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March 14, 2022

ATTORNEY DOCKET NO.: JHU3260-9

Parent Application No.: 16/601,347

Parent Filing Date: October 14, 2019

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 April 20, 2020 at Reel 052447, Frame 0640.

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

Pages of Specification	74
Pages of Claims	2
Pages of Abstract	1
Sheets of Drawing	75
Pages of Power of Attorney (from Parent)	2
(with Statement under 37 CFR 3.73(c))	
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); and
 - Nine (9) U.S. Patents;
 - Twelve (12) U.S. Patent Application Publications;
 - Two (2) Foreign Patent Documents; and
 - Thirty-Two (32) Non Patent Literature Documents.
- Sequence Listing in .txt format entitled “JHU3260-9_ST25.txt” (460 kB).

Under 35 USC § 120, this application is a **continuation** application of U.S. Application Serial No. 16/601,347 filed October 14, 2019, now issued as U.S. patent 11,274,156; which is a continuation application of U.S. Application Serial No. 15/362,632 filed November 28, 2016, now issued as U.S. patent 10,442,860; which is a divisional application of U.S. Application Serial No. 15/231,309 filed August 8, 2016, now issued as U.S. patent 9,850,306; which is a continuation application of U.S. Application Serial No. 14/645,282 filed March 11, 2015, now issued as U.S. patent 9,441,044; 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.

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

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The filing fee is calculated as follows:

	Number Filed		Number Extra		Rate			Fee	
					Large Entity	Small Entity		Large Entity	Small Entity
Total Claims	11-20	=	0	X		\$50	=		\$.00
Independent Claims	1-3	=	0	X		\$240	=		\$.00
Multiple Dependent Claims Present: ____ Yes <u>x</u> No						\$430			N/A
Fee for Each Additional 66 Sheets in Excess of 133 Pages						\$210			\$ 210.00
					FILING FEE				\$ 80.00
					SEARCH FEE				\$ 350.00
					EXAM FEE				\$ 400.00
					TOTAL FEE				\$1,040.00

The fees for this filing total \$1,040.00, which consist of \$80.00 for the electronic filing fee, \$350.00 for the search fee, \$400.00 for the exam fee and \$210.00 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)

March 14, 2022
 Date

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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. 16/601,347 filed October 14, 2019, now issued as U.S. patent 11,274,156; which is a continuation application of U.S. Application Serial No. 15/362,632 filed November 28, 2016, now issued as U.S. patent 10,442,860; which is a divisional application of U.S. Application Serial No. 15/231,309 filed August 8, 2016, now issued as U.S. patent 9,850,306; which is a continuation application of U.S. Application Serial No. 14/645,282 filed March 11, 2015, now issued as U.S. patent 9,441,044; 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.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] The material in the accompanying sequence listing is hereby incorporated by reference into this application. The accompanying sequence listing text file, named JHU3260-9_ST25.txt, was created on March 14, 2022 and is 460 kB in size. The file can be accessed using Microsoft Word on a computer than uses Windows OS.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

[0003] 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

[0004] The immune system provides the human body with a means to recognize and defend itself against microorganisms and substances recognized as foreign or potentially harmful. While passive immunotherapy of cancer with monoclonal antibodies and passive transfer of T cells to attack tumor cells have demonstrated clinical efficacy, the goal of active therapeutic vaccination to induce these immune effectors and establish immunological memory against tumor cells has remained challenging. Several tumor-specific and tumor-associated antigens have been identified, yet these antigens are generally weakly immunogenic and tumors employ diverse mechanisms to create a tolerogenic environment that allows them to evade immunologic attack. Strategies to overcome such immune tolerance and activating robust levels of antibody and/or T cell responses hold the key to effective cancer immunotherapy.

SUMMARY OF THE INVENTION

[0005] 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.

[0006] 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)

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Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2); (iii) Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL); (iv) Transforming growth factor-beta receptor (TGF- β R); (v) Programmed death-1 (PD-1); and (vi) Receptor activator of nuclear factor- κ B (RANK).

[0007] 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.

[0008] In one aspect, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: (i) CD4; (ii) CD25 (IL-2 α receptor; IL-2 α R); (iii) cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); (iv) Interleukin-10 (IL-10); (v) Transforming growth factor-beta receptor (TGF- β R); (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- κ B (RANK); or (x) Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL).

[0009] 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- κ B (RANK) ligand (RANKL).

[0010] 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- β RII, TGF- β RIIb, or TGF- β RIII. In another aspect, the immunomodulatory moiety includes an extracellular

ligand-binding domain of TGF- β RII. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of TGF- β .

[0011] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- β RII. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

[0012] 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- β RII. In another aspect, the molecule includes PD-1 ectodomain, immunoglobulin Fc region, and TGF β RII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

[0013] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- κ B (RANK) or Receptor activator of nuclear factor- κ B ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- β RII. In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and TGF β RII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

[0014] 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).

[0015] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.

[0016] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- κ B (RANK) or Receptor activator of nuclear factor- κ B ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Programmed Death-1 (PD-1). In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and PD-1 ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 25 or 26.

[0017] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Receptor activator of nuclear factor- κ B ligand (RANKL). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Receptor activator of nuclear factor- κ B ligand (RANKL).

[0018] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36.

[0019] 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.

[0020] 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.

[0021] 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.

[0022] In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 46 or 47.

[0023] In one aspect, the targeting moiety includes transforming growth factor- β (TGF- β) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 48 or 49.

[0024] 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.

[0025] 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.

[0026] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25 (IL-2 α receptor; IL-2 α R), 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.

[0027] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C γ 1), 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.

[0028] 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- κ B (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.

[0029] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

[0030] In one aspect, the targeting moiety includes a sequence from transforming growth factor- β (TGF- β) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or

ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

[0031] In one aspect, the targeting moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

[0032] In various aspects, the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

[0033] 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.

[0034] 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.

[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 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.

[0036] 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.

[0037] 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.

[0038] 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.

[0039] 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.

[0040] 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

[0041] 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).

[0042] 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 (Δ C terminus): TGF β RII lacking the last 38 amino acids from the C-terminus (SEQ ID NO: 81) and TGF- β R-IIB (Δ C terminus): TGF β RIIB lacking the last 38 aa from the C-terminus (SEQ ID NO: 82); (ii) TGF- β R-II (Δ cyt): TGF β RII lacking the kinase domain & juxtamembrane region (SEQ ID NO: 83) and TGF- β R-IIB (Δ cyt): TGF β RIIB 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).

[0043] In addition, Figures 1A-1C also show exemplary kinase-deficient mutants, deletion mutants, or point mutants of Transforming growth factor beta Receptor II (TGF β -RII) or TGF β -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 (Δ i) - TGF- β R-II (Δ i2) contains a deletion of amino acids 498 to 508 and is inactive as a kinase (SEQ ID NO: 91).

[0044] Figure 2 shows exemplary fusion proteins including anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), including anti-HER2/neu heavy chain + TGF β -RII ECD fusion amino acid sequence (SEQ ID NO: 1) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

[0045] Figure 3 shows exemplary fusion proteins including anti-EGFR1 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), including anti-EGFR1 heavy chain + TGF β -RII ECD fusion amino acid sequence (SEQ ID NO: 2) and anti-EGFR1 light chain amino acid sequence (SEQ ID NO: 71).

[0046] Figure 4 shows exemplary fusion proteins including anti-CD20 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), including anti-CD20 heavy chain+ TGF β -RII ECD fusion amino acid sequence (SEQ ID NO: 3) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0047] Figure 5 shows exemplary fusion proteins including anti-VEGF antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), including anti-VEGF heavy chain+ TGF β -RII ECD fusion amino acid sequence (SEQ ID NO: 4) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

[0048] Figure 6 shows exemplary fusion proteins including anti-human CTLA-4 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), including Anti-CTLA-4 heavy chain + TGF β -RII Extracellular domain fusion amino acid sequence (SEQ ID NO: 5) and Anti-CTLA-4 light chain (SEQ ID NO: 74).

[0049] Figure 7 shows exemplary fusion proteins including IL-2, Fc, and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), including IL-2 + Fc + TGF β -RII Extracellular domain (SEQ ID NO: 6) and TGF β -RII Extracellular domain + Fc + IL-2 (SEQ ID NO: 7). The linker GGGGSGGGGSGGGGS (SEQ ID NO: 104) is

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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.

[0050] Figures 8A-8B show exemplary fusion proteins including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), including anti-CD25 (Daclizumab) heavy chain and TGF β -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 TGF β -RII Extracellular domain (SEQ ID NO: 9) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76).

[0051] Figure 9 shows exemplary fusion proteins including anti-CD4 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), including anti-CD4 heavy chain and TGF β -RII Extracellular domain (SEQ ID NO: 10) and anti-CD4 light chain (SEQ ID NO: 77).

[0052] Figure 10 shows exemplary fusion proteins including Programmed Death-1 (PD-1) Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ectodomain), including PD-1 ectodomain + Fc + TGF β RII ectodomain (SEQ ID NO: 11) and TGF β RII ectodomain + Fc + PD-1 ectodomain (SEQ ID NO: 12). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

[0053] Figure 11 shows exemplary fusion proteins including Receptor activator of nuclear factor-kB (RANK) Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ectodomain), including RANK ectodomain + Fc + TGF β RII ectodomain (SEQ ID NO: 13) and TGF β RII ectodomain + Fc + RANK ectodomain (SEQ ID NO: 14). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

[0054] 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).

[0055] 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).

[0056] 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).

[0057] Figure 15 shows exemplary fusion proteins including anti-CD20 antibody and PD-1 Ectodomain, including anti-CD20 heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 17) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0058] 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).

[0059] 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).

[0060] 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).

[0061] 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.

[0062] 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).

[0063] 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.

[0064] 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).

[0065] 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).

[0066] 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).

[0067] 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).

[0068] 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).

[0069] 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).

[0070] 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).

[0071] 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.

[0072] 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).

[0073] 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).

[0074] Figure 32 shows exemplary fusion proteins including anti-tumor necrosis factor (TNF α) antibody and PD-1 ligand, including anti-TNF α heavy chain + PD-1L (SEQ ID NO: 37) and anti-TNF α 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).

[0075] Figure 33 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand, including TNFR2 ECD + IgG C γ 1 + PD-L1 (SEQ ID NO: 38) and PD-L1 + IgG C γ 1 -TNFR2 ECD (SEQ ID NO: 39).

[0076] 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).

[0077] 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).

[0078] 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 GGGGSGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0079] 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).

[0080] Figure 38 shows exemplary fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C γ 1), and a sequence from PD-1 ligand (PD-L1) including Oncostatin M signal peptide + CTLA-4 ECD + IgG C γ 1 + PD-L1 (SEQ ID NO: 46) and PD-1L1 + IgG C γ 1 + CTLA-4 ECD (SEQ ID NO: 47). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

[0081] Figure 39 shows exemplary fusion proteins including a sequence of transforming growth factor- β (TGF- β), Immunoglobulin Fc (IgG C γ 1), and a sequence of PD-1 ligand (PD-L1) including TGF- β -1 + Fc + PD-L1 (SEQ ID NO: 48), and PD-1L1 + Fc + TGF- β -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.

[0082] Figure 40 shows exemplary immunomodulatory moiety that binds Transforming growth factor-beta receptor (TGF- β R) including Transforming growth factor-beta (TGF- β 1, TGF- β 2, or TGF- β 3 or a fragment thereof, TGF- β 1 full sequence (SEQ ID NO: 102), and mature (active) TGF- β 1 sequence (Ala 279 – Ser 390; 112 amino acids) (SEQ ID NO: 103).

[0083] 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-TNF α heavy chain + TGF- β 1 (SEQ ID NO: 50) and anti-TNF α 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).

[0084] Figure 42 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain (TNFR2 ECD), immunoglobulin Fc (IgG C γ 1), and a sequence from transforming growth factor- β (TGF- β) including TNFR2 ECD + IgG C γ 1 + TGF- β 1 (SEQ ID NO: 51), and TGF- β 1 + IgG C γ 1 + TNFR2 ECD (SEQ ID NO: 52).

[0085] 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 TGF β 1 sequence (SEQ ID NO: 53) and anti-CD20 light chain sequence (SEQ ID NO: 72).

[0086] 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- β 1 (SEQ ID NO: 54) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 44A), and anti-CD25 (Basiliximab) heavy chain and TGF- β 1 (SEQ ID NO: 55) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 44B).

[0087] Figure 45 shows exemplary fusion proteins including IL-2, Fc, and a sequence from transforming growth factor- β (TGF- β), including TGF- β 1 + Fc + IL-2 (SEQ ID NO: 56) and IL-2 + Fc + TGF- β 1 (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.

[0088] 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).

