS TO THE CLAIMS

Please amend claims 1, 2 and 12 as proposed below.

Please cancel claim 3 without prejudice or disclaimer.

This listing of claims will replace all prior versions, and listings of claims in the application.

Listing of the Claims

1. (Currently Amended) An isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:

(a) the targeting moiety is a polypeptide that specifically binds Programmed Death-1 ligand (PD-1 ligand); and

(b) the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor (TGFβR).

2. (Currently Amended) The molecule of claim 1, wherein the ~~targeting moiety~~ polypeptide comprises an antibody, antibody fragment, antigen-binding domain of an immunoglobulin, single chain variable fragment (scFv), Fc-containing polypeptide, or fusion protein that specifically binds PD-1 ligand or a fragment thereof.

3. (Canceled)

4. (Original) The molecule of claim 3, wherein the molecule comprises the amino acid sequence corresponding to the heavy chain or light chain of an antibody that specifically binds PD-L1.

5. (Original) The molecule of claim 1, wherein the targeting moiety comprises an amino acid sequence of the extracellular domain of PD-1.

6. (Original) The molecule of claim 5, wherein the targeting moiety comprises the amino acid sequence corresponding to SEQ ID NO: 93 or a binding fragment thereof.

7. (Original) The molecule of claim 1, wherein the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor selected from the group consisting of SEQ ID NO: 79-91.

8. (Original) The molecule of claim 1, wherein the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor II (TGFβRII) or a binding fragment thereof.

9. (Original) The molecule of claim 7, wherein the immunomodulatory moiety comprises the amino acid sequence corresponding to SEQ ID NO: 87 or a binding fragment thereof.

10. (Original) The molecule of claim 1, wherein the molecule comprises the amino acid sequence set forth in SEQ ID NOs: 87 and SEQ ID NO:93 or a binding fragment thereof.

11. (Original) The isolated molecule of claim 1, wherein the targeting moiety is fused to the immunomodulatory moiety via a linker.

12. (Currently Amended) The isolated molecule of claim 11, wherein the targeting moiety is an Fc-containing polypeptide ~~further~~ comprising a linker at the CH3 region of the Fc.

13. (Original) The isolated molecule of claim 11, wherein the linker is (GGGGS)n and wherein n is 1, 2, 3, 4, 5, 6, 7 or 8.

14. (Original) The isolated molecule of claim 13, wherein the linker comprises the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO:104).

15. (Original) The molecule of claim 10, wherein the molecule comprises the amino acid sequence set forth in SEQ ID NOs: 11 or SEQ ID NO:12 or a binding fragment thereof.

16. (Withdrawn) A method of treating a neoplastic disease or cancer comprising: administration to a subject in need thereof a molecule of claim 1.

17. (Withdrawn) The method of claim 16, further comprising administering an additional cancer therapy.

18. (Withdrawn) The method of claim 17, wherein the cancer therapy is selected from the group consisting of a chemotherapeutic molecule, immunotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent, ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, and radiofrequency ablation.

19. (Withdrawn) The method of claim 16, further comprising administration in combination with a vaccine.

20. (Withdrawn) The method of claim 16, wherein the subject is human.

REMARKS

Claims 1-20 were pending prior to this Response with claims 16-20 having been withdrawn from consideration. By the present communication, claims 1, 2 and 12 have been amended and claim 3 has been canceled without prejudice or disclaimer. Support for the amendments can be found generally throughout the specification and claims as originally filed. No new matter has been added. After entry of the amendments, claims 1-20 will be pending and under consideration.

Rejections under 35 USC § 112, second paragraph

Claim 12 stands rejected under 35 USC § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. Applicants respectfully traverse this rejection.

The Examiner states that the phrase "further comprising a linker at the CH3 domain region of the Fc" in claim 12 lacks sufficient antecedent basis.

Applicants have amended claim 12 to provide antecedent basis for the phrase. Applicants submit that these amendments render the rejection moot and respectfully request withdrawal of the rejection.

Rejections under 35 USC § 112, first paragraph

Claims 1, 2, 7, 8 and 11-14 stand rejected under 35 USC § 112, first paragraph for allegedly failing to comply with the written description requirement. Applicants respectfully traverse this rejection.

The Examiner states that the specification discloses that ""targeting moiety" refers to a molecule that has the ability to localize and bind to a specific molecule or cellular component" and that the specification further discloses that the targeting moiety can be an antibody or a fragment of an antibody. The Examiner then states that the specification discloses that the targeting moiety includes an antibody, antibody fragment or peptide that specifically binds to PD-1, PDL1 or PD-L2. However, the Examiner states that the specification only reduces to

practice two species, specifically, SEQ ID NO: 11 (fusion protein comprising PD-1 ectodomain Fc and TGFβRII extracellular domain) and SEQ ID NO: 12 (a fusion protein comprising TGFBRII extracellular domain Fc and PD-1 ectodomain). Additionally, the Examiner states that there are no structural formulas disclosed in the specification of any targeting moieties that bind PD-L1 and there is no limitation placed on the structure or any chemical limitation on a targeting moiety that binds PD-L1. The Examiner concludes that the specification provides adequate written description support for the presently claimed genus.

Applicants respectfully disagree. M.P.E.P.§ 2163 provides guidance for evaluating compliance with the written description requirement. As is true when conducting any written description analysis, the "requirement must be applied in the context of the particular invention and state of the knowledge...As each field evolves between what is known and what is added by each inventive contribution." M.P.E.P. 2163; Capon v. Eshhar, 418F.3d 1349, 1357 (Fed. Cir. 2005).

Case law and the M.P.E.P. acknowledge that analysis of compliance with the written description requirement is a fact-based inquiry. Further, "[w]hat constitutes a 'representative number' is an inverse function of the skill and knowledge in the art. Satisfactory disclosure of a 'representative number' depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed." The M.P.E.P. details numerous cases in which the disclosure of a single species was held to be sufficient to support a claim directed to a genus. See In re Rasmussen, 650 F.2d 1212 (CCPA 1981); In re Herschler, 591 F.2d 696, 697 (CCPA 1979); In re Smythe, 480 F.2d 1376 (CCPA 1973).

Fortunately, the Federal Circuit has repeatedly and consistently stated the test to be applied when determining if the written description requirement has been met. Since its inception, the Court of Appeals for the Federal Circuit has frequently addressed the "written description" requirement of § 112. A fairly uniform standard for determining compliance with the "written description" requirement has been maintained throughout. "Although [the

applicant] does not have to describe exactly the subject matter claimed, ... the description must clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed. Vas-Cath, Inc. v. Mahurkar 935 F2d. 1555, 1562-1563 (Fed. Cir. 1991) citations omitted.

The Court has further stated that "[w]hen the original specification accomplishes that, regardless of how it accomplishes it, the essential goal of the description requirements is realized." In re Wright, 866 F.2d 422, 424 (Fed. Cir. 1989) emphasis in original.

In assessing whether skilled artisans would have recognized that Applicant was in possession of the claimed invention, the disclosure of the specification must be interpreted against the knowledge and the level of skill in the art at the time of filing. The C.C.P.A. has stated that "[n]ot every last detail is to be described, else patent specifications would turn into production specifications, which they were never intended to be." In re Gay, 309 F.2d 769, 135 USPQ 311, 316 (C.C.P.A. 1962). Thus, the specification need only describe the claimed subject matter to one of ordinary skill in the art.

Moreover, the Federal Circuit has held with regard to the written description requirement:

> A claim will not be invalidated on section 112 grounds simply because the embodiments of the specification do not contain examples explicitly covering the full scope of the claim language. That is because the patent specification is written for a person of skill in the art, and such a person comes to the patent with the knowledge of what has come before. Placed in that context, it is unnecessary to spell out every detail of the invention in the specification; only enough must be included to convince a person of skill in the art that the inventor possessed the invention and to enable such a person to make and use the invention without undue experimentation. [79 USPQ2d at 1007 (quoting LizardTech, Inc., v. Earth Res. Mapping, Inc., 424 F.3d 1336, 1345, 76 USPQ2d 1724 (Fed. Cir. 2005))].

The specification need only describe the invention to one of ordinary skill in the art and not the general public. Applicants submit that the specification clearly sets forth and defines to the skilled artisan the presently claimed subject matter.

The application provides for targeted immunomodulatory antibodies and fusion proteins that counter act or reverse immune tolerance (see paragraphs [0006] and [0102]). The application also provides numerous examples of such fusion proteins including a fusion protein comprised of a PD-L1 targeting moiety and an extracellular domain of TGFBR as an immunomodulatory moiety (see at least paragraphs [0007], [0010], [0011], [0021], [0025], [0110], [0111], [0114], [0116], [0117], [0118], [0122], [0134], [0165], [0168], [0170], [0172], [0173], [0176], [0178] and [0180]). Additionally, the application provides:

> "In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide or polypeptide that specifically binds a component of a regulatory T cell, myeloid suppressor cell or dendritic cells" paragraph [0170].

Paragraph [0170] further recites examples of molecules that the targeting moiety specifically binds, including PD-L1.

As such, the application provides written description support for the targeting moiety to be an anti-PD-L1 antibody or antibody fragment or a polypeptide which binds PD-L1. As noted by the Examiner, the application provides specific support for the targeting moiety to be the ectodomain the PD-1. Applicants submit that methods of producing antibodies and antibody fragments are well known in the art and considered routine by one of skill in the art. Additionally Applicants submit, that antibodies specific for PD-L1 are known in the art and commercially available (see Appendix B). Therefore, the application and general knowledge in the art provide specific support for the targeting moiety being anti-PD-L1 antibody or antibody fragment or a polypeptide which binds PD-L1.

Further, Applicants have provided a declaration by Dr. Atul Bedi with this Response in which provides specific examples of the claimed molecules and data demonstrating the efficacy of the molecules. Specifically, the declaration describes two molecules, an anti-PD-L1-TGFβRII and a TGFβRII*ecd*-Fc-PD1*ecd* and demonstrates that both molecules were efficacious at simultaneously binding and inhibiting the activity of both PD-L1 and TGFβ.

Applicants submit that the application does not have to reduce to practice every example of a genus. Applicants further submit that the application as filed and general knowledge in the art provide sufficient written description support for the presently claimed invention. Applicants respectfully request reconsideration and withdrawal of the rejection.

Rejections under 35 USC § 103(a)

Claims 1-5, 7-9 and 11 stand rejected under 35 USC § 103(a) as allegedly being unpatentable over Langermann et al. (U.S. Patent No. 8,114,845; hereafter "Langermann") and Sun et al. (U.S. Patent No. 7,795,389; hereafter "Sun"). Applicants respectfully traverse this objection.

The U.S. Supreme Court decision in KSR International v. Teleflex Inc. modified the standard for establishing a prima facie case of obviousness. KSR, 550 US 398, 82 USPQ 2d 1385 (2007). Under the KSR rule, three basic criteria are considered. First, some suggestion or motivation to modify a reference or to combine the teachings of multiple references still has to be shown. Second, the combination has to suggest a reasonable expectation of success. Third, the prior art reference or combination has to teach or suggest all of the recited claim limitations. Factors such as the general state of the art and common sense may be considered when determining the feasibility of modifying and/or combining references.

The 2010 KSR Guidelines Update provides additional guidance in view of decisions by the United States Court of Appeals for the Federal Circuit (Federal Circuit) since KSR. However, familiar lines of argument still apply, including teaching away from the claimed invention by the prior art, lack of a reasonable expectation of success, and unexpected results. Indeed, such arguments may have even taken on added importance in view of the recognition in KSR of a variety of possible rationales. See Federal Register, Vol. 75, No. 169, September 1, 2010, page 53645. Applicants respectfully submit that the Office has not met the burden of establishing a prima facie case of obviousness for the reasons discussed below.

The Examiner states that Langermann discloses a fusion protein that specifically binds to a PD-1 ligand which can be PD-L1 or PD-L2; that the fusion protein may comprise an antibody or the

amino acid sequence of the extracellular domain of PD-1; that the fusion protein may comprise a linker and that the fusion protein increases T cell response and therefore may be used to treat diseases such as cancer. The Examiner states that Langermann does not disclose a targeting moiety fused to an immunomodulatory moiety comprised of the extracellular domain of TGFβRII. The Examiner states that Sun discloses fusion proteins comprising soluble cell surface type II TGF-β receptors and that these fusion proteins can be used to treat cancer. The Examiner concludes that it would be obvious to combine the disclosures of the Langermann and Sun to arrive at the presently claimed invention.

Applicants respectfully disagree. Langermann discloses targeting moieties which bind PD-L1. Sun teaches TGF-β antagonists comprising soluble cell surface type II TGF-β receptors and fusion proteins comprising said soluble cell surface type II TGF-β receptors. However, neither Langermann nor Sun disclose or suggest a molecule comprising a PD-1 ligand targeting moiety fused with an immunomodulatory moiety where the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of transforming growth factor-beta receptor II as is presently claimed.

Applicants submit that the presently claimed molecule has unexpected and surprising properties. As provided in the declaration by Dr. Atul Bedi submitted herewith, the presently claimed molecule targets PD-L1 on a tumor cell or tumor infiltrating T cell and simultaneously traps and disables TGF-β within the localized microenvironment of the targeted cell, thereby enabling activation of ADCC (antibody-dependent cell cytotoxicity) and/or adaptive antitumor immune responses. The ability to sequester and disable PD-L1 and TGF-β in the immediate microenvironment of the targeted tumor cell or T cell enables interruption of ligand driven autocrine/paracrine communication that promotes tumor induced immune tolerance, tumor metastases and treatment resistance.

The present invention is directed to an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety is a polypeptide that specifically binds Programmed Death-1 ligand (PD-1 ligand); and the immunomodulatory moiety

comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor (TGFβR). The presently claimed molecule counteracts tumor-induced immune tolerance and activates antitumor immune responses by targeting PD-L1 and simultaneously binding and disabling TGFβ within the localized targeted cell microenvironment. As described in the declaration, two examples of the presently claimed molecule were produced (anti-PD-L1-TGFβRII and TGFβRII*ecd*-Fc-PD1*ecd*).

As described in the declaration, the α-PD-L1-TGFβRII molecule was examined to characterize its bifunctional target-binding ability. Appendix A, Figure 2 shows that the α-PD-L1-TGFβRII molecule simultaneously binds PD-L1 and TGF-β. Additionally, the anti-tumor efficacy of the molecule was evaluated in mice bearing MDA-MB-231-Luc breast cancer. As shown in Appendix A, Figures 3B-C, treatment of tumor-bearing mice with α-PD-L1-TGFβRII was significantly more effective at inhibiting tumor growth compared to treatment with either α-PD-L1 alone, non-specific IgG-TGFβRII alone, or even the combined administration of α-PD-L1 and non-specific IgG-TGFβRII. Most significantly, these data show the surprising result that the a-PD-L1-TGFβRII molecule has significantly superior antitumor efficacy compared to combined administration of α-PD-L1 with a non-specific IgG-TGFβRII which is not targeted to PD-L1-expressing tumor or tumor infiltrating T cells (Appendix A, Figure 3B,C).

Further, as described in the declaration, the TGFβRII*ecd*-Fc-PD1*ecd* molecule was examined to characterize its bifunctional binding ability. Appendix A, Figure 4 shows that the TGFβRII*ecd*-Fc-PD1*ecd* molecule binds both PD-L1 and TGF-β. Additionally, the anti-tumor efficacy of the molecule was evaluated in mice bearing MDA-MB-231-Luc breast cancer. As shown in Appendix A, Figures 5A-C, treatment of tumor-bearing mice with the TGFβRII*ecd*-Fc-PD1*ecd* molecule was significantly more effective at inhibiting tumor growth compared with IgG-PD1*ecd* alone or the combined administration of IgG-PD1*ecd* and TGFβRII*ecd*-Fc. Moreover, treatment with the TGFβRII*ecd*-Fc-PD1*ecd* molecule was effective at inhibiting lung metastases of MDA-MB-231-luc tumors, whereas either IgG-PD1*ecd* alone or the combination of IgG-PD1*ecd* and TGFβRII*ecd*-Fc failed to inhibit lung metastases. Most significantly, these data show the surprising

result that the TGFβRIIecd-Fc-PD1*ecd* molecule was significantly superior at inhibiting tumor growth and lung metastases compared to combined administration of IgG-PD1*ecd* with TGFβRII*ecd*-Fc which is not targeted to PD-L1-expressing tumor or tumor infiltrating T cells (Appendix A, Figures 5A-D).

These data demonstrate that the presently claimed molecule has unexpected and surprising properties and demonstrates that the presence of both a PD-L1 antagonist and the ECD of TGFβR on the same molecule is critical for anti-tumor efficacy while maintaining a clinically relevant therapeutic index. The significantly superior anti-tumor efficacy of the presently claimed molecule is crucially dependent on the physical linkage of the PD-L1-targeting moiety with the ligand-binding sequence of TGFβRII*ecd*, which endows it with the unique ability to block PD-L1/PD-1 interaction and efficiently counteract autocrine and paracrine TGF-β signaling by localizing the TGFβRII*ecd* to the immediate microenvironment of PD-L1-expressing tumor and tumor-infiltrating immune cells.

Applicants submit that based on the disclosure in Langermann and Sun, one of skill in the art would have no motivation to combine a PD-L1 antagonist and the ECD of TGFβR in a single molecule. Further, Applicants submit that one of skill in the art would not have predicted the surprising and unexpected properties of the presently claimed molecule based on the disclosure in Langermann and Sun. Accordingly, applicants submit that Langermann and Sun does not disclose or suggest the presently claimed invention. Therefore, Applicants respectfully request reconsideration and withdrawal of the rejection.

Claims 6 and 10 stand rejected under 35 USC § 103(a) as allegedly being unpatentable over Langermann et al. (U.S. Patent No. 8,114,845; hereafter "Langermann") and Sun et al. (U.S. Patent No. 7,795,389; hereafter "Sun") in further view of Langermann (U.S. Patent Appl. Pub. No. 2013/0017199; hereafter "Langermann 2"). Applicants respectfully traverse this objection.

The Examiner states that the Langermann and Sun patents apply as previously described. The Examiner states that Langermann 2 discloses SEQ ID NO: 45 which is 100% identical to SEQ ID NO: 93 of the presently claimed invention.

Applicants respectfully disagree. As discuss above, neither Langermann nor Sun disclose or suggest the instant invention. The Examiner relies on Langermann 2 to cure the deficiencies of Langermann and Sun. Langermann 2 is directed to compositions for increasing IFNγ producing cells and decreasing the number of regulatory T cells at the tumor site. Langermann 2 also discloses the sequence of the extracellular domain of PD-1. However, Langermann 2 does not disclose or suggest an isolated molecule comprising an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety is a polypeptide that specifically binds Programmed Death-1 ligand (PD-1 ligand); and the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor (TGFβR) as is presently claimed. As such Langermann 2 fails to cure the deficiencies of Langermann and Sun.

Applicants submit that none of Langermann, Sun or Langermann 2 disclose or suggest the present invention and that one of skill in the art would not arrive at the instant invention based on the disclosure of Langermann, Sun or Langermann 2, either alone or in combination. Therefore, Applicants respectfully request reconsideration and withdrawal of the rejection.

Claim 12 stands rejected under 35 USC § 103(a) as allegedly being unpatentable over Langermann et al. (U.S. Patent No. 8,114,845; hereafter "Langermann") and Sun et al. (U.S. Patent No. 7,795,389; hereafter "Sun") in view of Scaria (U.S. Patent Appl. Pub. No. 2011/0052585; hereafter "Scaria"). Applicants respectfully traverse this objection.

The Examiner states that the Langermann and Sun patents apply as previously described. The Examiner states that the references do not disclose a molecule comprising a linker at the CH3 region of the Fc. The Examiner states that Scaria discloses fusion proteins comprising the CH3

domain of an IgG and that the fusion protein may comprise a soluble cytokine receptor fragment linked via linker to the CH3 domain of the Fc.

Applicants respectfully disagree. As discuss above, neither Langermann nor Sun disclose or suggest the instant invention. The Examiner relies on Scaria to cure the deficiencies of Langermann and Sun. Scaria discloses a delivery method for IL-17 and IL-23 receptor constructs or proteins expressed from such constructs (see Scaria paragraph [0018]). Scaria additionally discloses that the interleukin receptor can be linked to a multimerization domain via linker including Gly9 (GGGGGGGGG) (see Scaria paragraph [0115]). Scaria does not disclose or suggest an isolated molecule comprising an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety is a polypeptide that specifically binds Programmed Death-1 ligand (PD-1 ligand); and the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor (TGFβR) as is presently claimed. As such Scaria fails to cure the deficiencies of Langermann and Sun.

Applicants submit that none of Langermann, Sun or Scaria disclose or suggest the present invention and that one of skill in the art would not arrive at the instant invention based on the disclosure of Langermann, Sun or Scaria, either alone or in combination. Therefore, Applicants respectfully request reconsideration and withdrawal of the rejection.

Claims 13 and 14 stand rejected under 35 USC § 103(a) as allegedly being unpatentable over Langermann et al. (U.S. Patent No. 8,114,845; hereafter "Langermann") and Sun et al. (U.S. Patent No. 7,795,389; hereafter "Sun") in view of Krauss et al. (U.S. Patent Appl. Pub. No. 2006/0193849; hereafter "Krauss"). Applicants respectfully traverse this objection.

The Examiner states that the Langermann and Sun patents apply as previously described. The Examiner states that Krauss discloses fusion proteins comprising a linker of $(GGGGS)_3$.

Applicants respectfully disagree. As discuss above, neither Langermann nor Sun disclose or suggest the instant invention. The Examiner relies on Krauss to cure the deficiencies of Langermann and Sun. Krauss is directed to antibody engineering modifications to improve the

stability of whole scFv molecules. Krauss also discloses a linker comprised of $(GGGGS)_3$. However, Krauss does not disclose or suggest an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety is a polypeptide that specifically binds Programmed Death-1 ligand (PD-1 ligand); and the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor (TGFβR) as is presently claimed. As such Krauss fails to cure the deficiencies of Langermann and Sun.

Applicants submit that none of Langermann, Sun or Krauss disclose or suggest the present invention and that one of skill in the art would not arrive at the instant invention based on the disclosure of Langermann, Sun or Krauss, either alone or in combination. Therefore, Applicants respectfully request reconsideration and withdrawal of the rejection.

CONCLUSION

Applicant provides that the claims are ready for examination. The Examiner is invited to contact Applicant's undersigned representative if there are any questions relating to this application.

The Commissioner is authorized to charge the total amount of $300.00 for a Two Month Extension of Time fee, small entity, to Deposit Account No. 07-1896. No additional fees are believed to be due with the filing of this paper. However, the Commissioner is hereby authorized to charge any fees associated with the filing submitted herewith, or credit any overpayment to Deposit Account No. 07-1896 referencing the above-identified attorney docket number.

Respectfully submitted,

Date: July 21, 2017

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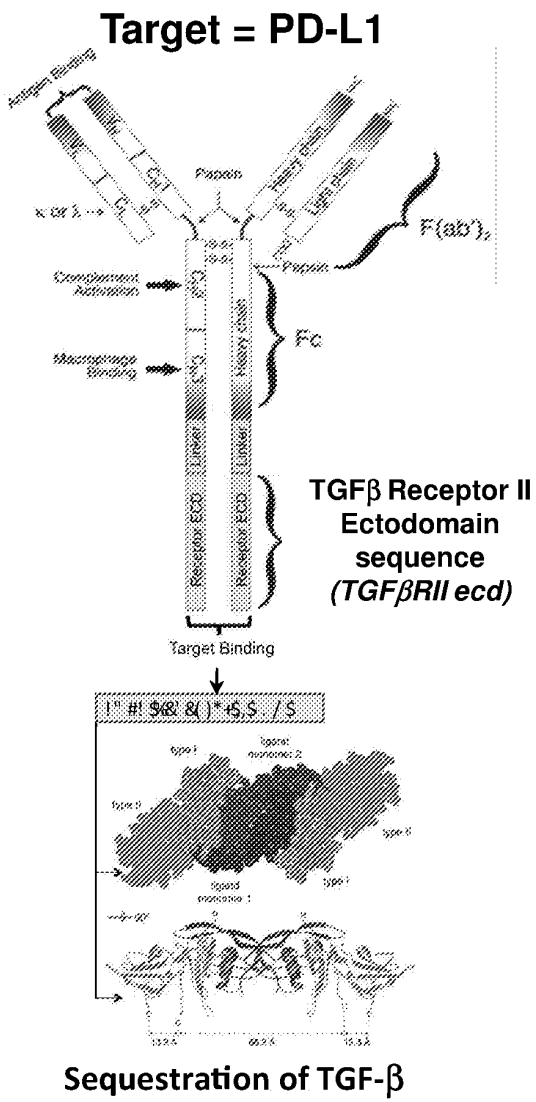
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Appendix A