[0089] Figure 47 shows exemplary Fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C γ 1), and a sequence from transforming growth factor- β (TGF- β) including Oncostatin M signal peptide + CTLA-4 ECD + IgG C γ 1 + TGF- β 1 (SEQ ID NO: 59), and TGF- β 1 + IgG C γ 1 + CTLA-4 ECD (SEQ ID NO: 60). The IgG sequence shown can have optional C to S conversion in (bold underlined). The

linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

[0090] Figure 48 shows exemplary fusion proteins including an antibody that binds TNF- α , and a sequence of RANK ectodomain, including anti-TNF α heavy chain + RANK ectodomain (SEQ ID NO: 61) and anti-TNF α 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).

[0091] Figure 49 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain (TNFR2 ECD), immunoglobulin Fc (IgG C γ 1), and a sequence from RANK ectodomain including TNFR2 ECD + IgG C γ 1 + RANK ectodomain (SEQ ID NO: 62), and RANK ectodomain + IgG C γ 1 + TNFR2 ECD (SEQ ID NO: 63).

[0092] Figure 50 shows exemplary Fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C γ 1), and a sequence from RANK ectodomain including Oncostatin M signal peptide + CTLA-4 ECD + IgG C γ 1 + RANK ectodomain (SEQ ID NO: 64), and RANK ectodomain + IgG C γ 1 + CTLA-4 ECD (SEQ ID NO: 65). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

[0093] Figure 51 shows exemplary fusion proteins including a sequence from transforming growth factor- β (TGF- β), immunoglobulin Fc (IgG C γ 1), and a sequence from RANK ectodomain including TGF- β + IgG C γ 1 + RANK ectodomain (SEQ ID NO: 66), and RANK ectodomain + IgG C γ 1 + TGF- β (SEQ ID NO: 67).

[0094] Figure 52 shows exemplary fusion proteins including a sequence from PD-1 ligand (PD-L1), immunoglobulin Fc (IgG C γ 1), and a sequence from RANK ectodomain including PD-L1 + IgG C γ 1 + RANK ectodomain (SEQ ID NO: 68), and RANK ectodomain + IgG C γ 1 + PD-L1 (SEQ ID NO: 69).

[0095] 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 μ M) for 4h. The surface exposure of CRT was determined by immunofluorescence cytometry of untreated control or doxorubicin-treated cells stained with Dylight-labeled anti-CRT antibody or an isotype control (IgG1) antibody. (FIG. 53B) Priming of tumor-reactive immune responses by MB49 tumor cells treated with doxorubicin *ex vivo* or *in vivo*. 5x10⁶ MB49 cells that were pre-treated *ex vivo* with doxorubicin (10 μ M) for 4h were injected into one flank of syngeneic immunocompetent C57BL/6 mice. Alternatively, C57BL/6 mice were injected with 5x10⁵ live MB49 tumor cells and then administered intratumoral doxorubicin (10 μ g) 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 (5x10⁶) that were pre-treated *in vitro* with doxorubicin (10 μ M) for 4h were injected subcutaneously into one flank of syngeneic immunocompetent C57BL/6 mice. Naïve or vaccinated mice were challenged with untreated live MB49 tumor cells injected into the opposite flank with or without pre-treatment with an anti-CD8 antibody (Clone GK2.43)(5 μ g 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 5x10⁵ live syngeneic MB49 tumor cells and then administered intratumoral doxorubicin (10 μ g) at d3, d7, or d10 following 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⁺FoxP3⁺ cells (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

5×10^5 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 5×10^6 doxorubicin-killed MB49 cells with or without intravenous adoptive transfer of 5×10^6 $CD4^+CD25^+$ 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 5×10^6 doxorubicin-killed MB49 cells (left flank) with or without pre-treatment with either an anti-CD8 antibody (Clo^{ne} GK2.43)(5 μ g x 2 doses, iv) to deplete $CD8^+$ T cells or adoptive transfer of 5×10^6 $CD4^+CD25^+$ 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.

[0096] 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 5×10^5 live MB49 tumor cells were evaluated utilizing ELISA. (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 /10⁶ cells in supernatants was measured by ELISA. (FIG. 54C) TGF β RII: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 TGF β RII:Fc (0-400 ng/ml) for 24h followed by measurement of TGF- β (pg/ml/10⁶ 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 TGF β RII:Fc (1 μ g 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 TGF β RII: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 TGF β RII:Fc (1 μ g 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).

[0097] 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 x 10⁵ 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. C57BL/6 mice injected s.c. with 5 x 10⁵ 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 x 10⁵ 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 x10⁵ 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.

[0098] 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 x10⁵ 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 x10⁵ 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.

[0099] 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 cytometric analysis of tumor cells labeled with an anti-human MICA/B MAbs (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 2×10^5 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 μ g 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 2×10^5 CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. $CD4^+CD25^+FoxP3^+$ 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 $IFN-\gamma^+CD8^+$ T cells. Balb/C mice injected with 2×10^5 CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of $IFN-\gamma$ expression in $CD8^+$ T cells from the tumor and draining lymph node (DLN) in response to *in vitro* stimulation with either CT26 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone. (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 2×10^5 CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) and/or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting $CD4^+$ T cells and/or $CD8^+$ T cells on the *in vivo* antitumor efficacy of irinotecan.

DETAILED DESCRIPTION OF THE INVENTION

[0100] 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.

[0101] 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.

[0102] 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:

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(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 cytorreduction 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.

[0103] 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.

[0104] 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.

[0105] 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.

[0106] 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.

[0107] 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- κ B ligand (RANKL), a ligand which engages Receptor activator of nuclear factor- κ B (RANK) and promotes osteoclast differentiation, Treg development, and tumor metastases. In addition, Tregs express other surface molecules; (v) LAG-3, a CD4-related molecule that binds MHC class II; (vi) glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); and (vii) IL-10. The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to bind a targeted molecule expressed by Tregs or myeloid suppressor cells while concurrently sequestering and inhibiting one or more immunosuppressive molecule that promotes their development, survival or function. In one aspect, the targeted immunostimulatory antibodies and/or fusion proteins directly deplete the number of Tregs.

[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 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).

[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 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.

[0110] 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- κ B (RANK) ligand (RANKL); (iv) Vascular endothelial growth factor (VEGF); (v) Transforming growth factor-beta receptor (TGF- β R); (vi) Programmed death-1 (PD-1); and (vii) Receptor activator of nuclear factor- κ B (RANK).

[0111] 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.

[0112] In one aspect, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: (i) CD4; (ii) CD25 (IL-2 α receptor; IL-2 α R); (iii) cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); (iv) Interleukin-10 (IL-10); (v) Transforming growth factor-beta receptor (TGF- β R); (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- κ B (RANK); (x) Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL); (xi) LAG-3; or (xii) glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18).

[0113] 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- κ B (RANK) ligand (RANKL); or (iv) vascular endothelial growth factor (VEGF).

[0114] 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- β RII, TGF- β RIIb, or TGF- β RIII. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- β RII. 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 .

[0115] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- β RII. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

[0116] 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- β RII. In another aspect, the molecule includes PD-1 ectodomain, immunoglobulin Fc region, and TGF β RII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

[0117] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- κ B (RANK) or Receptor activator of nuclear factor- κ B ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- β RII. In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and TGF β RII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

[0118] 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).

[0119] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4. In an additional aspect, the immunomodulatory moiety

includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.

[0120] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- κ B (RANK) or Receptor activator of nuclear factor- κ B ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Programmed Death-1 (PD-1). In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and PD-1 ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 25 or 26.

[0121] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Receptor activator of nuclear factor- κ B ligand (RANKL). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes a RANKL-binding amino acid sequence corresponding to SEQ ID NO: 95, 96, 97, or 98. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Receptor activator of nuclear factor- κ B ligand (RANKL).

[0122] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36.

[0123] 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.

[0124] 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.

[0125] The targeted immunosuppressive antibodies and/or fusion proteins of the invention provides their ability to inhibit the activity of targeted pro-inflammatory cytokines or immune cells while simultaneously promoting immune tolerance via the targeted delivery of immunosuppressive molecules that facilitate the development and/or function of regulatory T cells. These molecules of the present invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) The molecules of the invention enable targeted delivery of immunosuppressive molecules to immune cells or pro-inflammatory molecules in the milieu of the affected cell, tissue or organ; (ii) The molecules of the invention can couple the inhibition of the targeted pro-inflammatory molecule or immune cell with the

simultaneous delivery of an immunosuppressive molecule that promotes immune tolerance, thereby improving the suppression of immune effector cells; and (iii) The molecules of the invention can provide a mechanism of simultaneously engaging two independent or synergistic mechanisms of immune tolerance or immune suppression.

[0126] 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.

[0127] 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.

[0128] 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.

[0129] 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.

[0130] In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 46 or 47.

[0131] In one aspect, the targeting moiety includes transforming growth factor- β (TGF- β) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 48 or 49.

[0132] 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.

[0133] 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.

[0134] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25 (IL-2 α receptor; IL-2 α R), 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.

[0135] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C γ 1), 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.

[0136] 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- κ B (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.

[0137] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear

factor- κ B (RANK) or Osteoprotegerin (OPG). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

[0138] In one aspect, the targeting moiety includes a sequence from transforming growth factor- β (TGF- β) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

[0139] In one aspect, the targeting moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

[0140] In various aspects, the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

[0141] 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.

[0142] 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.

[0143] In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. In one aspect, the neoplastic disease is a non-T cell malignancy which does not express CD4 on the tumor cell. In one embodiment, the method includes administration to a subject in need thereof an antibody that targets and depletes CD4⁺ regulatory T cells (Tregs) in combination with a cytotoxic anticancer therapy. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In various aspects, the cytotoxic anticancer therapy includes a chemotherapeutic

molecule, tumor-targeted antibody, small molecule kinase inhibitor, hormonal agent, or tumor-targeted cytotoxic agent, anti-angiogenic agent or any combination thereof. In another aspect, the cytotoxic anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

[0144] 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.

[0145] 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.

[0146] In one aspect the chemotherapeutic agent is a topoisomerase-interacting agent, anthracycline, doxorubicin, mitoxantrone, camptothecin, camptothecin analogue, irinotecan, epipodophylotoxin, etoposide, alkylating agent, cyclophosphamide, cisplatin, cisplatin analogue, oxaliplatin, antimetabolite, fluoropyrimidine analogue, 5-fluorouracil, gemcitabine, azacytidine, antimicrotubule agent, taxane, paclitaxel, or docetaxel.

[0147] 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.

[0148] 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.

[0149] 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.

[0150] 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.

[0151] 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.

[0152] 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.

[0153] 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.

[0154] 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).

[0155] 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.

[0156] As used herein, “subject,” including grammatical variations thereof, means a human or vertebrate animal including a dog, cat, horse, cow, pig, sheep, goat, chicken, monkey, rat, and mouse.

[0157] 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.

[0158] 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.

[0159] For example, in various embodiments, a targeting moiety is specific for or binds to a molecule or component, which includes but is not limited to, epidermal growth factor receptor (EGFR, EGFR1, ErbB-1, HER1), ErbB-2 (HER2/neu), ErbB-3/HER3, ErbB-

4/HER4, EGFR ligand family; insulin-like growth factor receptor (IGFR) family, IGF-binding proteins (IGFBPs), IGFR ligand family (IGF-1R); platelet derived growth factor receptor (PDGFR) family, PDGFR ligand family; fibroblast growth factor receptor (FGFR) family, FGFR ligand family, vascular endothelial growth factor receptor (VEGFR) family, VEGF family; HGF receptor family; TRK receptor family; ephrin (EPH) receptor family; AXL receptor family; leukocyte tyrosine kinase (LTK) receptor family; TIE receptor family, angiopoietin 1, 2; receptor tyrosine kinase-like orphan receptor (ROR) receptor family; discoidin domain receptor (DDR) family; RET receptor family; KLG receptor family; RYK receptor family; MuSK receptor family; Transforming growth factor alpha (TGF- α), TGF- α receptor; Transforming growth factor-beta (TGF- β), TGF- β receptor; Interleukin 13 receptor alpha2 chain (IL13Ralpha2), Interleukin-6 (IL-6), IL-6 receptor, Interleukin-4, IL-4 receptor, Cytokine receptors, Class I (hematopoietin family) and Class II (interferon/IL-10 family) receptors, tumor necrosis factor (TNF) family, TNF- 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-Lfucosyltransferase (LDLR/FUT) fusion protein, HLA-A2, arginine to isoleucine exchange at residue 170 of the alpha-helix of the alpha2-domain in the HLA-A2 gene (HLA-A*201-R170I), MLA-A11, heat shock protein 70-2 mutated (HSP70-2M), KIAA0205, MART2, melanoma ubiquitous mutated 1,2, 3 (MUM-1, 2,3), prostatic acid phosphatase (PAP), neo-PAP, Myosin class 1, NFYC, OGT, OS-9, pml-RARalpha fusion protein, PRDX5, PTPRK, K-ras (KRAS2), N-ras (NRAS), HRAS, RBAF600, SIRT2, SNRPD1, SYT-SSX1 or -SSX2 fusion protein, Triosephosphate Isomerase, BAGE, BAGE-1, BAGE-2,3,4,5, GAGE-1,2,3,4,5,6,7,8, GnT-V (aberrant N-acetyl glucosaminyl transferase V, MGAT5), HERV-K-MEL, KK-LC, KM-HN-1, LAGE, LAGE-1, CTL-recognized antigen on melanoma (CAMEL), MAGE-A1 (MAGE-1), MAGE-A2, MAGE-