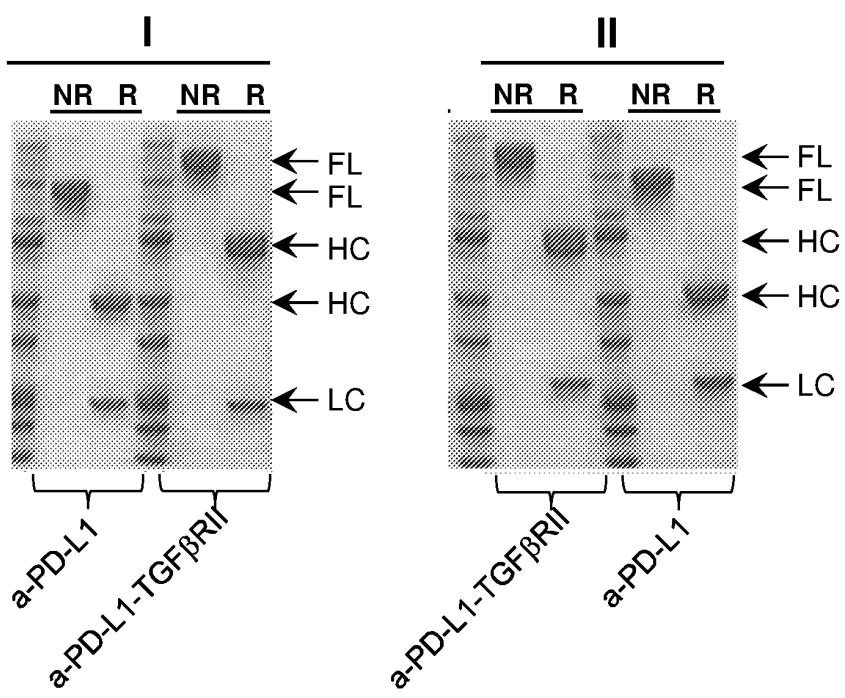
Appendix A

FIGURE 1

A. Anti-PD-L1-TGFβRII



B.



C.

a-PD-L1-TGFβRII SEC-HPLC Analysis

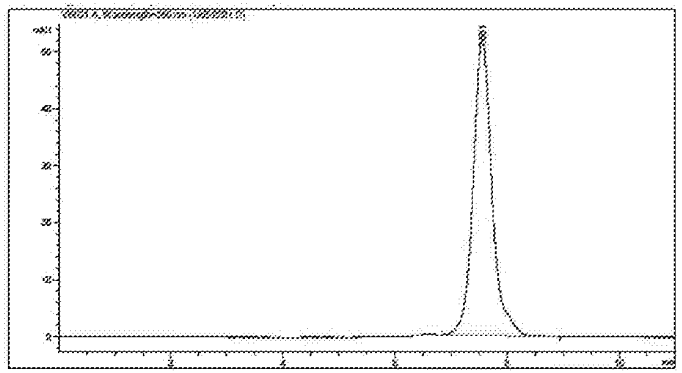
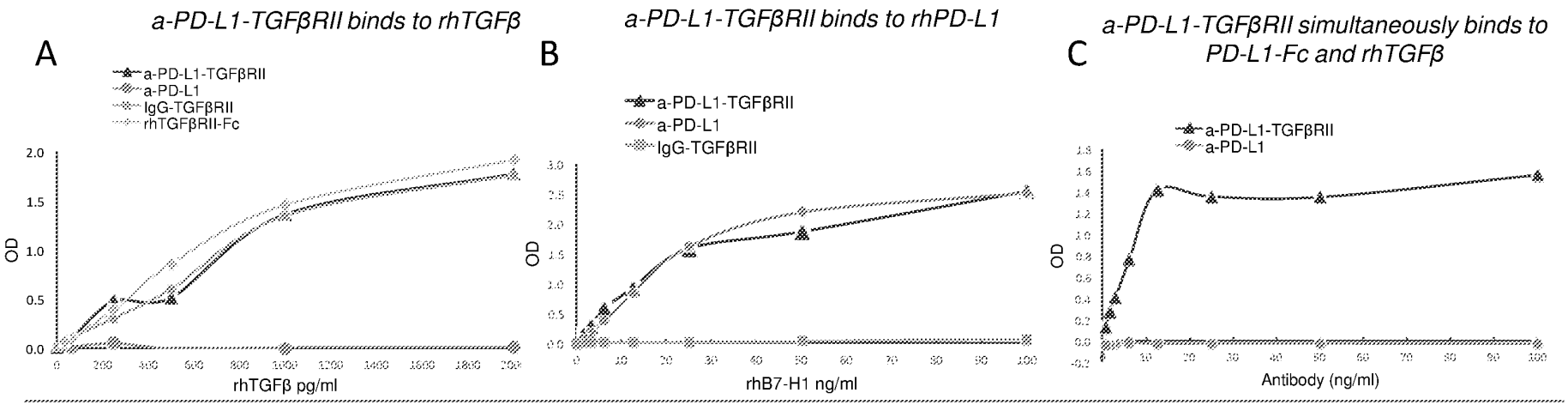
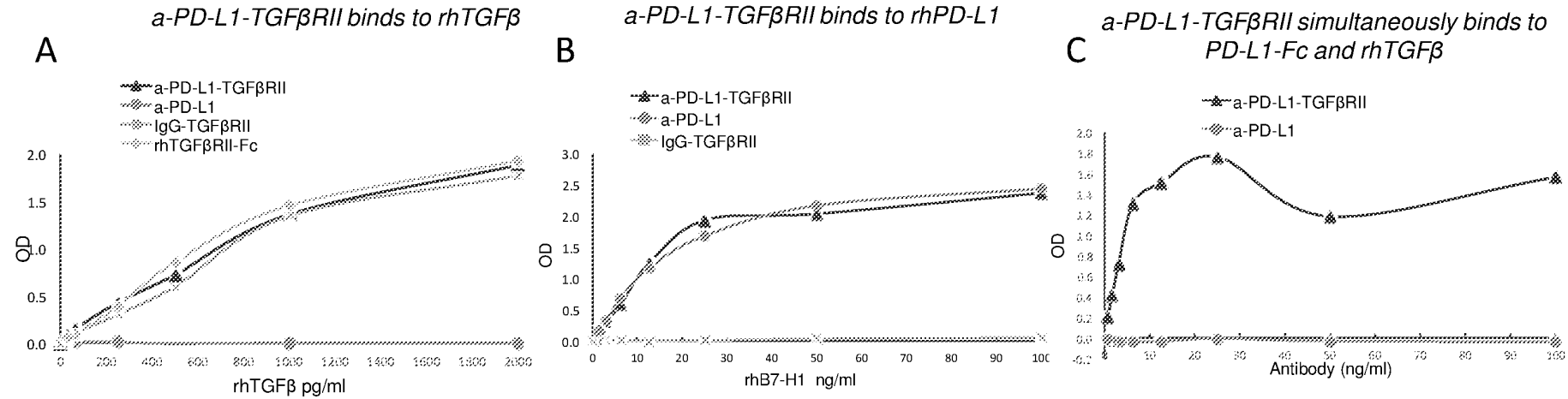


FIGURE 2

I. *Anti-PDL1-TGFβRII*

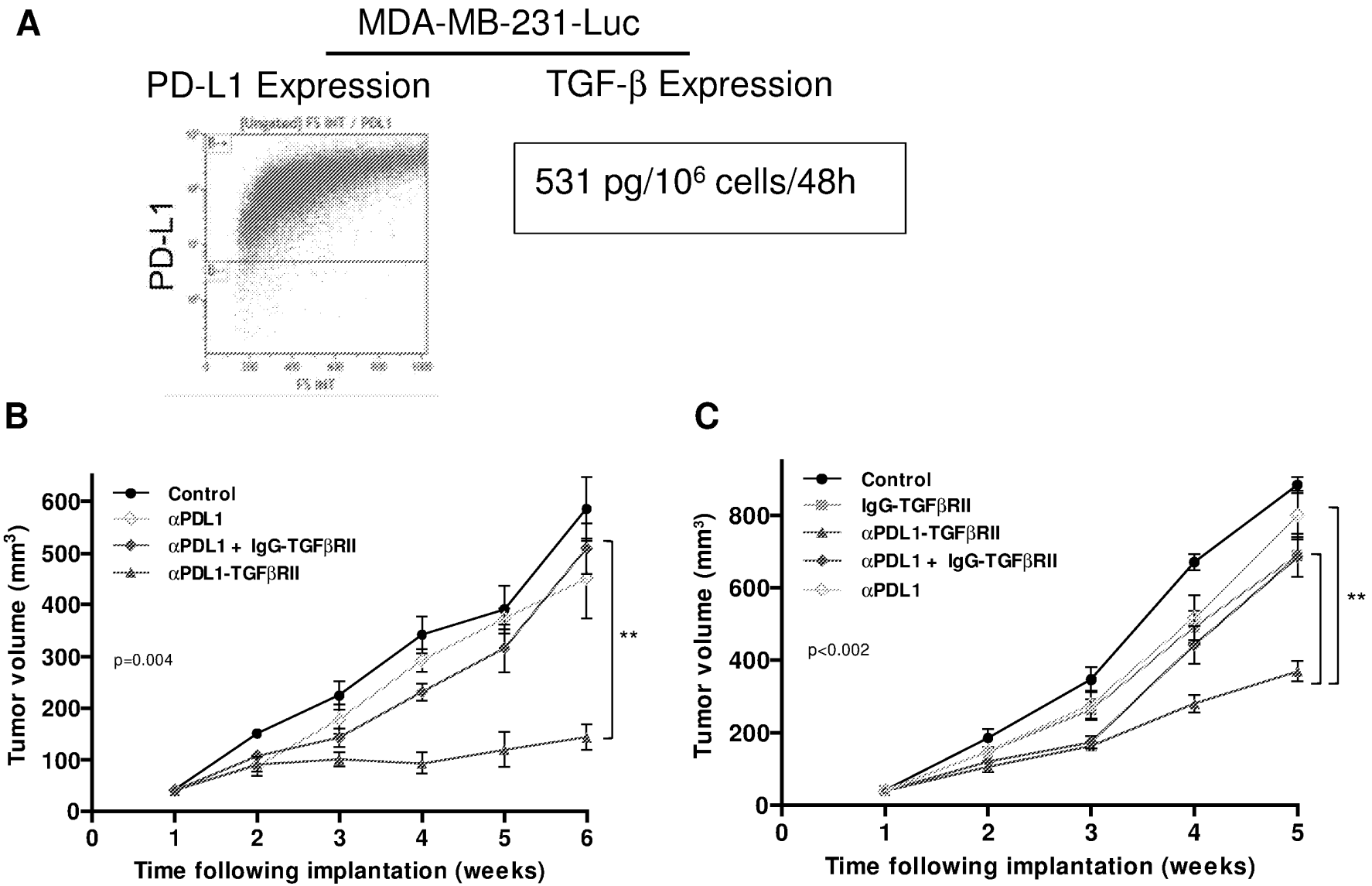


II. *Anti-PDL1-TGFβRII*



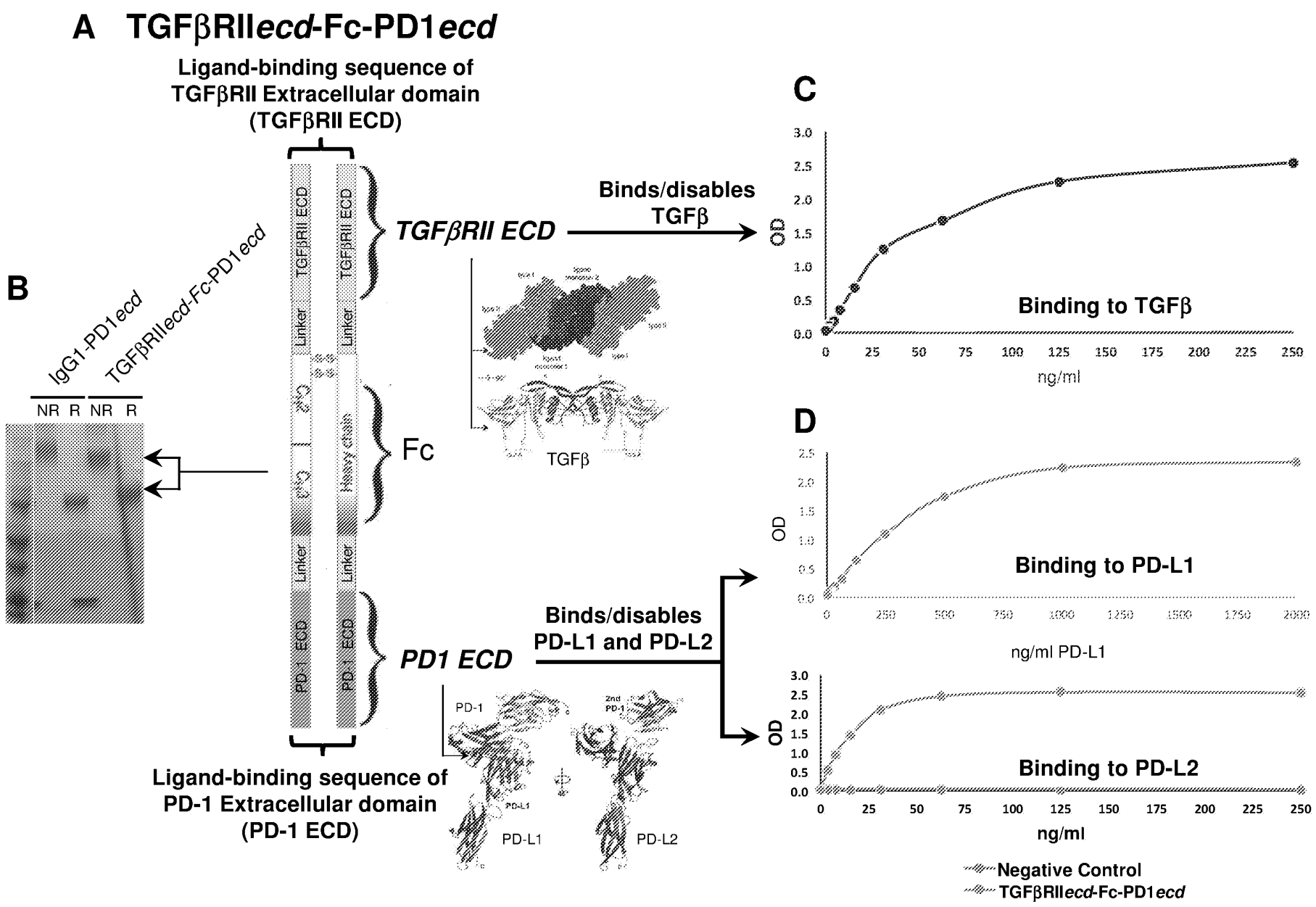
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FIGURE 3



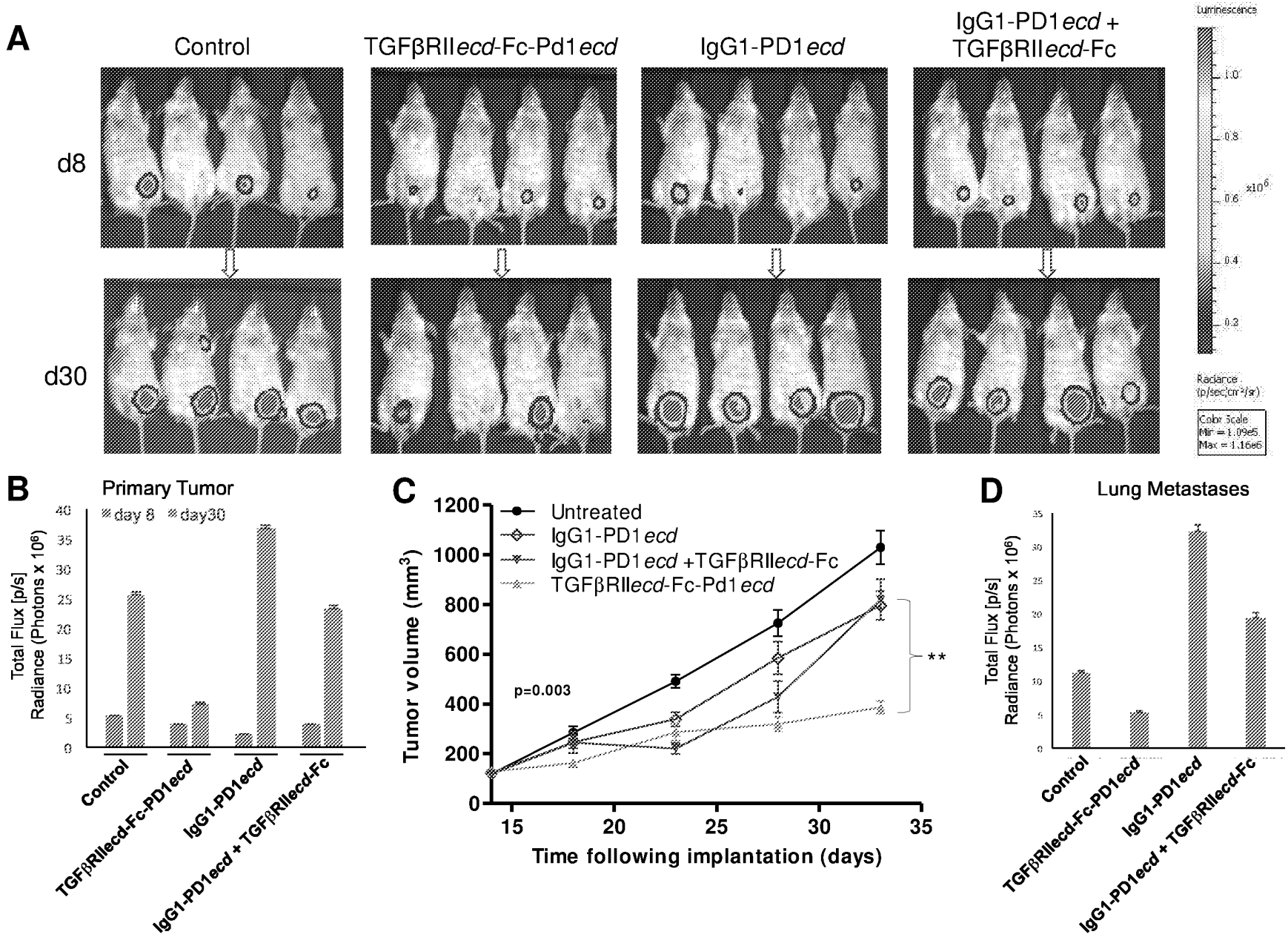
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FIGURE 4



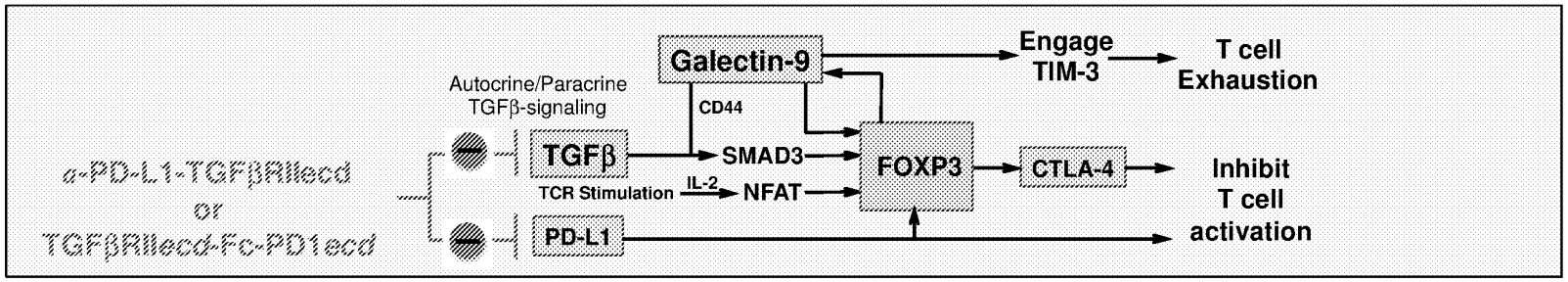
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FIGURE 5



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FIGURE 6



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Appendix B

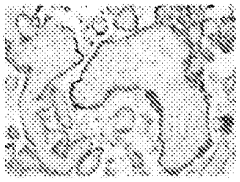
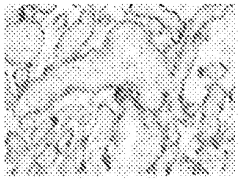
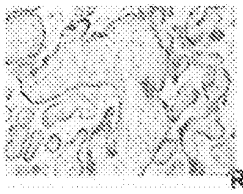
Appendix B

PD-L1

The immune system employs a series of checkpoints to protect normal, healthy tissue from an immune response. These consist of receptors on the surface of activated T cells and their corresponding ligands on the surface of antigen presenting cells. A key immune checkpoint is triggered when PD-1 (programmed cell death protein 1) engages its ligand PD-L1. As a result of this interaction, T cell activation is attenuated and an active immune response is prevented[1].

This mechanism is often co-opted by tumors. PD-L1 is upregulated in several tumor types and contributes to the malignancy of these cancers by interacting with PD-1 (/browse/?Ntk=Products&N=4294956287&Ntt=pd+1) and inhibiting T cell activation. In this way, the tumors avoid detection and destruction by the immune system[1-3]. Accordingly, PD-1 and PD-L1 have garnered much attention for their roles in tumor immunology and as immune-based therapeutic targets[4,5].

CST PD-L1 product comparison for IHC-P analysis

Product Name	PD-L1 (E1L3N®) XP® Rabbit mAb #13684 (/product/productDetail.jsp?productId=13684)	PD-L1 (405.9A11) Mouse mAb #29122 (/product/productDetail.jsp?productId=29122)	PD-L1 (Extracellular Domain Specific) (E1J2J) Rabbit mAb #15165 (/product/productDetail.jsp?productId=15165)
Clone	E1L3N®	405.9A11	E1J2J
Epitope	Intracellular	Intracellular	Extracellular
Antibody host species	Rabbit	Mouse	Rabbit
IHC analysis of paraffin-embedded human placenta			
Recommended usage for IHC	Preferred clone based on sensitivity	Use when multiplexing with rabbit antibodies	Use for studying the extracellular domain of PD-L1

PD-L1 (E1L3N®) XP® Rabbit mAb (/product/productDetail.jsp?productId=13684)

PD-L1 (Extracellular Domain Specific) (E1J2J) Rabbit mAb (/product/productDetail.jsp?productId=15165)

PD-L1 (405.9A11) Mouse mAb (/product/productDetail.jsp?productId=29122)

PD-L1 Conjugates (/browse/antibody-conjugates?N=4294956287+102260&Ntk=Products&N

Additional PD-L1 Products (/browse/?Ntk=Products&N=4294956287&Ntt=pd+l1

PD-L1 IHC Controls (/product/productDetail.jsp?productId=13747)

PD-L1 (E1L3N®) Antibody - Target Specificity

Expand

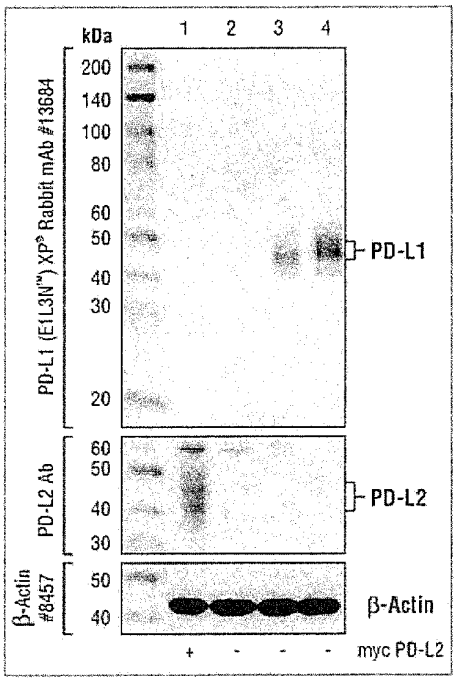
{}

Recognizes PD-L1 and does not cross-react with other B7 family members

Request Your Free Tumor Immunology Poster



Technical Support



Western blot analysis of lysates from:

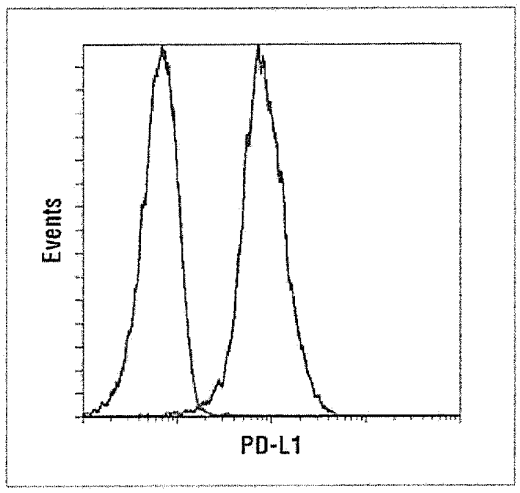
1. COS cells transfected with PD-L2
2. COS cells mock transfected
3. KARPAS-299 cells
4. SUP-M2 cells

Our scientists are at the bench daily to produce and validate our antibodies, so they have hands-on experience and knowledge of each antibody's performance.