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A3, MAGE-A4, MAGE-AS, MAGE-A6, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-3, MAGE-B1, MAGE-B2, MAGE-B5, MAGE-B6, MAGE-C1, MAGE-C2, mucin 1 (MUC1), MART-1/Melan-A (MLANA), gp100, gp100/Pmel17 (SILV), tyrosinase (TYR), TRP-1, HAGE, NA-88, NY-ESO-1, NY-ESO-1/LAGE-2, SAGE, Sp17, SSX-1,2,3,4, TRP2-INT2, carcino-embryonic antigen (CEA), Kallikrein 4, mammaglobin-A, OA1, prostate specific antigen (PSA), prostate specific membrane antigen, TRP-1/gp75, TRP-2, adipophilin, interferon inducible protein absent in melanoma 2 (AIM-2), BING-4, CPSF, cyclin D1, epithelial cell adhesion molecule (Ep-CAM), EpcA3, fibroblast growth factor-5 (FGF-5), glycoprotein 250 (gp250) intestinal carboxyl esterase (iCE), alpha-feto protein (AFP), M-CSF, mdm-2, MUC1, p53 (TP53), PBF, FRAME, PSMA, RAGE-1, RNF43, RU2AS, SOX10, STEAP1, survivin (BIRC5), human telomerase reverse transcriptase (hTERT), telomerase, Wilms' tumor gene (WT1), SYCP1, BRDT, SPANX, XAGE, ADAM2, PAGE-5, LIP1, CTAGE-1, CSAGE, MMA1, CAGE, BORIS, HOM-TES-85, AF15q14, HCA66I, LDHC, MORC, SGY-1, SPO11, TPX1, NY-SAR-35, FTHL17, NXF2 TDRD1, TEX 15, FATE, TPTE, immunoglobulin idiotypes, Bence-Jones protein, estrogen receptors (ER), androgen receptors (AR), CD40, CD30, CD20, CD19, CD33, CD4, CD25, CD3, cancer antigen 72-4 (CA 72-4), cancer antigen 15-3 (CA 15-3), cancer antigen 27-29 (CA 27-29), cancer antigen 125 (CA 125), cancer antigen 19-9 (CA 19-9), beta-human chorionic gonadotropin, 1-2 microglobulin, squamous cell carcinoma antigen, neuron-specific enolase, heat shock protein gp96, GM2, sargramostim, CTLA-4, 707 alanine proline (707-AP), adenocarcinoma antigen recognized by T cells 4 (ART-4), carcinoembryonic antigen peptide-1 (CAP-1), calcium-activated chloride channel-2 (CLCA2), cyclophilin B (Cyp-B), human signet ring tumor-2 (HST-2), Human papilloma virus (HPV) proteins (HPV-E6, HPV-E7, major or minor capsid antigens, others), Epstein-Barr virus (EBV) proteins (EBV latent membrane proteins—LMP1, LMP2; others), Hepatitis B or C virus proteins, and HIV proteins. A composition of the invention can further include the foregoing as a peptide/polypeptide and/or encoding the same.

[0160] 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.

[0161] 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 (anti-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) pancreatic carcinoma 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.

[0162] In one embodiment, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: CD4; CD25 (IL-2 α receptor; IL-2 α R); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF- β R); 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- κ B (RANK); Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); or Interleukin-4 receptor (IL-4R). In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

[0163] 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; TNFR4); 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.

[0164] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide, or peptide that specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a cytokine, cytokine receptor, co-stimulatory molecule, or co-inhibitory molecule that modulates the immune system. In another aspect, the targeting moiety specifically binds one of the following molecules: CD4; CD25 (IL-2 α receptor; IL-2 α R); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF- β R); 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- κ B (RANK); Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); Interleukin-4 receptor (IL-4R); tumor necrosis factor (TNF) superfamily; tumor necrosis factor- 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; TNFR4); OX40 ligand (OX40L; CD40); CD40 ligand (CD40L); CTLA-4; B7 family; B7-1 (CD80); B7-2 (CD86); B7-H3; B7-H4; GITR/AITR; GITRL/AITRL; BTLA; CD70; CD27; LIGHT; or HVEM. In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

[0165] Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as Zanolimumab (anti-CD4 mAb), Keliximab (anti-CD4 mAb); Ipilimumab (MDX-101; anti-CTLA-4 mAb); Tremilimumab (anti-CTLA-4 mAb); (Daclizumab (anti-CD25/IL-2R mAb); Basiliximab (anti-CD25/IL-2R mAb); MDX-1106 (anti-PD1 mAb); antibody to GITR; GC1008 (anti-TGF- 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- α 4 integrin); Vedolizumab (anti- α 4 β 7 integrin mAb); T1h (anti-CD6 mAb); Epratuzumab (anti-CD22 mAb); Efalizumab (anti-CD11a mAb); and Atacicept (extracellular domain of transmembrane activator and calcium-modulating ligand interactor fused with Fc).

[0166] 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- β R); 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- κ B (RANK); or Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL); or vascular endothelial growth factor (VEGF). In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR; TNFRSF18); GITRL/AITRL; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNFR4); 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 immunomodulatory moiety comprises an extracellular domain of CTLA-4. In one aspect, the immunomodulatory moiety is an agonist that increases the function of the bound molecule. In another aspect, the immunomodulatory moiety is an antagonist that inhibits the function of the targeted molecule.

[0167] 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- β RII, TGF- β RIIb, or TGF- β RIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor- κ B (RANK); vascular endothelial growth factor receptor (VEGFR1 or VEGFR2); or IL-10R. In another aspect, the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: tumor necrosis factor receptor 2 (TNFR2); 4-1BB (CD137); OX40 (CD134; TNFR4); 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.

[0168] 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.

[0169] 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.

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

[0171] 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.

[0172] 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- β RII, TGF- β RIIb, or TGF- β RIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor- κ B (RANK); or IL-10R. In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL. In another aspect, the specific targeted component of a regulatory T cell, myeloid suppressor cell, or dendritic cell is one of the following molecules: CD4; CD25 (IL-2 α receptor; IL-2 α R); 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- κ B (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); or Interleukin-4 receptor (IL-4R).

[0173] 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; TNFR4); CD40; IL-12R; or glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR; TNFRSF18); and the immunomodulatory moiety comprises a different molecule selected from the following: (i) an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF- β RII, TGF- β RIIb, or TGF- β RIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor- κ B (RANK); or IL-10R; or (ii) Fc-containing polypeptide or ligand that binds one of the following: CTLA-4; 4-1BB (CD137); OX40 (CD134; TNFR4); CD40; IL-12R; or GITR (AITR; TNFRSF18).

[0174] 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; TNFR4), 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- β RII, TGF- β RIIb, or TGF- β RIII), Programmed Death-1 (PD-1), Receptor activator of nuclear factor- κ B (RANK), or IL-10R. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is an immunomodulatory targeting moiety and Y is a different immunomodulatory moiety.

[0175] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds one of the following molecules: CD4; CD25 (IL-2 α receptor; IL-2 α R); 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.

[0176] 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; TNFR4), CD40, IL-12R or GITR/AITR (TNFRSF18).

[0177] 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; TNFR4), CD40, IL-12R or GITR/AITR (TNFRSF18).

[0178] 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; TNFR4), 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.

[0179] Antibodies: In one embodiment, the targeting moiety or fusion protein is an immunoglobulin. As used herein, the term "immunoglobulin" includes natural or artificial mono- or polyvalent antibodies including, but not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as

used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin ion can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2) or subclass of immunoglobulin molecule.

[0180] 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.

[0181] An antigen-binding antibody fragment, including single-chain antibody, may include the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. An antigen-binding fragment can also include any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. Also included is a Fc fragment, antigen-Fc fusion proteins, and Fc-targeting moiety. The antibody may be from any animal origin including birds and mammals. In one aspect, the antibody is, or derived from, a human, murine (*e.g.*, mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken. Further, such antibody may be a humanized version of an antibody. The antibody may be monospecific, bispecific, trispecific, or of greater multispecificity.

[0182] 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.

[0183] 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.

[0184] 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* (SEQ ID NO: 123) 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.

[0185] For example, a hybridoma can produce the polypeptides corresponding to SEQ. ID. NO: 1-69.

[0186] 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).

[0187] An intact antibody is one which includes an antigen-binding variable region as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variant thereof for any other modified Fc (*e.g.*, glycosylation or other engineered Fc).

[0188] The intact antibody may have one or more "effector functions" which refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region or any other modified Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor (BCR)); and cross-presentation of antigens by antigen presenting cells or dendritic cells. In one embodiment, the targeting antibody or Fc-containing fusion protein facilitates focused or preferential delivery of a immunomodulatory moiety to a target cell. In another aspect, a targeting antibody can induce death of the targeted cell or sensitize it to immune cell-mediated cytotoxicity. In another aspect, the Fc-fusion protein or antibody can facilitate delivery of the immunomodulatory moiety or immunogenic apoptotic material from antibody-bound tumor targets, or both, to an antigen presenting cells (APC) via interactions between their Fc and Fc receptors (on APC).

[0189] Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different “classes.” There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into “subclasses” (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy-chain constant domains that 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.

[0190] 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.

[0191] 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.

[0192] 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.

[0193] 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.

[0194] 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.

[0195] 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.

[0196] 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.

[0197] 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.

[0198] 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.

[0199] 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.

[0200] 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.

[0201] 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.

[0202] 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

[0203] 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.

[0204] 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.

[0205] 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.

[0206] 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.

[0207] 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 /10⁶ 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 TGF β RII and the Fc portion of the murine IgG1 heavy chain (TGF β RII:Fc). This fusion protein interferes with the binding of TGF- β to endogenous TGF β RII and functions as a stable TGF- β antagonist. ELISA assays confirmed the ability of TGF β RII: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 TGF β RII:Fc (1 μ g 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 TGF β RII: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 TGF β RII:Fc (1 μ g intratumoral). In contrast to treatment with either doxorubicin or TGF β RII:Fc alone, combined treatment with both agents was able to arrest the growth of MB49 tumors. These results indicate that tumor cell autonomous expression of TGF- 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.

[0208] 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).

[0209] 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.

[0210] 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. NKG2D (NK group 2, member D) is a lectin-like type II transmembrane stimulatory receptor used by NK cells, $\gamma\delta$ -TCR⁺ T cells and $\alpha\beta$ -TCR⁺ T cells for immune surveillance of tumors. Expression of mouse and human ligands for NKG2D is upregulated in transformed epithelial cell lines in response to genotoxic stress or stalled DNA replication, via activation of a DNA damage checkpoint pathway initiated by ATM (ataxia telangiectasia, mutated) or ATR (ATM- and Rad3-related) protein kinases. Treatment of CT26 mouse colon cancer cells with genotoxic chemotherapeutic agents resulted in upregulation of mouse NKG2D ligands of the retinoic acid inducible gene family (Rae1) (FIG. 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 CD8⁺ T cells, we investigated whether DNA damage-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 bearing CT26 tumors were administered an anti-CD4 antibody (Clone GK1.5) to deplete CD4⁺ T cells and/or an anti-CD8 antibody (Clone GK2.43) to deplete CD8⁺ T cells and then treated with irinotecan. Flow cytometric analyses confirmed the loss of CD4⁺CD25⁺FoxP3⁺ T cells in the spleen and draining lymph node of mice treated with anti-CD4 antibody (FIG. 57D). Antibody-mediated depletion of CD4⁺ T cells enhanced the percentage of tumor-reactive IFN-γ⁺CD8⁺ T cells in irinotecan-treated animals (FIG. 57E). Whereas treatment of CT26 tumor-bearing mice with irinotecan only slowed tumor growth, depletion of CD4⁺ T cells enhanced the response to irinotecan and arrested tumor growth (FIG. 57F). The ability of CD4⁺ T cell depletion to augment the antitumor efficacy of irinotecan was mediated by CD8⁺ T cells since antibody-mediated depletion of CD8⁺ T cells completely negated the *in vivo* antitumor effect of chemotherapy in CD4⁺ T cell-depleted mice (FIG. 57F).

[0211] 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

[0212] Exemplary Targeted Immunomodulatory Antibodies and Fusion Proteins

[0213] 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.

[0214] 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 (TGF β -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 (TGF β -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 (TGF β -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 (TGF β -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 (TGF β -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 (TGF β -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).

[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 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).

[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 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).