PD-L1 (E1L3N®) Antibody - Flow Validation

Expand

Demonstrates consistent, reliable results in immunohistochemistry, flow cytometry, western blotting and immunoprecipitation.

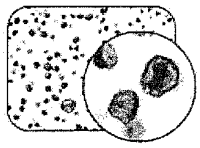
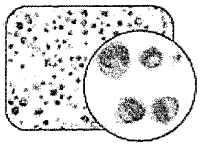
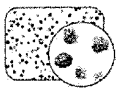
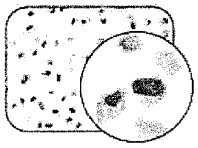
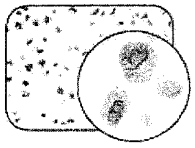
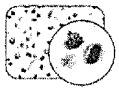


Flow cytometric analysis of untreated SUP-M2 cells using **PDL1 (E1L3N®) XP® Rabbit mAb #13864 (/product/productDetail.jsp?productId=13684)** (blue) compared to concentration matched **Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (/product/productDetail.jsp?productId=3900)** (red). **Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 647 Conjugate) #4414 (/product/productDetail.jsp?productId=4414)** was used as a secondary antibody.

PD-L1 (E1L3N®) Antibody Comparative Data: IHC

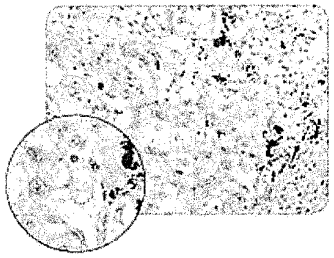
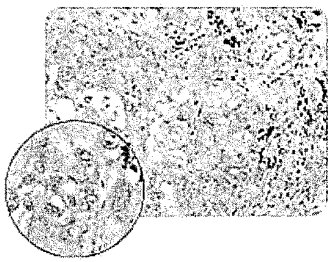
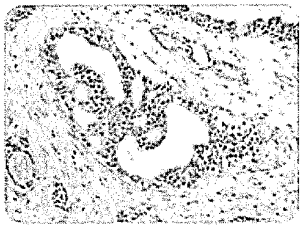
Expand

KARPAS-299 and PC-3 Cell Staining

	CST PD-L1 (E1L3N®) XP® Rabbit mAb #13684 1:200	Company 1 Rabbit mAb 1:8000	Company 2 Mouse mAb 1:50
KARPAS-299			
	Strong, membrane associated staining	Weaker membrane staining than **#13684 (/products/primary-antibodies/13684)**	No membrane staining
PC-3			
	No staining	Diffuse, Non-specific staining	Diffuse, Non-specific staining

IHC analysis demonstrates that CST **PD-L1 (E1L3N®) XP® Rabbit mAb #13684 (/products/primary-antibodies/13684)** at 1:200 gives a strong signal in KARPAS-299, which are known to be PD-L1 high expressing cells, and no staining in PC-3 cells. In contrast, Company 1 Rabbit mAb and Company 2 Mouse mAb stain both KARPAS-299 and PC-3 cells meaning that they are not specific for PD-L1.

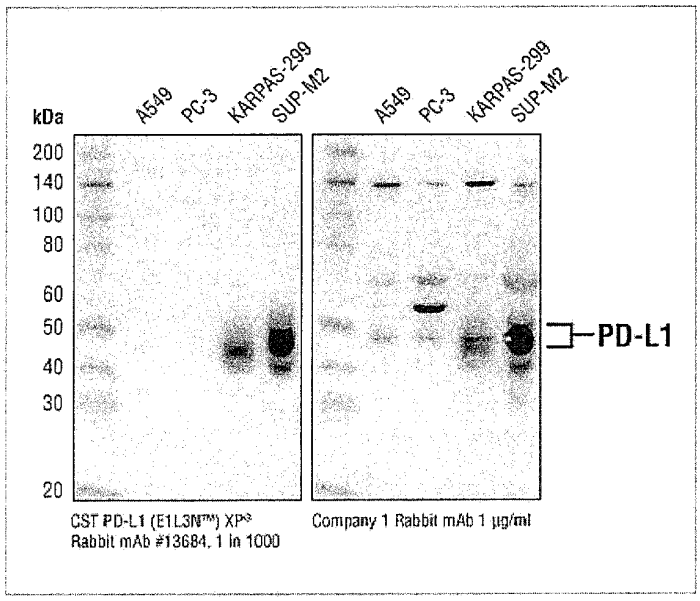
Lung and Breast Carcinoma

	CST PD-L1 (E1L3N®) XP® Rabbit mAb #13684 1:200	Company 1 Rabbit mAb 1:8000
Lung Carcinoma		
	Predominantly membrane staining in lung carcinoma and no staining in stromal cells.	Staining in cytoplasm and membrane in both lung carcinoma and stromal cells.
Breast Carcinoma		
	No staining in PD-L1 negative breast carcinoma.	Non-specific nuclear and cytoplasmic staining in the PD-L1 negative breast carcinoma.

In IHC analysis of human lung and breast carcinoma CST **PD-L1 (E1L3N®) XP® Rabbit mAb #13684 (/products/primary-antibodies/13684)** demonstrates appropriate staining in lung carcinoma with no staining in surrounding stromal cells, and no staining in breast carcinoma. Company 1 Rabbit mAb, however, demonstrates staining in both lung carcinoma and surrounding stromal cells, and inappropriate staining in breast carcinoma. This antibody is detecting a protein that is not PD-L1.

PD-L1 (E1L3N®) Antibody Comparative Data: WB

Expand



Western blot analysis using CST **PD-L1 (E1L3N®) XP® Rabbit mAb #13684 (/products/primary-antibodies/13684)** results in detection of a band at the appropriate molecular weight for PD-L1 in KARPAS-299 and SUP-M2 cells, which are known to have high PD-L1 expression, and no protein in A549 and PC-3, cells demonstrating that **#13684 (/products/primary-antibodies/13684)** is specific for PD-L1. At the dilution recommended by the supplier, Company 1 Rabbit mAb produces multiple signals at various molecular weights in A549, PC-3, KARPAS-299, and SUP-M2 cells, demonstrating that this antibody is not specific for PD-L1.

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: 877-678-8324

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Bedi and Ravi	Art Unit:	1647
Application No.:	15/231,309	Examiner:	Shafer, Shulamith H.
Filing Date:	August 8, 2016	Conf. No.	3446
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

Mail Stop AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Dear Sir:

I, Dr. Atul Bedi, declare and state that:

1. I am a named inventor of the subject matter described and claimed in U.S. Patent Application Serial No. 15/231,309, filed on August 8, 2016 entitled, "Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins." I am a physician-scientist and medical oncologist, and an Associate Professor of Otolaryngology/Head & Neck Surgery and Oncology at the Johns Hopkins University School of Medicine. A copy of my CV is attached herewith.

2. I understand that claims 1-5, 7-9 and 11 of the patent application have been rejected as being obvious under 35 U.S.C. §103 given the disclosures of Langermann et al. (U.S. Patent No. 7,820,165; hereafter "Langermann") and Sun et al. (U.S. Patent No. 7,795,389; hereafter "Sun"); claims 6 and 10 have been rejected as being obvious given the disclosures of Langermann and Sun in view of Langermann et al. (U.S. Patent Appl. Pub. No. 2013/0017199; hereafter "Langermann 2"); claim 12 has been rejected as being obvious given the disclosures of Langermann and Sun in view of view of Scaria (U.S. Patent Appl. Serial No. 2011/0052585; hereafter "Scaria") and claims 13 and 14 have been rejected as being obvious given the

disclosures of Langermann and Sun in view of Krauss et al. (U.S. Patent Appl. Serial No. 2006/0193849; hereafter "Krauss").

3. I disagree with the Examiner's assertion that Langermann and Sun render the present invention obvious. Langermann allegedly teaches targeting moieties which bind PD-1 ligand. However, Langermann does not teach a molecule comprising a PD-1 ligand targeting moiety fused with an immunomodulatory moiety where the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of transforming growth factor-beta receptor II.

4. Sun allegedly teaches TGF-beta antagonists comprising soluble cell surface type II TGF-beta receptors and fusion proteins comprising said soluble cell surface type II TGF-beta receptors. However, Sun does not teach a molecule comprising PD-1 ligand targeting moiety fused to a ligand-binding fragment of a TGF-beta receptor (TGFβR).

5. The present invention provides a molecule that counteracts tumor-induced immune tolerance and enhances antitumor immune responses by simultaneously targeting PD-L1 and binding and disabling TGFβ within the localized microenvironment of tumor-infiltrating T cells and tumor cells. The claimed invention is directed to a molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein: the targeting moiety specifically binds PD-1 ligand; and the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain (ECD) of TGFβR. The anti-PD-L1-TGFβRII molecule is, as an illustrative example, a bifunctional antibody that targets and sequesters PD-L1 and simultaneously inactivates TGF-β by an extracellular domain sequence of TGF-β Receptor II (TGF-βRIIecd) fused to the C-terminus of the heavy chain of an anti-PD-L1 antibody via a flexible linker sequence, $(GGGGS)_3$ (see Appendix A, Figure 1A). Appendix A, Figure 1B is an SDS-PAGE comparison of two different anti-PD-L1 antibodies and their corresponding anti-PD-L1-TGFβRII products (I and II) under reducing and non-reducing conditions showing the expected higher molecular weight of the heavy chain of anti-PD-L1-TGFβRII. Appendix A, Figure 1C shows SEC-HPLC analysis of purified anti-PD-L1-TGFβRII. In contrast to anti-PD-L1 antibody, the

anti-PD-L1-TGFβRII antibody exhibited simultaneous binding to PD-L1 and TGF-β1 (Appendix A, Figure 2).

6. The present invention demonstrates that a fusion protein comprising the ligand-binding sequence of the extracellular domain of TGFβRII linked to the C-terminus of IgG1 Fc binds TGF-β1 (IgG-TGFβRII) with efficiency that is equivalent to that of a fusion protein of the same ligand-binding sequence of the extracellular domain of TGFβRII linked to the N-terminus of IgG1 Fc (TGFβRII-Fc)(Appendix A, Figure 2A). This enabled the design of a bifunctional fusion protein comprising the same ligand-binding sequence of the extracellular domain of TGFβRII linked to the C-terminus of the heavy chain of an anti-PD-L1 antibody (anti-PD-L1-TGFβRII). Furthermore, we show that anti-PD-L1-TGFβRII binds TGF-β1 with efficiency equivalent to that of TGFβRII-Fc (Appendix A, Figure 2A). The comparative ability of anti-PD-L1-TGFβRII and anti-PD-L1 antibody to bind TGF-β was evaluated by ELISA, wherein recombinant human TGFβ (rhTGFβ1; 0-2000 pg/ml) was added to plates coated with anti-PD-L1-TGFβRII or anti-PD-L1 antibody (1 μg/ml), which was detected by biotinylated anti-TGFβ1 and HRP-Avidin (R&D systems). Plates coated with non-specific IgG-TGFβRII and rhTGFβRII-Fc served as positive controls to analyze the binding ability of the test samples to TGFβ. In contrast to the respective a-PD-L1 antibody that failed to bind TGFβ, each of the corresponding anti-PD-L1-TGFβRII products (I and II) exhibited binding to TGFβ with an efficiency that was similar to the positive controls (IgG-TGFβRII and TGFβRII-Fc coated plates)(Appendix A, Figure 2A). The data show the optical density (OD) values (mean of three replicate wells for each assay condition) from a representative of two independent experiments.

7. Additionally, we show that anti-PD-L1-TGFβRII also binds PD-L1, with efficiency equivalent to anti-PD-L1 antibody (Appendix A, Figure 2B). The comparative ability of anti-PD-L1-TGFβRII and anti-PD-L1 antibody to bind PD-L1 was evaluated by ELISA, wherein biotinylated recombinant human PD-L1 (rh B7-H1-biotin; 0-100 ng/ml) was added to plates coated with anti-PD-L1-TGFβRII or anti-PD-L1 antibody (1 μg/ml), followed by detection with HRP-Avidin. Plates coated with non-specific IgG-TGFβRII showed no binding to PD-L1 and served as a negative control to analyze the binding ability of the test samples. Each of

the corresponding anti-PD-L1-TGFβRII products (I and II) exhibited specific binding to rhPD-L1 with an efficiency that was similar to the respective a-PD-L1 antibody (Appendix A, Figure 2B). The data show the optical density (OD) values (mean of three replicate wells for each assay condition) from a representative of two independent experiments.

8. In another experiment, we show that anti-PD-L1-TGFβRII simultaneously binds TGF-β1 and PD-L1 (Appendix A, Figure 2C). We show each of these results for two different anti-PD-L1-TGFβRII molecules (I and II), and their corresponding anti-PD-L1 antibodies. The ability of anti-PD-L1-TGFβRII to simultaneously bind both PD-L1 and TGF-β was evaluated by a bispecific ELISA, wherein anti-PD-L1-TGFβRII or anti-PD-L1 antibody (0-100 ng/ml) was added to PD-L1-Fc coated plates (1 μg/ml), followed by rhTGFβ1 (100 ng/ml) that was detected by a biotinylated anti-human TGF-β1 antibody (R&D systems). In contrast to the respective a-PD-L1 antibody, each of the corresponding anti-PD-L1-TGFβRII products (I and II) exhibited simultaneous binding to PD-L1 and TGFβ (Appendix A, Figure 2C). The data show the optical density (OD) values (mean of three replicate wells for each assay condition) from a representative of two independent experiments.

9. Most significantly, we have demonstrated that the antitumor efficacy of the a-PD-L1-TGFβRII molecule of my invention exceeds that of either the corresponding anti-PD-L1 antibody, or the combination of the corresponding anti-PD-L1 antibody and non-specific IgG-TGFβRII (the same TGFβRII sequence linked to a non-specific IgG heavy chain)(Appendix A, Figure 3). The comparative antitumor efficacy of a-PD-L1-TGFβRII, a-PD-L1 antibody, non-specific IgG-TGFβRII, and the combination of a-PD-L1 antibody and IgG-TGFβRII against human cancers expressing both PD-L1 and TGFβ was evaluated in NSG (NOD/Shi-scid IL-2rg^{null}) mice reconstituted with human CD34+ hematopoietic stem cells (HSC). MDA-MB-231 is a metastatic human Triple-Negative Breast Cancer (TNBC) cell line with mesenchymal-like morphology (Basal B-like). MDA-MB-231-Luc (D3H2LN) is a TNBC subline with enhanced primary tumor growth and lung metastases that was derived from a metastatic deposit of bioluminescent MDA-MB-231 cells stably expressing firefly luciferase. MDA-MB-231-Luc express high TGF-β (531 pg/10^6 cells/48h) and Programmed death-1 ligand 1 (PD-L1)

(Appendix A, Figure 3A). NSG mice (6-8 week old) were irradiated at 200 cGy, followed by adoptive transfer of CD34+ human hematopoietic cells (10^5/mouse) from a normal donor (HLA-A2 matched to the TNBC line). Mice were tested for engraftment of human CD3+ T cells in peripheral blood obtained via tail-bleed at 10 weeks following injection of CD34+ cells. Mice demonstrating evidence of human CD3+ cell engraftment were injected with TNBC cells (1.5 x10^6 cells in 50% PBS/50% matrigel; mammary fat pad implantation). At 10 days following tumor cell inoculation, mice were randomized and treated i.p. with 5mg/kg/week with the following (either alone or in combination, as indicated): (i) anti-PD-L1-TGFβRII; (ii) anti-PD-L1 antibody; (iii) Control non-specific IgG-TGFβRII (anti-gp120-TGFβRII); (iv) a-PD-L1 mAb + IgG-TGFβRII; (vi) Vehicle alone (Control). Independent experiments were conducted to compare two different anti-PD-L1-TGFβRII antibody-ligand traps with their respective a-PD-L1 antibodies (I and II). Tumor size was evaluated weekly and tumor volume calculated [length × width x height]. In vivo tumor growth curves (mean + SEM) in both the experiments (I and II) demonstrated that treatment of tumor-bearing mice with a-PD-L1-TGFβRII was significantly more effective at inhibiting the progression of MDA-MB-231-luc tumors compared with the respective α-PD-L1 alone (I, $p<0.001$ and II, $p<0.001$), IgG-TGFβRII alone, and the combination of α-PD-L1 and non-specific IgG-TGFβRII (I, $p=0.004$ and II, $p<0.002$)(Appendix A, Figure 3B,C).

10. The TGFβRII*ecd*-Fc-PD1*ecd* molecule is, as another illustrative example, a bifunctional fusion molecule that targets and sequesters PD-L1 via an extracellular domain sequence of PD-1 (PD1*ecd*) and simultaneously inactivates TGF-β by an extracellular domain sequence of TGF-β Receptor II (TGF-βRII*ecd*) fused to the IgG Fc sequence via a flexible linker sequence, $(GGGGS)_3$ (see Appendix A, Figure 4A). Appendix A, Figure 4B is an SDS-PAGE of TGFβRII*ecd*-Fc-PD1*ecd* under reducing and non-reducing conditions showing the expected molecular weight of the monomer and dimer (indicated). ELISA demonstrated that TGFβRII*ecd*-Fc-PD1*ecd* exhibited binding to TGF-β1, PD-L1 and PD-L2 (Appendix A, Figure 4C, D).

11. Most significantly, we have demonstrated that the TGFβRII*ecd*-Fc-PD1*ecd* molecule of the present invention exhibits superior antitumor efficacy compared to the non-

specific IgG-PD1*ecd* (the same PD1*ecd* sequence linked to a non-specific control IgG), non-specific TGFβRII*ecd*-Fc (the same TGFβRII*ecd* sequence linked to IgG Fc), or the combined administration of IgG-PD1*ecd* and TGFβRII*ecd*-Fc (Appendix A, Figure 5). The comparative antitumor efficacy of TGFβRII*ecd*-Fc-PD1*ecd*, non-specific IgG-PD1*ecd*, non-specific TGFβRII*ecd*-Fc, and the combination of non-specific IgG-PD1*ecd* and TGFβRII*ecd*-Fc against human cancers expressing both PD-L1 and TGFβ was evaluated in NSG (NOD/Shi-scid IL-2rg^{null}) mice reconstituted with human CD34+ hematopoietic stem cells (HSC). MDA-MB-231-Luc (D3H2LN) is human Triple-Negative Breast Cancer (TNBC) sub-line with enhanced primary tumor growth and lung metastases that was derived from a metastatic deposit of bioluminescent MDA-MB-231 cells stably expressing firefly luciferase. MDA-MB-231-Luc express high TGF-β (531 pg/10^6 cells/48h) and Programmed death-1 ligand 1 (PD-L1) (Appendix A, Figure 3A).

12. NSG mice (6-8 week old) were irradiated at 200 cGy, followed by adoptive transfer of CD34+ human hematopoietic cells (10^5/mouse) from a normal donor (HLA-A2 matched to the TNBC line). Mice were tested for engraftment of human CD3+ T cells in peripheral blood obtained via tail-bleed at 10 weeks following injection of CD34+ cells. Mice demonstrating evidence of human CD3+ cell engraftment were injected with TNBC cells (1.5 x10^6 cells in 50% PBS/50% matrigel; mammary fat pad implantation). At 10 days following tumor cell inoculation, mice were randomized and treated i.p. with 5mg/kg/week of the following (either alone or in combination, as indicated): (i) TGFβRII*ecd*-Fc-PD1*ecd*; (ii) non-specific IgG-PD1*ecd* (anti-gp120-PD1*ecd*) ; (iii) non-specific TGFβRII*ecd*-Fc; (iv) IgG-PD1*ecd* + TGFβRII*ecd*-Fc; (vi) Vehicle alone (Control). The following parameters were used to evaluate and compare the antitumor efficacy of treatment groups: (1) Tumor size was evaluated and tumor volume calculated [length × width x height]; (2) Bioluminescent Imaging of primary and metastatic tumors: Tumor burden in mice bearing MDA-MB-231-Luc was assessed by visualization of *in vivo* luciferase activity using a Xenogen IVIS Spectrum system. To detect metastases, the lower portion of each animal was shielded before re-imaging to minimize bioluminescence from the primary tumor. Lungs were harvested and imaged *ex vivo* to confirm

in vivo observations. Photon flux was used to quantify the differences in tumor burden between treatment groups.

13. *In vivo* bioluminescent imaging (Appendix A, Figure 5A, B) and tumor growth curves (mean + SEM)(Appendix A, Figure 5C) of primary tumors demonstrated that treatment of tumor-bearing mice with TGFβRII*ecd*-Fc-PD1*ecd* was significantly more effective at inhibiting the progression of MDA-MB-231-luc tumors compared with IgG-PD1*ecd* alone ($p<0.0001$) or the combination of IgG-PD1ecd and TGFβRII*ecd*-Fc ($p<0.003$)(Appendix A, Figure 5A,B,C). *In vivo* bioluminescent imaging of lung metastases demonstrated that treatment of MDA-MB-231-luc-bearing mice with treatment TGFβRII*ecd*-Fc-PD1*ecd* was effective at inhibiting lung metastases of MDA-MB-231-luc tumors, whereas either IgG-PD1*ecd* alone or the combination of IgG-PD1*ecd* and TGFβRIIecd-Fc failed to inhibit lung metastases compared to untreated animals (Appendix A, Figure 5D).

14. PD-L1 is overexpressed on tumor cells as well as tumor-infiltrating T cells, where it cooperates with TGF-β to inhibit T-cell activation and induce and maintain immunosuppressive regulatory T cells (Tregs). PD-L1 and TGF-β entrain independent but cooperative mechanisms of immune tolerance that involve autocrine/paracrine signaling in the tumor immune microenvironment (Appendix A, Figure 6). The data demonstrate that the bifunctional molecules of my invention (anti-PD-L1-TGFβRII and TGFβRII*ecd*-Fc-PD1*ecd*) have superior anti-tumor efficacy via their unique ability to localize the TGFβRII*ecd* to the immediate microenvironment of PD-L1 expressing tumor-infiltrating immune cells and tumor cells. Unlike anti-TGF-β antibodies or non-targeted TGFβRII*ecd*-Fc, anti-PD-L1-TGFβRII and TGFβRII*ecd*-Fc-PD1*ecd* are therefore able to efficiently interrupt autocrine and paracrine TGF-β signaling in the tumor immune microenvironment via their ability to efficiently compete with native cellular TGFβRII for TGF-β binding on the surface of PD-L1-expressing cells. My invention (e.g. anti-PD-L1-TGFβRII and TGFβRII*ecd*-Fc-PD1*ecd*) is unique in its ability to not only block PD-L1/PD-1 interaction, but simultaneously counteract autocrine/paracrine TGF-β signaling in the localized microenvironment of tumor infiltrating T cells. *The superior anti-tumor efficacy of anti-PD-L1-TGFβRII and TGFβRIIecd-Fc-PD1ecd is crucially dependent on*

the physical linkage of the PD-L1-targeting moiety with the ligand-binding sequence of TGFβRIIecd, and cannot be achieved by the simultaneous administration of a PD-L1-targeting antibody and a non-targeted TGFβRIIecd-Fc which is not preferentially localized to PD-L1-expressing tumor or tumor-infiltrating immune cells.

15. This data demonstrates that anti-PD-L1-TGFβRII and TGFβRII*ecd*-Fc-PD1*ecd* have unexpected and surprising properties and demonstrates that the presence of both a PD-L1 antagonist and the ligand-binding sequence of a TGF-β receptor on the **same molecule** is significantly more effective at inhibiting tumor growth and metastases than the simultaneous administration of a PD-L1 antagonist and non-targeted TGF-β antagonist.

16. In my opinion, one of skill in the art would have no motivation to combine a PD-L1 antagonist and the ECD of TGFβRII in a fusion protein since, prior to my invention, there was no reason to believe that the fusion protein would be more effective than the simultaneous administration of a PD-L1 antagonist and non-targeted TGF-β antagonist. Moreover, it would not be obvious to one of skill in the art that the TGF-β binding properties of the ECD of TGFβRII would be preserved when linked to the C-terminus of an a-PD-L1 antibody or fusion protein. Further, one of skill in the art would not have predicted the surprising and unexpected properties of the presently claimed molecule based on the disclosures in Langermann and Sun.