[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 Programmed death-1 (PD-1). SEQ ID NO: 37 provides a fusion protein including anti-tumor necrosis factor (TNF α) 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 C γ 1 + 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 C γ 1 -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 C γ 1), and a sequence from PD-1 ligand (PD-L1) (Oncostatin M signal peptide + CTLA-4 ECD + IgG C γ 1 + 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 C γ 1), and a sequence from the extracellular domain of CTLA-4: (PD-L1 + IgG C γ 1 + CTLA-4 ECD; FIG. 38). SEQ ID NO: 48 provides a fusion protein including Transforming growth factor- β (TGF- β), immunoglobulin Fc (IgG C γ 1), and a sequence from PD-1 ligand 1 (PD-L1) (TGF β -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 C γ 1), and Transforming growth factor beta (TGF- β) (PD-L1 + Fc + TGF β -1; FIG. 39).

[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 includes a molecule that specifically binds to Transforming growth factor-beta receptor (TGF- β R). 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 C γ 1 + 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 C γ 1 + 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 C γ 1), and a sequence from a sequence from transforming growth factor- β (TGF- β) (Oncostatin M signal peptide + CTLA-4 ECD + IgG C γ 1 + TGF- β 1; FIG. 47). SEQ ID NO: 60 provides a fusion protein including a sequence from Transforming growth factor- β (TGF- β), immunoglobulin Fc (IgG C γ 1), and a sequence from the extracellular domain of CTLA-4: (TGF- β 1 + IgG C γ 1 + CTLA-4 ECD) (FIG. 47).

[0219] 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- κ B 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 C γ 1 + 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 C γ 1 + TNFR2 ECD; FIG. 49). SEQ ID NO: 64 provides a fusion protein including the extracellular domain of CTLA-4, immunoglobulin Fc (IgG C γ 1), and a sequence from a sequence from RANK Ectodomain (Oncostatin M signal peptide + CTLA-4 ECD + IgG C γ 1 + RANK Ectodomain; FIG. 50). SEQ ID NO: 65 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG C γ 1), and a sequence from the extracellular domain of CTLA-4: (RANK Ectodomain + IgG C γ 1 + 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 C γ 1), and an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK) (TGF- β + IgG C γ 1 + RANK Ectodomain; FIG. 51). SEQ ID NO: 67 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG C γ 1), and a sequence from transforming growth factor- β (TGF- β): (RANK Ectodomain + IgG C γ 1 + 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 C γ 1), and an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK) (PD-L1 + IgG C γ 1 + RANK Ectodomain; FIG. 52). SEQ ID NO: 69 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG C γ 1), and a sequence from Programmed death-1 ligand 1 (PD-L1): (RANK Ectodomain + IgG C γ 1 + PD-L1) (FIG. 52).

[0220] 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 fusion molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:
 - a) the targeting moiety comprises a polypeptide that specifically binds a component of a tumor cell that is a tumor cell surface molecule; and
 - b) the immunomodulatory moiety comprises a ligand binding amino acid sequence of the extracellular domain of interleukin-10 receptor (IL-10R).
2. The fusion molecule of claim 1, wherein the targeting moiety specifically binds a tumor cell surface molecule selected from the group consisting of a tumor antigen, growth factor receptor, cytokine receptor, or co-inhibitory ligand.
3. The fusion molecule of claim 1, wherein the targeting moiety comprises an antigen-binding domain of an immunoglobulin, antibody, bispecific or multispecific antibody, antibody fragment, single chain variable fragment (scFv), bivalent or multivalent scFv, or Fc-containing polypeptide.
4. The fusion molecule of claim 1, wherein said immunomodulatory moiety is fused to the C-terminus or N-terminus of said targeting moiety.
5. The fusion molecule of claim 1, wherein the targeting moiety is fused to the immunomodulatory moiety via a linker.
6. The fusion molecule of claim 5, wherein the linker is (GGGGS)_n and wherein n is 1, 2, 3, 4, 5, 6, 7, or 8.
7. The fusion molecule of claim 5, wherein the targeting moiety is an Fc-containing polypeptide comprising a linker at the CH3 region of the Fc.
8. The fusion molecule of claim 3, wherein the targeting moiety that binds interleukin-4 receptor (IL4R).
9. The fusion molecule of claim 1, wherein the targeting moiety binds Programmed Death-1 ligand 1 (PD-L1) and/or Programmed Death-1 ligand 2 (PD-L2).

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10. The fusion molecule of claim 9, wherein the targeting moiety comprises a ligand binding amino acid sequence of the extracellular domain of Programmed-Death 1 (PD-1).

11. The fusion molecule of claim 10, wherein the targeting moiety comprises the amino acid sequence corresponding to SEQ ID NO: 93 or a ligand binding fragment thereof.

**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 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.

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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 *ASTIPPHVQK* *SVNNDMIVTD* *NNGAVKFPQL* *CKFCDVRFST*
CDNQKSCMSN *CSITSICEKP* *QEVCAVVRK* *NDENITLETV* *CHDPKLPYHD* *FILEDAASPK*
CIMKEKKKPG *ETFFMCSCSS* *DECNDNIIFS* *EEYNTSNPDL* *LLVIFQVTGI* *SLPPLGVAI*
SVIIIFYCYR VNRQQLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELKGQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQVVG
TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC WDHDPEARLT AQCVAERFSE
LEHLDRLSGR SCSEEKIPED GSLNTTK

[*Italic* – Extracellular domain of Transforming growth factor beta Receptor II (TGF β RII)]
[Underlined = TGF β 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 *ASTIPPHVQK* *SDVEMEAQKD* *EIICPSCNRT* *AHPLRHINND*
MIVTDNNGAV *KFPQLCKFCD* *VRFSTCDNQK* *SCMSNCSITS* *ICEKPQEVCAV* *AVWRKNDENI*
TLETVCHDPK *LPYHDFILED* *AASPKCIMKE* *KKKPGETFFM* *CSCSSDECND* *NIIFSEYNT*
SNPDL *LLVIF* *QVTGISLLPP* *LGVAISVIII* *FYCYRVNRQQ* *KLSSTWETGK* *TRKLMEFSEH*
CAIILEDERS DISSTCANNI NHNTPELLIE LDTLVGKGRF AEVYKAKLKQ NTSEQFETVA VKI FP
YEEYASWKTE KDIFSDINLK HENILQFLTA EERKTELKGQ YWLITAFHAK GNLQEYLTRH
VISWEDLRKL GSSLARGIAH LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL
SLRLDPTLSV DDLANSQVVG TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR
CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC
WDHDPEARLT AQCVAERFSE LEHLDRLSGR SCSEEKIPED GSLNTTK

[*Italic* – Extracellular domain of Transforming growth factor beta Receptor IIB (TGF β RIIB)]
[Underlined = TGF β 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 (Δ C terminus): TGF β RII lacking the last 38 amino acids from the C-terminus (SEQ ID NO: 81):

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

FIG. 1A

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

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND
MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS ICEKPQEVCV AVWRKNDENI
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT
SNPDLLLLVIF QVTGISLLPP LGVAISVIII FYCYRVNRQQ KLSSTWETGK TRKLMEFSEH
CAIILEDERS DISSTCANNI NHNTELLPIE LDTLVGKGRF AEVYKAKLKQ NTSEQFETVA VKI FP
YEEYASWKTE KDIFSDINLK HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH
VISWEDLRKL GSSLARGIAH LHSHTPCGR PKMPIVHRDL KSSNILVKND LTCCCLDFGL
SLRLDPTLSV DDLANSQVG TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR
CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC
WDHDPPEARL

(ii) TGF- β R-II (Δ cyt): TGF β RII lacking the kinase domain & juxtamembrane region (SEQ ID NO: 83):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETTV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDLLLVIFQVTGI SLLPPLGVAI
SVIIIFYCYR VNRQQKLSS

TGF- β R-IIB (Δ cyt): TGF β RIIB lacking the kinase domain & juxtamembrane region (SEQ ID NO: 84):

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND
MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS ICEKPQEVCV AVWRKNDENI
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT
SNPDLLLLVIF 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 NDENITLETTV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDLLLVIFQ

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

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND
MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS 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 NDENITLETTV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS
DECNDNIIFS EEYNTSNPD

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

TIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND 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 NDENITLETTV CHDPKLPYHD
FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD

FIG. 1B

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3. Kinase-deficient mutants, deletion mutants, or point mutants of Transforming growth factor beta Receptor II (TGF- β -RII) or TGF- β -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 QEVCAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDL LLVIFQVTGI SLLPPLGVAI
SVIIIFYCYR VNRQQLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVRIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQVVG
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 (Δ i)

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

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDL LLVIFQVTGI SLLPPLGVAI
SVIIIFYCYR VNRQQLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQVVG
TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
HPCVESMKDA SGIQMVCELT TECWDHDPEA RLTAQCVAER FSELEHLDRL SGRSCSEKI
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):

GGGSGGGSGGGGS

FIG. 1C

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

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

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKG
RFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **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):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFS
SGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSLTLSKADYEEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-HER2 antibody variable region

Plain: Anti-HER2 antibody constant region

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 2

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

Anti-EGFR1 heavy chain + TGF β -RII ECD fusion amino acid sequence

(SEQ ID NO: 2):

QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPF
LSINKDNSKQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTQVSAASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQ
TYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST**
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

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

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGSG
SGTDFTLINSVESEDIADYYCQNNNWPTTFGAGTKLELKR
TVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-EGFR antibody variable region

Plain: Anti-EGFR antibody constant region

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 3

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

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

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKG
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
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):

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPRFSGSGS
GTSYSLTISRVEAEDAATYYCQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Underlined: Anti-CD20 antibody variable region

Plain: Anti-CD20 antibody constant region

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 4

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

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

EVQLVESGGGLVQPGGSLRLSCAASGYTFSTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFKR
RFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYGGSSHWYFDVWGQGTLLTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSS
LGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTCTPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVTLHQDWLNGKEYKCKVKS
NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST**
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

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

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSRFSGSG
SGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-VEGF antibody variable region

Plain: Anti-VEGF antibody constant region

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 5

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

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

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

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

EIVLTQSPGT LSLSPGERAT LSCRASQSVG SSYLAWYQQK PGQAPRLLIY GAFSRATGIP
DRESGSGSGT DFTLTISRLE PEDFAVYICQ QYGSSPWTFG QGTKVEIKRT VAAPSVFIFP
PSDEQLKSGT ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYLSSTL
TLSKADYEKH KVIACEVTHQ GLSSPVTKSF NRGEC

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

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

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 6

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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 *GGGGSGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS* **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 EYNTSNPD *GGGGSGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T

Bold: TGFβRII ectodomain

Italic: Linker (Optional; other linker sequences)

Plain: Fc

Underlined: IL-2 fragment

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

EPKSCDK SEQ ID NO: 105)

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

FIG. 7

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

Anti-CD25 (Daclizumab) heavy chain and TGF β -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 KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK *GGGGSGGGSGGGGS* **TIPPHVQK SVNNDMIVTD**
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCAVWRK NDENITLETV
CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD

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

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

Plain: Anti-CD25 antibody

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 8A

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Anti-CD25 (Basiliximab) heavy chain and TGF β -RII Extracellular domain

(SEQ ID NO: 9):

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDYGY YFDFWGQGT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTL PPSRDELTKNQ
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
FSCSVMEAL HNHYTQKSL LSPGK *GGGGSGGGSGGGGS* **TIPPHVQK SVNNDMIVTD**
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCAVVRK NDENITLET
CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD

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

QIVSTQSPAI MSASPGKVT MTCSASSRS YMQWYQQKPG TSPKRWIYDT SKLASGVPAR
FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE
QLKSGTASVV CLLNFPYPR AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK
ADYEKHKVYA CEVTHQGLSS PVTKSFNRGE

Plain: Anti-CD25 antibody

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 8B

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

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

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSISIDTSKNLFSCLKRSVTAADTAVYYCASNILKYLHWLLYWGQGVLTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVHLQDNLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRF**
STCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCI
EKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTLVLRGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

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

Italic: Linker

Bold: TGF β RII ectodomain

FIG. 9

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

PD-1 ectodomain + Fc + TGF β RII ectodomain (SEQ ID NO: 11):

PGWFLDSPDRPWNPPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSTYLCAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVW
RKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYN
TSNPD

TGF β RII ectodomain + Fc + PD-1 ectodomain (SEQ ID NO: 12):

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVW
RKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYN
TSNPD *GGGGSGGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS*
PGWFLDSPDRPWNPPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSTYLCAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV

Underlined: PD-1 ectodomain

Italic: Linkers

Plain: IgG1 Fc

Bold: TGF β RII ectodomain

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

FIG. 10

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

TGF β RII ectodomain – Fc - RANK ectodomain (SEQ. ID. NO: 13):

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVW
RKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYN
TSNPDGGGSGGGSGGGSGGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMEALHNHYTQKSLSLSPGK GGGSGGGSGGGSGGG QI
APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

RANK ectodomain – Fc - TGF β RII ectodomain (SEQ. ID. NO: 14):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
GGGSGGGSGGGSGGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNV FSCSVMEALHNHYTQKSLSLSPGKGGGSGGGSGGGSGGGSTIPPHVQKS
VNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITL
ETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

Bold: TGF β RII ectodomain

Plain: IgG1 Fc

Italics: Linker

Underlined: RANK ectodomain

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

FIG. 11

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

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

Italic: PD-1 ectodomain

Underlined Italic: ligand-binding domain

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

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

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

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

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

FIG. 12

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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
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **PGWFLDSPDR PWNPTFSPA 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):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSSGSR
SGTDFTLTISSLPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-HER2/neu Variable region

Plain: Anti-HER2/neu Constant region

Italic: Linker

Bold: PD-1 ectodomain

FIG. 13

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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):

QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPFTSR
LSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGTLLTVSAASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQ
TYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS**
ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT
YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV

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

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSG
SGTDFTLINSVESEDIADYYCQQNNNWPTTFGAGTKLELKR
TVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-EGFR Variable region

Plain: Anti-EGFR Constant region

Italic: Linker

Bold: PD-1 ectodomain

FIG. 14

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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
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS PGWFLDSPDR PWNPTFSPA LLVTEGDNA TFTCSFSNTS
ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCFRFVTQL PNGRDFHMSV VRARRNDSGT
YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFAQKPGSSPKPWIYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Underlined: Anti-CD20 Variable region

Plain: Anti-CD20 Constant region

Italic: Linker

Bold: PD-1 ectodomain

FIG. 15

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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
PLAPSSKSTSGGTAALGCLVKDYFPEPVTSVNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS
LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **PGWFLDSPDR PWNPTFSPA LLVTEGDNA TFTCSFSNTS**
ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT
YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV

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

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSG
SGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-VEGF Variable region

Plain: Anti-VEGF Constant region

Italic: Linker

Bold: PD-1 ectodomain

FIG. 16

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

Anti-(human CTLA-4) (human γ 1-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 ISYDGNKYY
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGO GTLTVVSSAS
TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL
YSLSSVTVTP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPELLGGPS
VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ
GNVFSCSVMH EALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS* **PGWFLDSPDR**
PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNMYRM SPSNQTDKLA AFPEDRSQPG
QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA ELRVTERAE
VPTAHPSPSP RPAGQFQTLV

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

EIVLTQSPGT LSLSPGERAT LSCRASQSVG SSYLAWYQQK PGQAPRLLIY GAFSRATGIP
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPWTFG QGTEVEIKRT VAAPSVFIFP
PSDEQLKSGT ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYSLSSTL
TLISKADYEKH KVIYACEVTHQ GLSSPVTKSF NRGEC

Underlined: Anti-CTLA-4 Variable region

Plain: Anti-CTLA-4 Constant region

Italic: Linker

Bold: PD-1 ectodomain

FIG. 17

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

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDYGY YFDFWQGGTT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL
FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV
VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTL PPSRDELTKNQ
VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
FSCSVMHEAL HNHYTQKSLS LSPGK *GGGGSGGGSGGGGS*
PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNT
SESFVLNWRMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRAR
RNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLV

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

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

Plain: Anti-CD25 antibody

Italic: Linker

Bold: PD-1 ectodomain

FIG. 18B

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

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

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T *GGGGSGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGYLCAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV

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

PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGYLCAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV *GGGGSGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T

Underlined: IL-2 fragment

Italic: Linker

Plain: Fc

Bold: human PD-1 ectodomain

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

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

FIG. 19

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

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

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSI SIDTSKNLFSLKLRSVTAADTAVYYCASNILKYLHWLLYWGQGVLT VSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
PGWFLDSPDRPWNPPTFSPALLV VTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRND SGTYLCGAISLAPKAQIKESLRAELRV TERRAEVP
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

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

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

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
GGGSGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK GGGSGGGSGGGGS
PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWRMSPSNQTDKLAAPEDR
SQPGQDCRFRTQLPNGRDFHMSVVRARRNDSGYLCAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV

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

PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWRMSPSNQTDKLAAPEDR
SQPGQDCRFRTQLPNGRDFHMSVVRARRNDSGYLCAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLVGGGSGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK GGGSGGGSGGGGS QI APPCTSEKHY EHLGRCCNKC
EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR
RACTAGYHW 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

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Moieties that bind Receptor activator of nuclear factor- κ B (RANK) ligand

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

1 MAPRARRRRP LFALLLLCAL LARLQVALQI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC
61 TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW
121 SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
181 RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPGLIILLFF ASVALVAII FGVCYRKKGK
241 ALTANLWHWI NEACGRLSGD KESSGDSCVS THTANFGQQG ACEGVLLLTLE EKTTFPEDMC
301 YPDQGGVCQG TCVGGGPYAQ GEDARMLSLV SKTEIEEDSF RQMPTEDEYM DRPSQPTDQL
361 LFLTEPGSKS TPPFSEPLEV GENDSLSQCF TGTQSTVGSE SCNCTEPLCR TDWTPMSEN
421 YLQKEVD SGH CPHWAASPS NWADVCTGCR NPPGEDCEPL VGSPKRGPLP QCAYGMGLPP
481 EEEASRTEAR DQPEDGADGR LPSSARAGAG SGSSPGGQSP ASGNVTGNSN STFISSGQVM
541 NFKGDIIVVY VSQTSQEGAA AAAEPMGRPV QEETLARDS FAGNGPRFPD PCGGPEGLRE
601 PEKASRPVQE QGGAKA

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

(ii) RANK extracellular domain (ectodomain) or fragment thereof (SEQ. ID. NO: 96):

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

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

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

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

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

(iv). Mutant of RANK or a fragment thereof which binds Receptor activator of nuclear factor- κ B (RANK) ligand (RANKL)

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

1 MNKLLCCALV FLDISIKWTT QETFPKYLH YDEETSHQLL CDKCPPGYL KQHCTAKWKT
61 VCAPCPDHYY TDSWHTSDEC LYCSPVCKEL QYVKQECNRT HNRVCECKEG RYLEIEFCLK
121 HRSCPPGFGV VQAGTPERN T VCKRCPDGGF SNETSSKAPC RKHTNCSVFG LLLTQKGNAT
181 HDNICSGNSE STQKCGIDVT LCEEAFRFA VPTKFTPNWL SVLVDNLPGT KVNAESVERI
241 KRQHSSQEQT FQLLKLWKHQ NKAQDIVKKI IQDIDLCE NS VQRHIGHANL TFEQLRSLME
301 SLPGKKVGAE DIEKTIKACK PSDQILKLLS LWRIKNGDQD TLKGLMHALK HSKTYHFPKT
361 VTQSLKKTIR FLHSFTMYKL YQKLFLEMIG NQVQSVKISC L

Bold: RANK ligand-binding domain

FIG. 22

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

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

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISA
DTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK
KVEPKSCDKTHTCTPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMH
EALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW
SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSSGSRSGTDF
LTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSSTLSKLTKADYERHKVYACEVTHQGLSSPVTKSFNRGEC

Underlined: Anti-HER2 antibody variable region

Plain: Anti-HER2 antibody constant region

Italics: Linker

Bold: RANK ectodomain

FIG. 23

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

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

QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGNTDYNTPTTSRLSINKD
NSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTVTSAASTKGPSVFPLAPSSKSTSGGTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKR
VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQVMHE
ALHNHYTQKSLSLSPGK **GGGGSGGGSGGGGS** **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW
SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFT
LSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKR
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC

Underlined: Anti-EGFR antibody variable region

Plain: Anti-EGFR antibody constant region

Italics: Linker

Bold: RANK ectodomain

FIG. 24

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

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

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKGKATLTA
DKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVM
HEALHNHYTQKSLSLSPGK **GGGGSGGGSGGGGS** **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW
SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGSGTSYSL
TISRVEAEDAATYYCQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSSTLSLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC

Underlined: Anti-CD20 antibody variable region

Plain: Anti-CD20 antibody constant region

Italics: Linker

Bold: RANK ectodomain

FIG. 25

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

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

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADF
KRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWFYFDVWGQGLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSFSVMHEALHNHYTQKSLSLSPGK
GGGSGGGSGGGSG **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP**
CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRRN
TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS
DAVCSSSLPA RKPPNEPHVY LPG

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

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSG
TDFTLTISLQPEDFATYYCQOYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEEKHKVYACEVTHQG
LSSPVTKSFNRGEC

Underlined: Anti-VEGF antibody variable region

Plain: Anti-VEGF antibody constant region

Italics: Linker

Bold: RANK ectodomain

FIG. 26

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

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

QVQLVESGGG VVQPGRLRL SCAASGFTFS SYTMHWVRQA PGKGLEWVTF ISYDGNKYY
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGO GTLVTVSSAS
TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL
YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPELLGGPS
VFLFPPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPPVL SDGSFFLYSK LTVDKSRWQQ
GNVFSCSVMH EALHNHYTQK SLSLSPGK *GGGSGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC**
EPGKYMSSKC TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR
RCACTAGYHW SQDCECCRN 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 DSTYSLSSLT
TLISKADYEKH KVIACEVTHQ GLSSPVTKSF NRGEK

Underlined: Anti-CTLA-4 antibody variable region

Plain: Anti-CTLA-4 antibody constant region

Italics: Linker

Bold: RANK ectodomain

FIG. 27

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Fusion proteins comprising anti-CD25 antibody and Receptor activator of nuclear factor- κ B (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 KVS NKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN
VFSCSVMEHA LHNHYTQKSL SLSPGK GGGGSGGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC**
EPGKYMSSKC TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR
RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKD TV CKPCLAGYFS DAFSSTDKCR
PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

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

Plain: Anti-CD25 antibody

Italics: Linker

Bold: RANK ectodomain

FIG. 28A

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

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

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

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

QIVSTQSPAI MSASPGEKVT MTCSASSRS YMQWYQQKPG TSPKRWIYDT SKLASGVPAR
FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE
QLKSGTASVV CLLNFFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDYSTY SLSSTLTLSK
ADYEKHKVYA CEVTHQGLSS PVTKSFNREGC

Plain: Anti-CD25 antibody

Italics: Linker

Bold: RANK ectodomain

FIG. 28B

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

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

APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGSGGGGSG
GGGSTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHN
HYTQKSLSLSPGK GGGSGGGSGGGSG **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW
SQDCECCRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
GGGSGGGSGGGSGTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSREMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
SCSVMHEALHNHYTQKSLSLSPGKGGGSGGGSGGGSGAPTSSSTKKTQLQLEHLLLDLQMLNGINNYKN
PKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMC
EYADETATIVEFLNRWITFCQSIISTLT

Bold: RANK ectodomain

Italics: Linker (Optional; any linker sequence)

Plain: Fc

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

(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

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

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

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSISIDTSKNLFSCLKRSVTAADTAVYYCASNILKYLHWLLYWGQGVLTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP**
CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRRN
TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS
DAVCSSSLPA RKPPNEPHVY LPG

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

Underlined: Anti-CD4 antibody variable region

Italic: Linker

Bold: PD-1 ectodomain

FIG. 30

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

MRIFAVFIFM TYWHLNNAFT VTVPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME
DKNIIQFVHG EEDLKVQHSS YRQARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG
ADYKRITVKV NAPYNKINQR ILVDPVTSE HELTCQAEY PKAEVIWTSS DHQVLSGKTT
TTNSKREEKL FNVSTLRLN TTTNEIFYCT FRRLDPEENH TAEIVIPELP 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 YRQARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV
NAPYNKINQR ILVDPVTSE HELTCQAEY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL
FNVSTLRLN TTTNEIFYCT FRRLDPEENH TAEIVI

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

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

RIFAVFIFM TYWHLNNAFT VTVPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME
DKNIIQFVHG EEDLKVQHSS YRQARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG
ADYKRITVKV NAPYNKINQR ILVDPVTSE HELTCQAEY PKAEVIWTSS DHQVLSGKTT
TTNSKREEKL FNVSTLRLN TTTNEIFYCT FRRLDPEENH TAEIVIPELP LAHPPNERTH
LVILGAILLC LGVALTFIFR LRKGRMMDVK KCGIQDTNSK KQSDTHLEET

Bold: PD-L1 extracellular domain (ectodomain)

Underlined Bold: Ligand Binding domain

FIG. 31

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

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

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSV
EG
RFTISRDNAKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGT
LVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVD**KKAE**PKSCDKHTCTPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
GGGSGGGSGGGGS
RIFAVFIFMTYWHLLNAFTVTVPKDLVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQF
VHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVDVPTSEHELTCAEGYPAEVIWTSDDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

Anti-TNF α light chain (SEQ ID NO: 78):

DIQMTQSPSSLSASVGRVTITCRASQGIRNYLAWYQQLP
GGKAPKLLIYAAS
TLQSGVPSRFS
SGSG
SGTDF
TLTIS
SLQPEDVATYYCQRYNRPYT
FGQGTKVEIK
RTVAAPS
VFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: anti-TNF α antibody variable region

Plain: anti-TNF α 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

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

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

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKEGCRLCAPLRKCRPGFGVARPGT
ETSDVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPLMPGSPPAEGSTGD *EPKSCDK*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
RIFAVFIFMTYWHLNNAFTVTVPKDLVVEYGSNMTIECKFPVEKQDLAALIVWEMEDKNIIQF
VHGEEDLVQHSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
YNKINQRIILVDPVTSEHELTCQAEQYPAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
NTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