17. I declare that all statements made herein are of my own knowledge and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

July 20, 2017
Date

Atul Bedi
Dr. Atul Bedi

Electronic Patent Application Fee Transmittal

Application Number:	15231309
Filing Date:	08-Aug-2016
Title of Invention:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS
First Named Inventor/Applicant Name:	Atul Bedi
Filer:	Lisa A. Haile/Shannon Warner
Attorney Docket Number:	JHU3260-6

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 2 months with $0 paid	2252	1	300	300
Miscellaneous:				
		Total in USD ($)		**300**

Electronic Acknowledgement Receipt

EFS ID:	29859973
Application Number:	15231309
International Application Number:	
Confirmation Number:	3446
Title of Invention:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Lisa A. Haile/Shannon Warner
Filer Authorized By:	Lisa A. Haile
Attorney Docket Number:	JHU3260-6
Receipt Date:	21-JUL-2017
Filing Date:	08-AUG-2016
Time Stamp:	17:15:51
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$300
RAM confirmation Number	072417INTEFSW00003745071896
Deposit Account	071896
Authorized User	Shannon Warner
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.16 (National application filing, search, and examination fees) 37 CFR 1.17 (Patent application and reexamination processing fees)	

37 CFR 1.19 (Document supply fees)

37 CFR 1.20 (Post Issuance fees)

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		JHU3260_6_Response_OA.pdf	1096102 6844d0a62444267ada74325661fe2c54132 02a6a	yes	16
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Amendment/Req. Reconsideration-After Non-Final Reject		1	1	
	Claims		2	4	
	Applicant Arguments/Remarks Made in an Amendment		5	16	
Warnings:					
Information:					
2	Applicant Arguments/Remarks Made in an Amendment	JHU3260_6_Appendices.pdf	1574136 7a73c97f14023bc92f3d5bd1e49a593a699a 2a5f	no	13
Warnings:					
Information:					
3	Affidavit-traversing rejectns or objectns rule 132	JHU3260_6_132_Declaration. pdf	1390026 126e140a6d1ab530f6518d3eac4a8e4717b 25d2e	no	8
Warnings:					
Information:					
4	Fee Worksheet (SB06)	fee-info.pdf	30875 529a3804603840ebb85d64880275f6dae5a 0e843	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	4091139		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/231,309	Filing Date 08/08/2016	☐ To be Mailed

ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	07/21/2017	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 19	Minus	** 20	= 0	X $40 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $210 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/POLIN ANG/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**
If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/08b (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Apt of 1995 no Persons are required to respond to a collection of information unless it contains a valid OMR control number

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	15/231,309
Filing Date	August 8, 2016
First Named Inventor	Atul Bedi
Art Unit	1647
Examiner Name	Shulamith H. Shafer
Attorney Docket Number	JHU3260-6

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	AA	ROSE-JOHN et al.: *"The IL-6/sIL-6R complex as a novel target for therapeutic approaches"*; Expert Opinion on Therapeutic Targets, 11:5, 613-624.	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.
[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.
This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO:**
Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Electronic Acknowledgement Receipt

EFS ID:	30554124
Application Number:	15231309
International Application Number:	
Confirmation Number:	3446
Title of Invention:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Lisa A. Haile/Tia de Langen
Filer Authorized By:	Lisa A. Haile
Attorney Docket Number:	JHU3260-6
Receipt Date:	03-OCT-2017
Filing Date:	08-AUG-2016
Time Stamp:	15:46:29
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		JHU3260-6_IDS.pdf	100727 f93373018a9cfa1390f4819b49b35ed421000e14	yes	3

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Transmittal Letter	1	2
	Information Disclosure Statement (IDS) Form (SB08)	3	3

Warnings:					
Information:					
2	Non Patent Literature	Rose_AA.pdf	393921 50151c4ce17dfd63a3ca63031cc01d3d2954edba	no	13
Warnings:					
Information:					
		Total Files Size (in bytes):	494648		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	The Johns Hopkins University	Art Unit:	1647
Application No.:	15/231,309	Examiner:	Shulamith H. Shafer
Filed:	August 8, 20116	Conf. No.:	3446
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

Mail Stop AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Dear Sir:

In accordance with 37 C.F.R. § 1.97(d), enclosed are references relating to the above-identified application. This IDS is accompanied by the statement specified in 37 C.F.R. § 1.97(e)(1) For the convenience of the Examiner, the cited reference is listed on the attached Form PTO/SB/08.

It is respectfully requested that this reference be considered in the examination of this application and its consideration be made of written record in the application file.

This Information Disclosure Statement is being submitted after the mailing date of a Final Office Action under 37 C.F.R. § 1.113 for the above-identified application. However, the item of information contained in the information disclosure statement was first cited in by the examiner in an Office Action from a counterpart U.S. application not more than three months prior to the filing of the information disclosure statement.

No fee is believed to be due in connection with this submission. However, the Commissioner is hereby authorized to charge any other fees associated with the filing submitted herewith, or credit any overpayments to Deposit Account No. 07-1896 referencing the above-identified attorney docket number.

Respectfully submitted,

Date: October 3, 2017

/Lisa A. Haile/

Lisa A. Haile, Ph.D., J.D.
Registration No. 38,347
Telephone: (858) 638-1456
Facsimile: (858) 638-5040

DLA PIPER LLP (US)
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133
USPTO Customer No.: 100899

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

100899 7590 10/16/2017

The John Hopkins University
c/o DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

EXAMINER
SHAFER, SHULAMITH H

ART UNIT	PAPER NUMBER
1647	

DATE MAILED: 10/16/2017

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/231,309	08/08/2016	Atul Bedi	JHU3260-6	3446

TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480	$0	$0	$480	01/16/2018

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PTOL-85 (Rev. 02/11)

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

100899 7590 10/16/2017

The John Hopkins University
c/o DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/231,309	08/08/2016	Atul Bedi	JHU3260-6	3446

TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480	$0	$0	$480	01/16/2018

EXAMINER	ART UNIT	CLASS-SUBCLASS
SHAFER, SHULAMITH H	1647	530-387100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:

☐ Issue Fee

☐ Publication Fee (No small entity discount permitted)

☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

☐ A check is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

☐ Applicant asserting small entity status. See 37 CFR 1.27

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

☐ Applicant changing to regular undiscounted fee status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/231,309	08/08/2016	Atul Bedi	JHU3260-6	3446

100899 7590 10/16/2017

The John Hopkins University
c/o DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

EXAMINER	
SHAFER, SHULAMITH H	

ART UNIT	PAPER NUMBER
1647	

DATE MAILED: 10/16/2017

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

PTOL-85 (Rev. 02/11)

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 15/231,309	Applicant(s) BEDI ET AL.	
	Examiner SHULAMITH H. SHAFER	Art Unit 1647	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *7/21/17 and telephonic interview 10/2/17*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *1,2 and 4-15*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☒ Interview Summary (PTO-413), Paper No./Mail Date *10/2/17* .
5. ☒ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/SHULAMITH H. SHAFER/
Primary Examiner, Art Unit 1647

Bicara Ex. 1010, Page 288 of 319
Bicara v. JHU - PGR2026-00025

Detailed Action

The present application is being examined under the pre-AIA first to invent provisions.

Status of Application, Amendments, And/Or Claims:

Applicants' response of 21 July 2017 is acknowledged. Claim 3 has been canceled. Claims 1, 2 and 12 have been amended and the amendment made of record.

Claims 1, 2 and 4-20 are pending in the instant application. Claims 16-20 stand withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim.

Claims 1, 2 and 4-15 are under consideration to the extent they read on the elected invention.

Withdrawn Rejections

Claim 3 has been canceled; all rejections of the claim are therefore moot.

Applicants' amendment to the claims has overcome the following rejections of record:

The rejection of Claim 12 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention

The rejection of Claims 1, 2, 7, 8, and 11-14 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement

The Declaration under 37 CFR 1.132 filed by Dr. Atul Bedi on 21 July 2017 is sufficient to overcome the following rejections of record:

The rejection of Claims 1, 2, 4, 5, 7-9 and 11 under 35 U.S.C. 103(a) as being unpatentable over Langermann et al. (the '845 patent) and Sun et al. (the '389 patent)

The rejection of Claims 6 and 10 under 35 U.S.C. 103(a) as being unpatentable over the '845 patent and the '389 patent as applied to claims 1 and 5, further in view of Langermann (USPGPUB 2013/0017199)

The rejection of Claim 12 under 35 U.S.C. 103(a) as being unpatentable over the '845 patent and the '389 patent as applied to claim 11 in view of Scaria et al. (US 2011/0052585)

The rejection of Claims 13 and 14 under 35 U.S.C. 103(a) as being unpatentable over the '845 patent and the '389 patent as applied to claim 11 in view of Krauss et al (USPGPUB 2006/0193849)

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in an interview with Dr. Lisa Haile on 2 October 2017.

Please amend Claim 4 so that the claim now reads as follows:

4. The molecule of claim 2, wherein the molecule comprises the amino acid sequence corresponding to the heavy chain or light chain of an antibody that specifically binds PD-L1.

Claims 16-20 are canceled.

Conclusion:

Claims 1, 2, and 4-15 are allowed with the aforementioned amendment.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SHULAMITH H. SHAFER whose telephone number is (571)272-3332. The examiner can normally be reached on Monday through Friday, 8 AM to 5 PM.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joanne Hama can be reached on 571-272-2911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

SHULAMITH H. SHAFER
Primary Examiner
Art Unit 1647

/SHULAMITH H. SHAFER/
Primary Examiner, Art Unit 1647

Examiner-Initiated Interview Summary	Application No. 15/231,309	Applicant(s) BEDI ET AL.	
	Examiner SHULAMITH H. SHAFER	Art Unit 1647	

All participants (applicant, applicant's representative, PTO personnel):

(1) *SHULAMITH H. SHAFER*. (3)_____.

(2) *Dr. Lisa Haile*. (4)_____.

Date of Interview: *02 October 2017*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☒112 ☐102 ☐103 ☐Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *4*.

Identification of prior art discussed: *N/A*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

In the amended claims of 7/21/17, claim 3 has been canceled. Claim 4 is listed as dependent from claim 3. Examiner contacted Dr. Haile to correct claim dependency. Dr. Haile noted that the proper claim dependency would recite "the molecule of claim 2"
The Examiner also noted that, as the election was made without traverse, non-elected claims 16-20 would be canceled.
.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☒ Attachment

/SHULAMITH H. SHAFER/ Primary Examiner, Art Unit 1647	

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L2	1	("9441044").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2017/10/02 15:12
L3	34	bedi adj atul or ravi adj rajani	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	OR	OFF	2017/10/02 15:55
L4	2436	"programmed death-1 ligand" or "PD-1 ligand" or CD279 or B7-H1 or B7-DC	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	OR	OFF	2017/10/02 15:58
L5	487	l4 same (fusion or conjugate or chimer? or hybrid)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	OR	OFF	2017/10/02 15:59
L6	310	l4 with (fusion or conjugate or chimer? or hybrid)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	OR	OFF	2017/10/02 15:59
L7	1425	"transforming growth factor beta receptor" or TGFbetaR or "TGF beta R"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	OR	OFF	2017/10/02 16:00
L8	310	l6 and l4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	OR	OFF	2017/10/02 16:00
L9	310	l6 same l4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	OR	OFF	2017/10/02 16:00
L10	36	l7 and l4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	OR	OFF	2017/10/02 16:00
L13	95	"transforming growth factor beta receptor".clm. or TGFbetaR.clm.	US-PGPUB; USPAT; USOCR; FPRS; JPO; DERWENT	OR	OFF	2017/10/02 16:09

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L11	190	"programmed death-1 ligand".clm. or "PD-1 ligand".clm. or CD279.clm. or B7-H1.clm. or B7-DC.clm.	US-PGPUB; USPAT	OR	OFF	2017/10/02 16:08
L12	345	"l1" with (conjugate or fusion or hybrid or chimer? or fus?).clm.	US-PGPUB; USPAT	OR	OFF	2017/10/02 16:09
L14	95	"transforming growth factor beta receptor".clm. or TGFbetaR.clm.	US-PGPUB; USPAT	OR	OFF	2017/10/02 16:10
L15	1	l12 and l14	US-PGPUB; USPAT	OR	OFF	2017/10/02 16:10

10/2/2017 4:10:58 PM
C:\Users\sshafer\Documents\EAST\Workspaces\15231309.wsp

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15231309	BEDI ET AL.
	Examiner	**Art Unit**
	SHULAMITH H SHAFER	1647