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

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

MRIFAVFIFMTYWHLNNAFTVTVPKDLVVEYGSNMTIECKFPVEKQDLAALIVWEMEDKNIIQF
FVHGEEDLVQHSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
PYNKINQRIILVDPVTSEHELTCQAEQYPAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
INTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFR
LRKGRMMDVKKCGIQDTNSKKQSDTHLEET *GGGGSGGGSGGGGS* *EPKSCDK*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKEGCRLCAPLRKCRPGFGVARPGT
ETSDVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPLMPGSPPAEGSTGD

Underlined: TNFR2 ligand-binding domain

Plain: human IgG1 heavy chain constant region (IgG C γ 1 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 GGGGSGGGSGGGGS (SEQ. ID. NO: 104))

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

FIG. 33

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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
TQTYICNVNHKPSNTKVDKKAEPKSCDKHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
GGGSGGGSGGGGS
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVDVPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Underlined: anti-CD20 antibody variable region

Plain: anti-CD20 antibody constant region

Italic: Linker

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

FIG. 34

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

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

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY
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 KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK GGGGSGGGGSGGGGS

RIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECK

FPVEKQLDLAALIVYWEMEDKNIIQFVHGEECLKVQHSSYRQRARLLKDQLSLGNA

ALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVDPVTSEHEL

TCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYC

TFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR

MMDVKKCGIQDTNSKKQSDTHLEET

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

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

Plain: anti-CD25 antibody

Italic: Linker (optional and may be replaced)

Human PD-1 ligand 1 or extracellular binding domain of PD-L1 (underlined)

FIG. 35A

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

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

RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECK

FPVEKQLDLAALIVWEMEDKNIIQFVHGEECLKVQHSSYRQARLLKDQLSLGNA

ALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVDPVTSEHEL

TCQAEYGPKAQEVITWSSDHQVLSGKTTTNSKREEKLFNVTSTLRINTTTNEIFYC

TFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR

MMDVKKCGIQDTNSKKQSDTHLEET

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

QIVSTQSPAI MSASPGKVT MTCSASSRS YMQWYQQKPG TSPKRWIYDT SKLASGVPAR
FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGK LEIKRTVAAP SVFIFPPSDE
QLKSGTASVV CLLNMFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK
ADYEKHKVYA CEVTHQGLSS PVTKSFNRGE

Plain: anti-CD25 antibody

Italic: Linker

Human PD-1 ligand 1 or extracellular binding domain of PD-L1 (underlined)

FIG. 35B

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

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

MRIFAVFIFMTYWHLLNNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNI IQFVHGEE
DLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKV
NAPYNKINQRILVVDPVTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTNSKREEKLFNVTSTLRINTT
TNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFR
LRKGRMDVKKCGIQDTNSKKQSDTHLEET GGGGSGGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHEALHNHYTQ
KSLSLSPGK GGGGSGGGGSGGGGS
APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

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

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
GGGGSGGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHEALHNHYTQ
KSLSLSPGK GGGGSGGGGSGGGGS
RIFAVFIFMTYWHLLNNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNI IQFVHGEE
LKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD
PVTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMDVKKCGIQDTNSKKQSDTHLEE
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

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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
NRVSISIDTSKNLFSCLKRSVTAADTAVYYCASNILKYLHWLLYWGQGVLTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
GGGSGGGSGGGGS
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVD PVTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTNSKREEKLFNVTSTLRI
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
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

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

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

MGVLLTQRTLLSLVLALLFSPMASMAMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRLQA
DSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPIYLGI
GNGTQIYVIDPEPCPDS QEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
RIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQDLAALIVWEMEDKNIIQF
VHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
YNKINQIRILVVDVPTSEHELTCAEGYPKAEVIWTSDDHQVLSGKTTTTNSKREEKLFNVTSTLR
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

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

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

MRIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQDLAALIVWEMEDKNIIQF
FVHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
PYNKINQIRILVVDVPTSEHELTCAEGYPKAEVIWTSDDHQVLSGKTTTTNSKREEKLFNVTSTLR
INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFR
LRKGRMMDVKKCGIQDTNSKKQSDTHLEET GGGGSGGGGSGGGGS
QEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRLQADSQVTEVCAATYMMGNELTFLDDSI
CTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPIYLGI GNGTQIYVIDPEPCPDS

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

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

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

Italic: Linker (optional)

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

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

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

FIG. 38

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

TGF- β -1 + Fc + PD-L1 (SEQ ID NO: 48):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS GGGGSGGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
RIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNI IQF
VHGEEDLKVOHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVDVPTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKRGR
MMDVKKCGIQDTNSKKQSDTHLEET

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

PD-L1 + Fc + TGF- β -1 (SEQ ID NO: 49):

MRIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNI IQF
FVHGEEDLKVOHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
PYNKINQRILVVDVPTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKG
RMMDVKKCGIQDTNSKKQSDTHLEET GGGGSGGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Underlined: TGF- β -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

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

TGF- β 1 full sequence (SEQ ID NO: 102):

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

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

(ii) Mature (active) TGF- β 1 sequence (Ala 279 – Ser 390; 112 aa)

(SEQ ID NO: 103):

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

FIG. 40

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

Anti-TNF α heavy chain + TGF- β 1 (SEQ ID NO: 50):

EVQLVESGGGLVQPGRSRLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSVEG
RFTISRDNAKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGTLLTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

GGGSGGGSGGGGS

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Anti-TNF α light chain (SEQ ID NO: 78):

DIQMTQSPSSLSASVGDRTITCRASQGIRNYLAWYQQLPKGKAPKLLIYAASLTQSGVPSRFSGSG
SGTDFTLTITSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: anti-TNF α antibody variable region

Plain: anti-TNF α antibody constant region

Italic: Linker

Bold: Mature (active) human TGF- β 1

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

FIG. 41

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

TNFR2 ECD + IgG C γ 1 + TGF- β 1 (SEQ ID NO: 51):

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSSDQVETQACTREQNRICTRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPMGSPSPAEGSTGD *EPKSCDK*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVHLQDNLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**E****M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPASAAAPCCVPQALEPLPIVYVGRKPKVEQLSNMIVRSCKCS

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

TGF- β 1 + IgG C γ 1 + TNFR2 ECD (SEQ ID NO: 52):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPASAAAPCCVPQALEPLPIVYVGRKPKVEQLSNMIVRSCKCS *GGGSGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVHLQDNLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
R**E****M**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSSDQVETQACTREQNRICTRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPMGSPSPAEGSTGD

Underlined: TNFR2 ligand-binding domain

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

Italic: Linker

Bold: Mature (active) human TGF- β 1

(Note: Can replace linker 1 with *GGGSGGGSGGGGS* 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

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Fusion proteins comprising anti-CD20 antibody and a sequence from transforming growth factor- β (TGF- β)

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

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKG
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK

GGGGSGGGGSGGGGS

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Underlined: anti-CD20 antibody variable region

Plain: anti-CD20 antibody constant region

Italic: Linker

Bold: Mature (active) human TGF- β 1

FIG. 43

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

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

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

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

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

Plain: anti-CD25 antibody

Italic: Linker

Bold: Mature (active) human TGF- β 1

FIG. 44A

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

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

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

QIVSTQSPAI MSASPGKVT MTCSASSSRS YMOWYQQKPG TSPKRWIYDT SKLASGVPAR
FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE
QLKSGTASVV CLLNFPYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK
ADYEKHKVYA CEVTHQGLSS PVTKSFNRGE

Plain: anti-CD25 antibody

Italic: Linker

Bold: Mature (active) human TGF- β 1

FIG. 44B

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

TGF- β 1 + Fc + IL-2 (SEQ ID NO: 56):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAA**PCCVPQ**ALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS *GGGGSGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMH~~E~~ALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T

IL-2 + Fc + TGF- β 1 (SEQ ID NO: 57):

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T *GGGGSGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMH~~E~~ALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAA**PCCVPQ**ALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Underlined: IL-2 fragment

Italic: Linker (optional)

Plain: Fc

Bold: Mature (active) human TGF- β 1

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

EPKSCDK SEQ ID NO: 105)

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

FIG. 45

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

Anti-CD4 heavy chain and TGF- β 1 (SEQ ID NO: 58)

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSIIDTSKNLFSCLKRSVTAADTAVYYCASNILKYLHWLLYWGQGLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS
ALDTNYCFSSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNP GASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTLVLRGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

Underlined: Anti-CD4 antibody variable region

Plain: Anti-CD4 antibody constant region

Italic: Linker

Bold: Mature (active) human TGF- β 1

FIG. 46

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Fusion proteins comprising the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C γ 1), and a sequence from a sequence from transforming growth factor- β (TGF- β)

Oncostatin M signal peptide + CTLA-4 ECD + IgG C γ 1 + TGF- β 1 (SEQ ID NO: 59):

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQA
DSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPYYLGI
GNGTQIYVIDPEPCPDSDQEPKSCDK THTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNP GASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Fusion proteins comprising a sequence from transforming growth factor- β (TGF- β), Immunoglobulin Fc (IgG C γ 1), and a sequence from the extracellular domain of CTLA-4

TGF- β 1 + IgG C γ 1 + CTLA-4 ECD (SEQ ID NO: 60):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNP GASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS EPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCVSMHEALHNHYTQKSLSLSPGK GGGGSGGGSGGGGS
AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSI
CTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPYYLGIGNGTQIYVIDPEPCPDSD

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

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

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

Italic: Linker (optional)

Bold: Mature (active) human TGF- β 1

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

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

FIG. 47

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

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

EVQLVESGGGLVQPGRSRLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSVEG
RFTISRDNAKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGLTVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG
TQTYICNVNHKPSNTKVD**KKAE**PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK
GGGSGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP**
CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRRN
TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS
DAVCSSSLPA RKPPNEPHVY LPG

Anti-TNF α light chain (SEQ ID NO: 78):

DIQMTQSPSSLSASVGRVTITCRASQGIRNYLAWYQQLPKGAPKLLIYAASLTQSGVPSRFGSG
SGTDFTLTISSLQPEDVATYYCQRYNRPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: anti-TNF α antibody variable region

Plain: anti-TNF α antibody constant region

Italic: Linker

Bold: RANK ectodomain

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

FIG. 48

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

TNFR2 ECD + IgG C γ 1 + RANK ectodomain (SEQ ID NO: 62):

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKEGCRLCAPLRKCRPGFGVARPGT
ETSDVVKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPLMGPSPPAEGSTGD *EPKSCDK*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS* **QI APPCTSEKHY**
EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA
VVAGNSTTPR RACTAGYHW SQDCECCRN 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 C γ 1 + TNFR2 ECD (SEQ ID NO: 63):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDW
NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRN TECAPGLGAQ
HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA
RKPPNEPHVY LPG
GGGGSGGGSGGGGS THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSREMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKEGCRLCAPLRKCRPGFGVARPGT
ETSDVVKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPLMGPSPPAEGSTGD

Underlined: TNFR2 ligand-binding domain

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

Italic: Linker

Bold: RANK ectodomain

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

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

FIG. 49

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Fusion proteins comprising the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C γ 1), and a RANKL-binding sequence of receptor activator of nuclear factor-kB (RANK)

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

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLGIGNGTQIYVIDPEPCPDSDQEPKSCDK THTCPPCPPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSFCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGGS QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW 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 C γ 1), and a sequence from the extracellular domain of CTLA-4

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

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

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

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

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

Italic: Linker (optional)

Bold: RANK ectodomain

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

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

FIG. 50

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

TGF- β 1 + Fc + RANK ectodomain (SEQ ID NO: 66):

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNP GASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS *GGGGSGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS* **QI APPCTSEKHY**
EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDW NEEDKCLLHK VCDTGKALVA
VVAGNSTTPR RACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS
DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

RANK ectodomain + Fc + TGF- β 1 (SEQ ID NO: 67):

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDW
NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRRN TECAPGLGAQ
HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA
RKPPNEPHVY LPG *GGGGSGGGSGGGGS*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNP GASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Underlined: Mature (active) human TGF- β 1

Italic: Linker (optional)

Plain: Fc

Bold: RANK ectodomain

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

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

FIG. 51

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

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

RIFAVFIFMTYWHLNNAFTVTVPKDLVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQFVHGEED
LKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD
PVTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEE
T GGGGSGGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHEALHNHYTQ
KSLSLSPGK GGGGSGGGGSGGGGS QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP
CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRRN
TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS
DAVCSSSLPA RKPPNEPHVY LPG

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

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
GGGGSGGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHEALHNHYTQ
KSLSLSPGK GGGGSGGGGSGGGGS
RIFAVFIFMTYWHLNNAFTVTVPKDLVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQFVHGEED
LKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD
PVTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEE
T

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

Italic: Linker (optional)

Plain: Fc

Underlined: RANK ectodomain

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

EPKSCDK SEQ ID NO: 105)

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

FIG. 52

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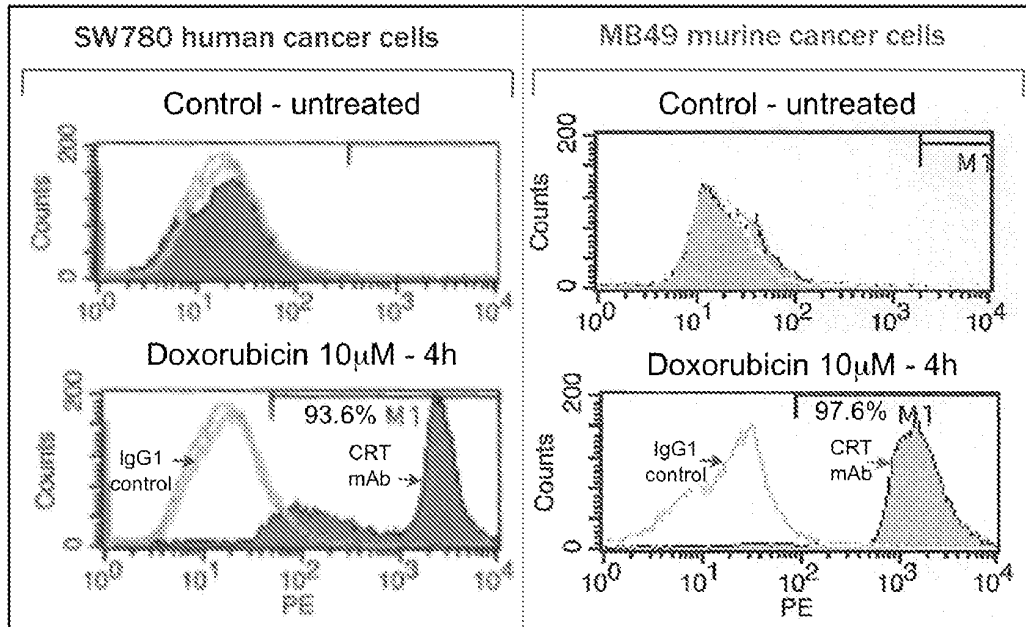


FIG. 53A

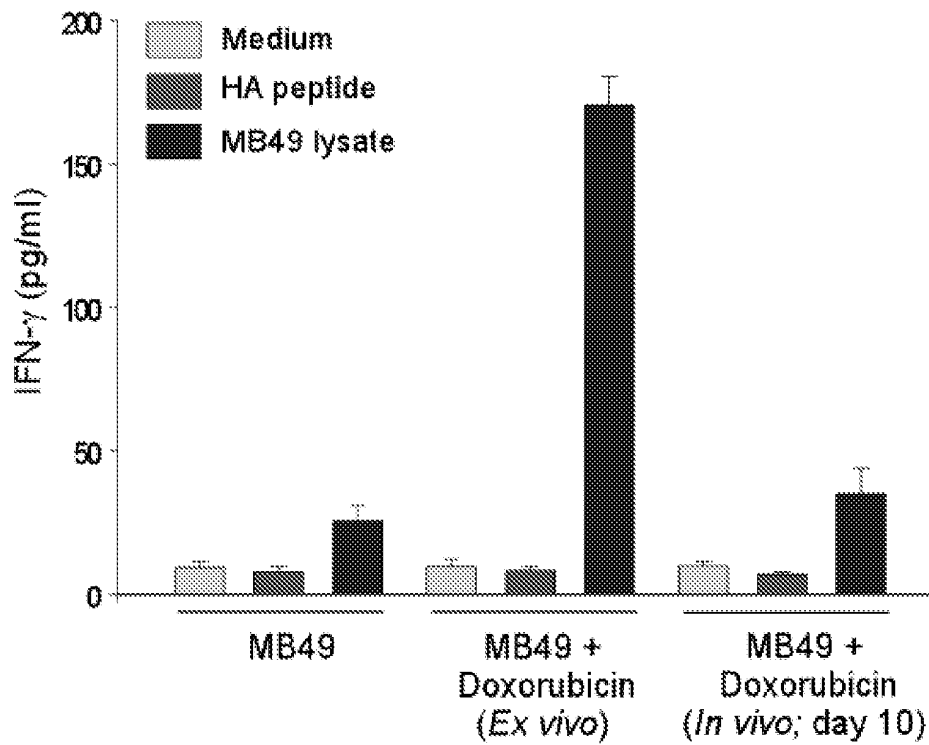


FIG. 53B

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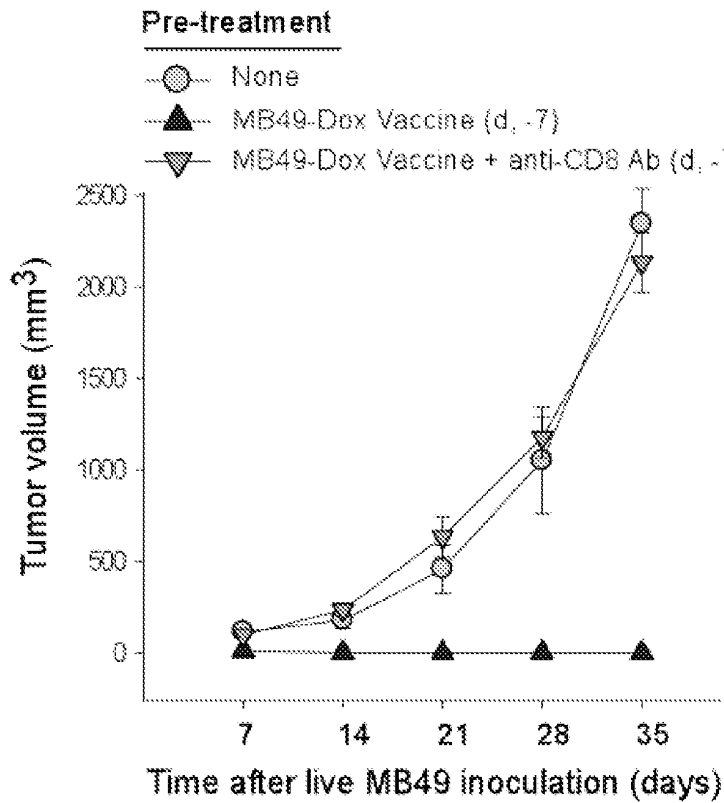


FIG. 53C

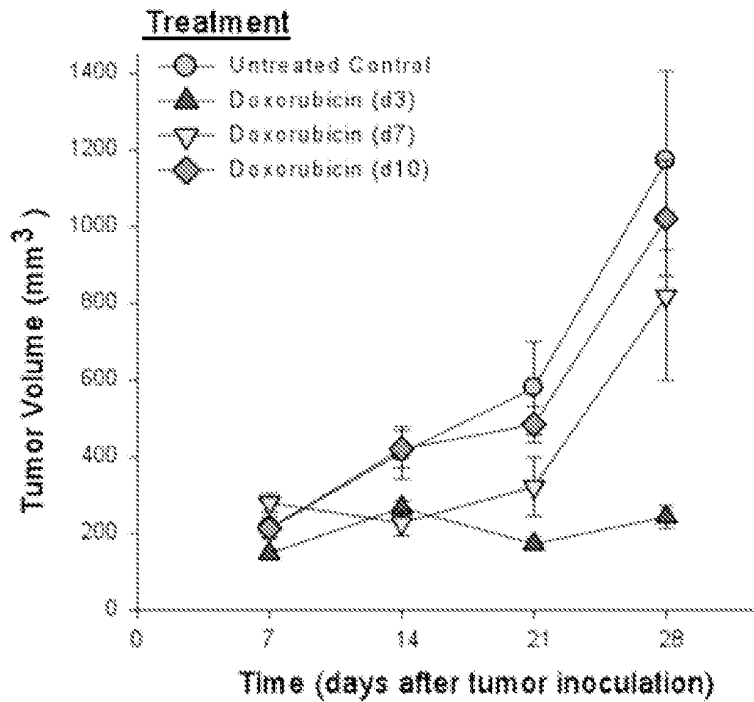


FIG. 53D

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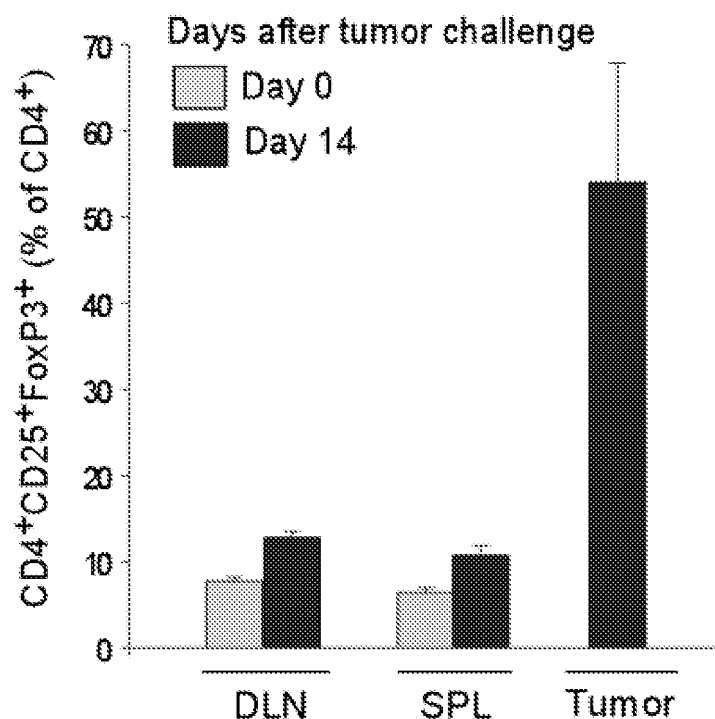


FIG. 53E

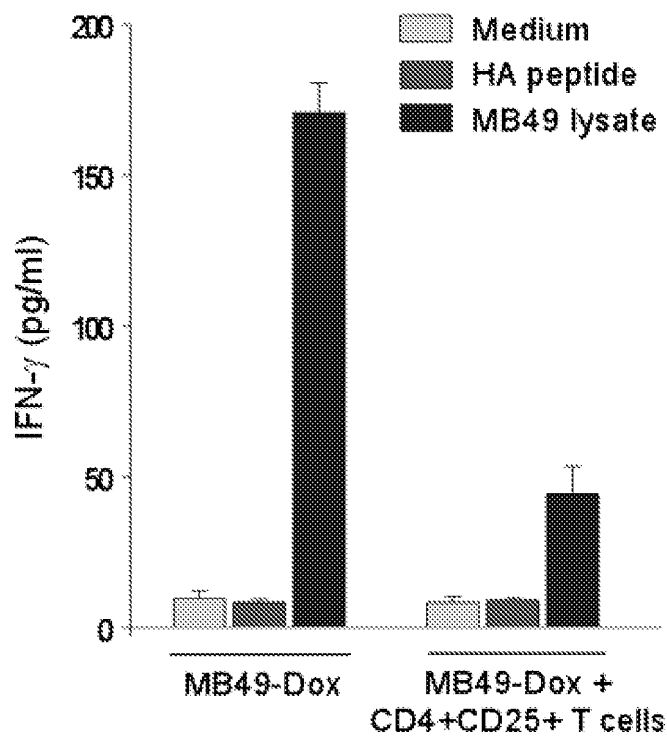


FIG. 53F

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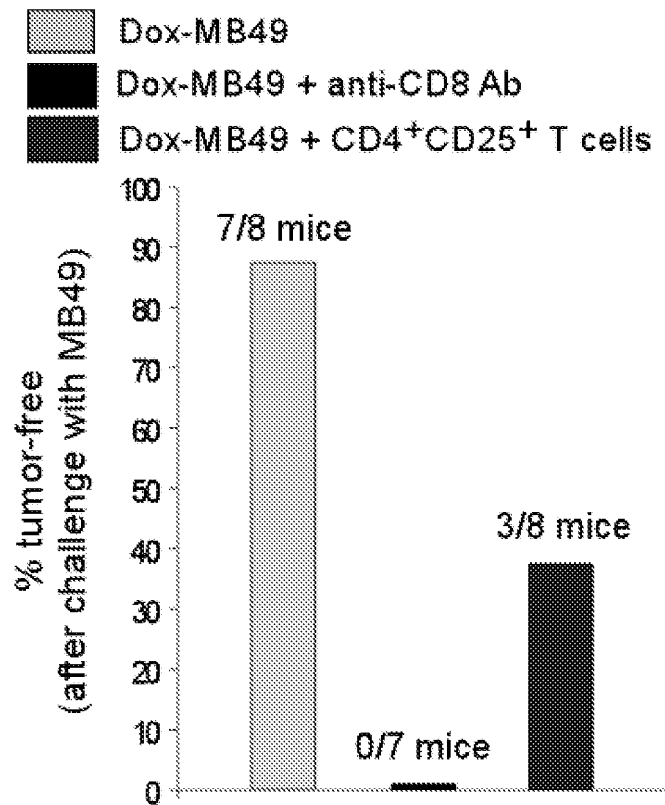