CPC						
Symbol					**Type**	**Version**
C07K		16	/	2827	F	2013-01-01
C07K		14	/	495	I	2013-01-01
C07K		14	/	70532	I	2013-01-01
C07K		14	/	70575	I	2013-01-01
C07K		14	/	70578	I	2013-01-01
C07K		14	/	70596	I	2013-01-01
C07K		14	/	71	I	2013-01-01
C07K		16	/	28	I	2013-01-01
C07K		2319	/	00	A	2013-01-01
C07K		16	/	2812	I	2013-01-01
C07K		16	/	2815	I	2013-01-01
C07K		16	/	2851	I	2013-01-01
A61K		2039	/	505	A	2013-01-01
C07K		2317	/	73	A	2013-01-01
C07K		2317	/	76	A	2013-01-01
A61K		39	/	3955	I	2013-01-01
A61K		45	/	06	I	2013-01-01
A61K		47	/	48384	I	2013-01-01
C07K		16	/	2863	I	2013-01-01
C07K		2317	/	622	A	2013-01-01
C07K		16	/	30	I	2013-01-01
C07K		2317	/	33	A	2013-01-01
C07K		2317	/	526	A	2013-01-01
C07K		2319	/	70	A	2013-01-01

CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
			/					
			/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	14	
/SHULAMITH H SHAFER/ Primary Examiner.Art Unit 1647	10/2/17	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	none

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15231309	BEDI ET AL.
	Examiner	**Art Unit**
	SHULAMITH H SHAFER	1647

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	14	
/SHULAMITH H SHAFER/ Primary Examiner.Art Unit 1647	10/2/17	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	none

U.S. Patent and Trademark Office

Part of Paper No. 20171002

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15231309	BEDI ET AL.
	Examiner	**Art Unit**
	SHULAMITH H SHAFER	1647

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION										
CLASS	SUBCLASS	CLAIMED					NON-CLAIMED					
		C	0	7	K	16 / 00 (2006.01.01)						
CROSS REFERENCE(S)		C	1	2	P	21 / 08 (2006.01.01)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)											

NONE (Assistant Examiner)	(Date)	Total Claims Allowed: 14	
/SHULAMITH H SHAFER/ Primary Examiner.Art Unit 1647 (Primary Examiner)	10/2/17 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure none

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15231309	BEDI ET AL.
	Examiner	**Art Unit**
	SHULAMITH H SHAFER	1647

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	1														
2	2														
3	4														
4	5														
5	6														
6	7														
7	8														
8	9														
9	10														
10	11														
11	12														
12	13														
13	14														
14	15														

NONE (Assistant Examiner) (Date)	**Total Claims Allowed:** 14	
/SHULAMITH H SHAFER/ Primary Examiner.Art Unit 1647 10/2/17 (Primary Examiner) (Date)	O.G. Print Claim(s) 1	O.G. Print Figure none

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15231309	BEDI ET AL.
	Examiner	Art Unit
	SHULAMITH H SHAFER	1647

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

SEARCH NOTES		
Search Notes	Date	Examiner
Inventor name search, PALM, EAST and STN, search history included	2/14/17	SHS
EAST search, search history included	2/14/17	SHS
STN search, search history included	2/14/17	SHS
Review prosecution history of parent applications, SN 13/582,717 and 14/645,282, now US 8,993,524 and 9,441,044	2/14/17	SHS
SEQ searches, see results in SCORE	2/13/17	SHS
Inventor name search update, PALM and EAST, search history included	10/2/17	SHS
EAST search update, search history included	10/2/17	SHS

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
	EAST search of claims, search history included	10/2/17	SHS

PTO/SB/08b (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	15/231,309
Filing Date	August 8, 2016
First Named Inventor	Atul Bedi
Art Unit	1647
Examiner Name	Shulamith H. Shafer
Attorney Docket Number	JHU3260-6

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	AA	ROSE-JOHN et al.: *"The IL-6/sIL-6R complex as a novel target for therapeutic approaches"*; Expert Opinion on Therapeutic Targets, 11:5, 613-624.	

Examiner Signature	/SHULAMITH H SHAFER/	Date Considered	10/20/2017

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.
[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.
This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO:
Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

WEST\278392372.1
331323-001150

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.H.S/

PATENT
Attorney Docket No.: JHU3260-6

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	1647
Application No.:	15/231,309	Examiner:	S.H. Shafer
Filed:	August 8, 2016	Conf. No.:	3446
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO NOTICE OF ALLOWANCE

Sir:

In response to the Notice of Allowance mailed October 16, 2017, attached is the completed Issue Fee Transmittal Form PTOL-85B.

The Commissioner is hereby authorized to charge $480.00 to Deposit Account No. 07-1896 for the required Issue Fees. The Commissioner is also authorized to charge any other fees associated with the filing submitted herewith or credit any overpayment to the aforementioned Deposit Account.

Respectfully submitted,

Date: November 16, 2017

Lisa A. Haile, J.D., Ph.D.
Registration No. 38,347
Telephone: (858) 677-1456
Facsimile: (858) 638-5040

USPTO Customer No. 100899
DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133

WEST\278852464.1
331323-001150

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

100899 7590 10/16/2017
The John Hopkins University
c/o DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

Aldon Griffis	(Depositor's name)
Aldon Griffis	(Signature)
November 16, 2017	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/231,309	08/08/2016	Atul Bedi	JHU3260-6	3446

TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480	$0	$0	$480	01/16/2018

EXAMINER	ART UNIT	CLASS-SUBCLASS
SHAFER, SHULAMITH H	1647	530-387100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 DLA Piper LLP (US)
2
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: The Johns Hopkins University
(B) RESIDENCE: (CITY and STATE OR COUNTRY): Baltimore, Maryland

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
☒ Issue Fee
☐ Publication Fee (No small entity discount permitted)
☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
☐ A check is enclosed.
☐ Payment by credit card. Form PTO-2038 is attached.
☒ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number 07-1896 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
☐ Applicant certifying micro entity status. See 37 CFR 1.29
NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
☐ Applicant asserting small entity status. See 37 CFR 1.27
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
☐ Applicant changing to regular undiscounted fee status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Lisa A. Haile/ Date November 16, 2017
Typed or printed name Lisa A. Haile, J.D., Ph.D. Registration No. 38,347

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Electronic Patent Application Fee Transmittal

Application Number:	15231309
Filing Date:	08-Aug-2016
Title of Invention:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS
First Named Inventor/Applicant Name:	Atul Bedi
Filer:	Lisa A. Haile/Aldon Griffis
Attorney Docket Number:	JHU3260-6

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
UTILITY APPL ISSUE FEE	2501	1	480	480

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**480**

Electronic Acknowledgement Receipt

EFS ID:	30972825
Application Number:	15231309
International Application Number:	
Confirmation Number:	3446
Title of Invention:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Lisa A. Haile/Aldon Griffis
Filer Authorized By:	Lisa A. Haile
Attorney Docket Number:	JHU3260-6
Receipt Date:	16-NOV-2017
Filing Date:	08-AUG-2016
Time Stamp:	15:19:52
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$480
RAM confirmation Number	111717INTEFSW00001896071896
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		C111230_P11123-11_20171116_OTH_DLAP_IssueFeeEFS.pdf	1678720 425d8eb18d048b7f43e72501a7efdc6fbac831db	yes	3
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Transmittal Letter		1	1	
	Miscellaneous Incoming Letter		2	2	
	Issue Fee Payment (PTO-85B)		3	3	
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30578 c506de9f4f4357ce84023dfe5c549fdc8e956d82	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1709298		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT
Attorney Docket No.: JHU3260-6

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	1647
Application No.:	15/231,309	Examiner:	S.H. Shafer
Filed:	August 8, 2016	Conf. No.:	3446
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

TRANSMITTAL SHEET

Sir:

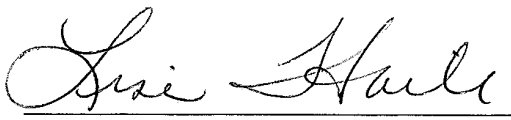
Transmitted herewith for the above-identified application, please find:

1. Response to Notice of Allowance mailed October 16, 2017 (1 page); and
2. Form PTOL-85B Issue Fee Transmittal (1 page).

The fees for this filing total $480.00, which consist of $480.00 for the Issue Fee pursuant to 37 CFR § 1.18(a). The Commissioner is hereby authorized to charge this fee and any other fees associated with the filing submitted herewith or credit any overpayment to Deposit Account No. 07-1896.

Respectfully submitted,

Date: November 16, 2017



Lisa A. Haile, J.D., Ph.D.
Registration No. 38,347
Telephone: (858) 677-1456
Facsimile: (858) 638-5040

USPTO Customer No. 100899
DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133

WEST\278852421.1
331323-001150

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/231,309	12/26/2017	9850306	JHU3260-6	3446

100899 7590 12/06/2017

The John Hopkins University
c/o DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Atul Bedi, Timonium, MD;
The Johns Hopkins University, Baltimore, MD;
Rajani Ravi, Ruxton, MD;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s):	Bedi, et al.	Assignee:	The Johns Hopkins University
Patent No.:	9,850,306	Appl. No.:	15/231,309 Conf. No. 3446
Issued:	December 26, 2017	Filed:	August 08, 2016
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

ATTN: CERTIFICATE OF CORRECTION BRANCH
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 C.F.R. §1.323

Sir:

The undersigned requests the issuance of a Certificate of Correction in the above-identified patent to correct the government support clause. Accompanying this Request is a completed form PTO/SB/44.

Applicants submit that the Request for Certificate of Correction does not raise issues of new matter.

In accordance with 37 C.F.R. §1.20(a), the amount of $150.00 in payment of the certificate of correction fee due is hereby paid electronically via USPTO EFS-Web.

Respectfully submitted,

Date: October 11, 2018

/Joseph Contrera/
Joseph Contrera
Reg. No. 44628

Customer No. 54110
Johns Hopkins Technology Transfer
1812 Ashland Avenue, Suite 110
Baltimore, MD 21205
Tel: 410-614-0300
Fax: 410-614-8425

PTO/SB/44 (09-07)
Approved for use through 08/31/2013. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 9,850,306

APPLICATION NO.: 15/231,309

ISSUE DATE : December 26, 2017

INVENTOR(S) : Atul Bedi, Rajani Ravi

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On page 1, after the title, please add the following paragraph:

STATEMENT OF GOVERNMENTAL INTEREST

This invention was made with government support under grant number CA123277, awarded by the National Institutes of Health. The government has certain rights in the invention.

MAILING ADDRESS OF SENDER (Please do not use customer number below):

Johns Hopkins Technology Ventures
1812 Ashland Avenue, Suite 110
Baltimore, MD 21205

This collection of information is required by 37 CFR 1.322, 1.323, and 1.324. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1.0 hour to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Attention Certificate of Corrections Branch, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	15231309
Filing Date:	08-Aug-2016
Title of Invention:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS
First Named Inventor/Applicant Name:	Atul Bedi
Filer:	Joseph Contrera/Cheryl Oliver
Attorney Docket Number:	JHU3260-6

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
CERTIFICATE OF CORRECTION	1811	1	150	150

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**150**

Electronic Acknowledgement Receipt

EFS ID:	33989847
Application Number:	15231309
International Application Number:	
Confirmation Number:	3446
Title of Invention:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Joseph Contrera/Cheryl Oliver
Filer Authorized By:	Joseph Contrera
Attorney Docket Number:	JHU3260-6
Receipt Date:	12-OCT-2018
Filing Date:	08-AUG-2016
Time Stamp:	08:01:14
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$150
RAM confirmation Number	101218INTEFSW08013800
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Certificate of Correction	P11123-11_COC.pdf	222466 d8681e92af87aff56c69a17955dce941d0c9630d	no	3
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30326 816667b032ce4e770904370518af0e9f15fa7e66	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	252792		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,850,306 B2
APPLICATION NO. : 15/231309
DATED : December 26, 2017
INVENTOR(S) : Atul Bedi and Rajani Ravi

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

Column 1, Line 5, please insert:
--STATEMENT OF GOVERNMENTAL INTEREST
This invention was made with government support under grant number CA123277, awarded by the National Institutes of Health. The government has certain rights in the invention.--

Signed and Sealed this
Thirteenth Day of November, 2018

Andrei Iancu
Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY.DOCKET NO./TITLE	REQUEST ID
15/231,309	08/08/2016	The Johns Hopkins University	JHU3260-6	103564

Acknowledgement of Loss of Entitlement to Entity Status Discount

The entity status change request below filed through Private PAIR on 01/17/2020 has been accepted.

CERTIFICATIONS:

Change of Entity Status:

X Applicant changing to regular undiscounted fee status.

NOTE: Checking this box will be taken to be notification of loss of entitlement to small or micro entity status, as applicable.

This portion must be completed by the signatory or signatories making the entity status change in accordance with 37 CFR 1.4(d)(4).

Signature:	/Aldon Griffis/
Name:	Aldon Griffis
Registration Number:	55949