FIG. 53G

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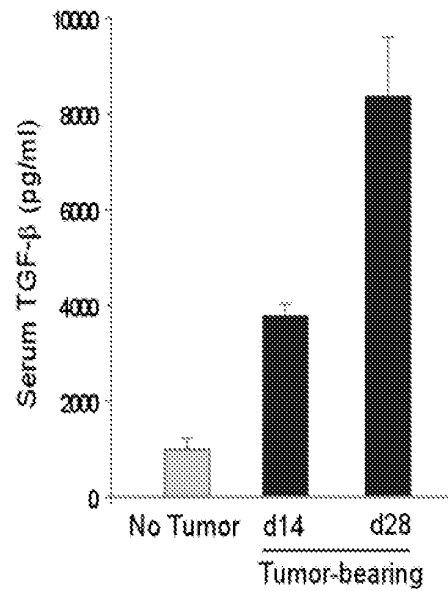


FIG. 54A

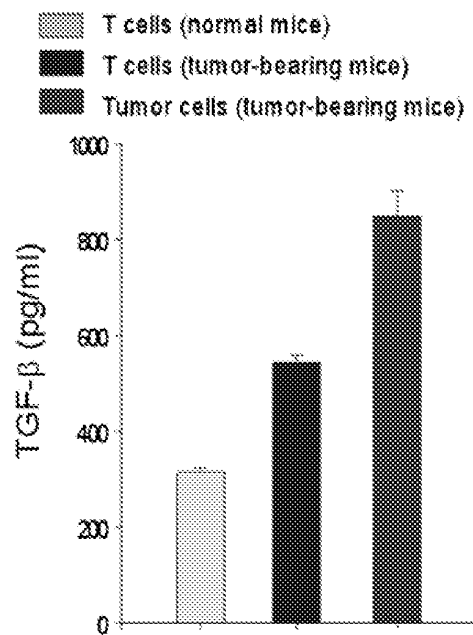


FIG. 54B

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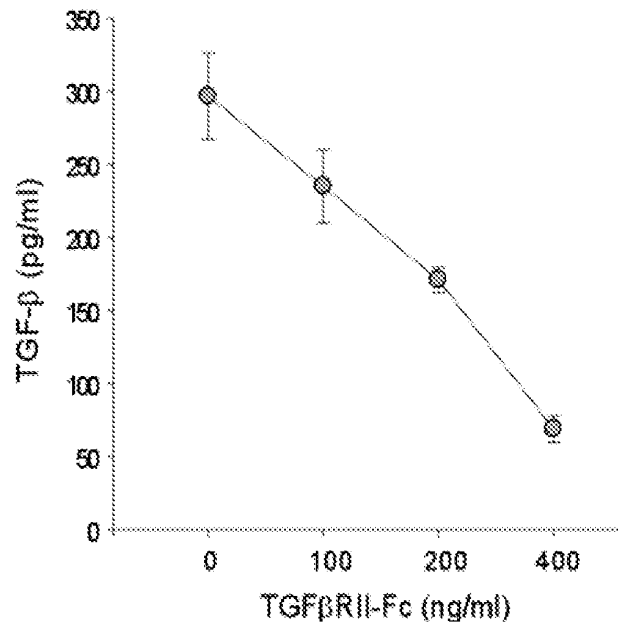


FIG. 54C

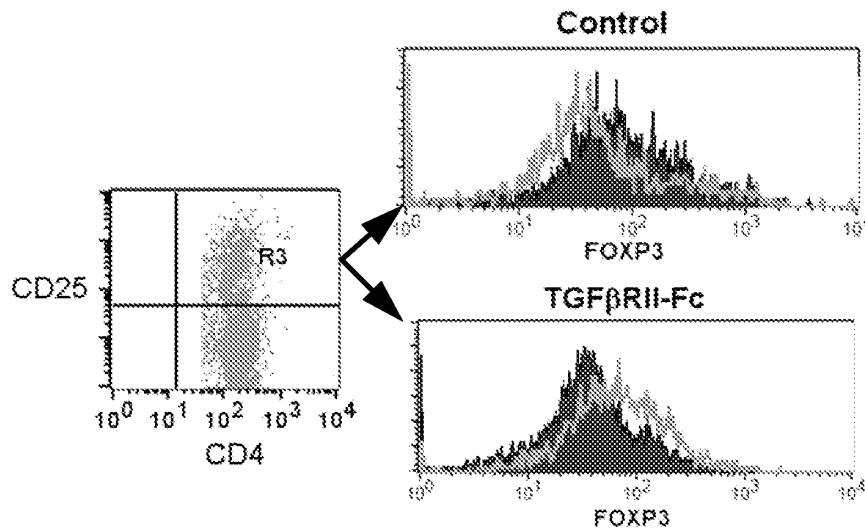


FIG. 54D

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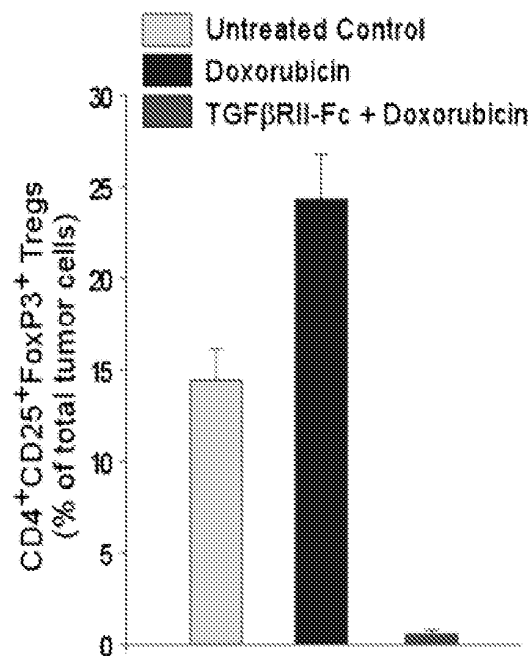


FIG. 54E

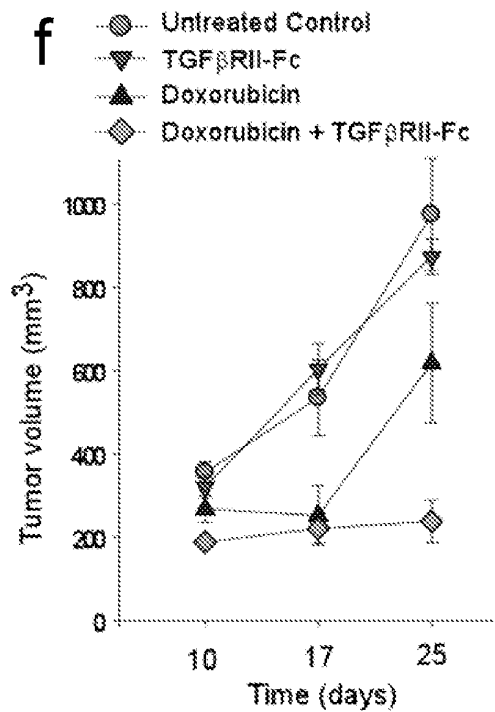


FIG. 54F

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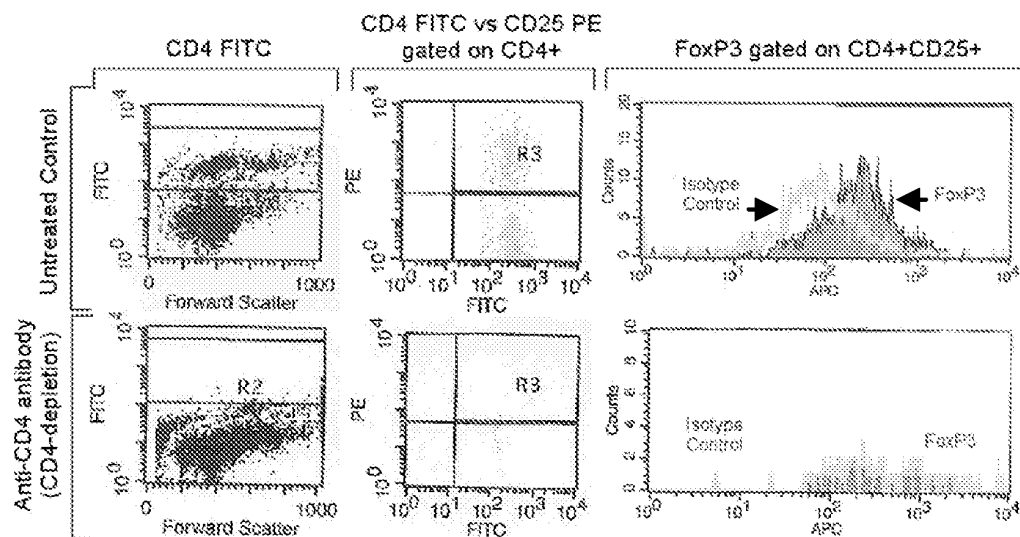


FIG. 55A

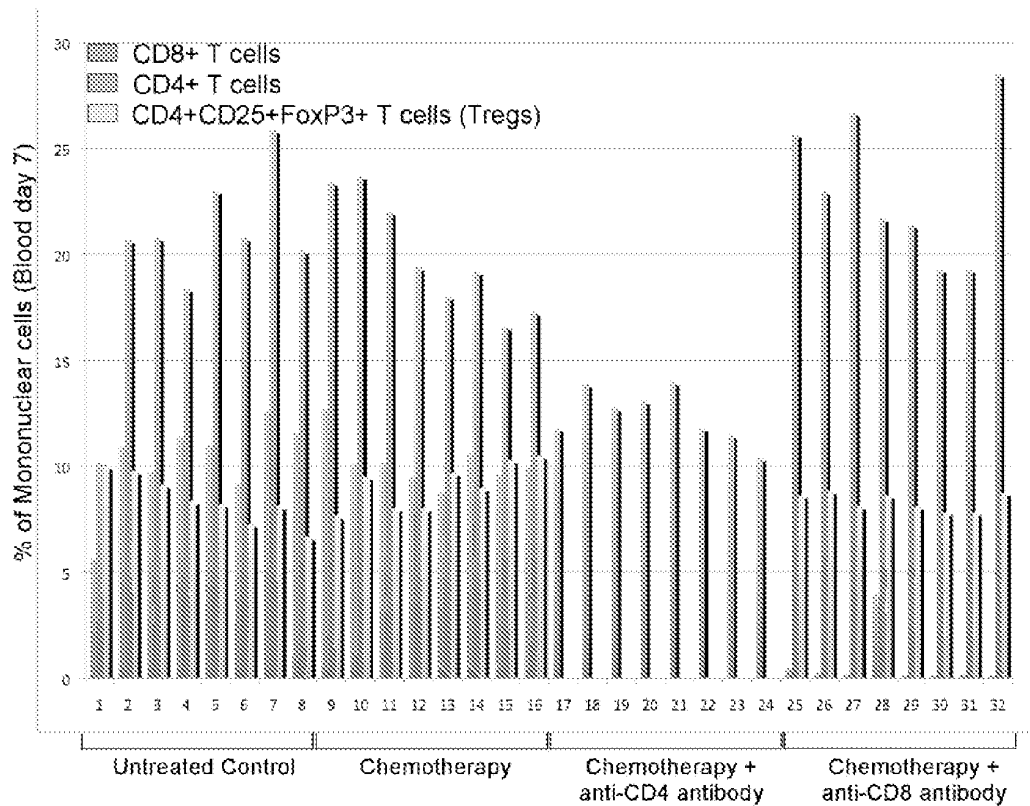


FIG. 55B

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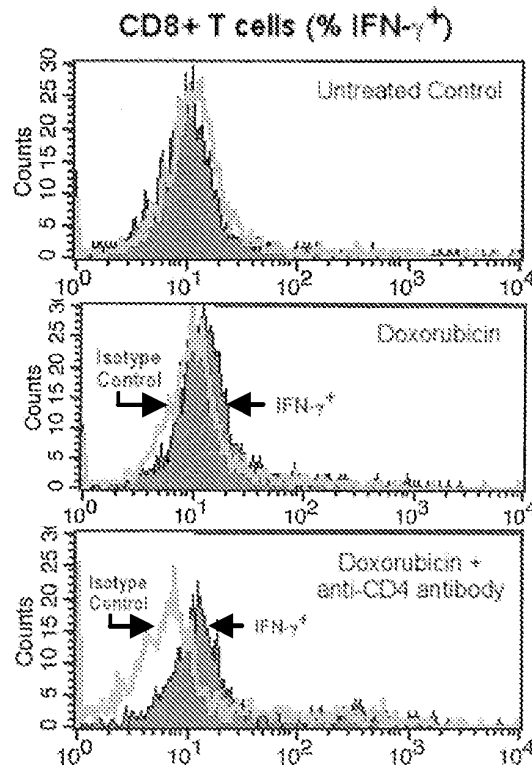


FIG. 55C

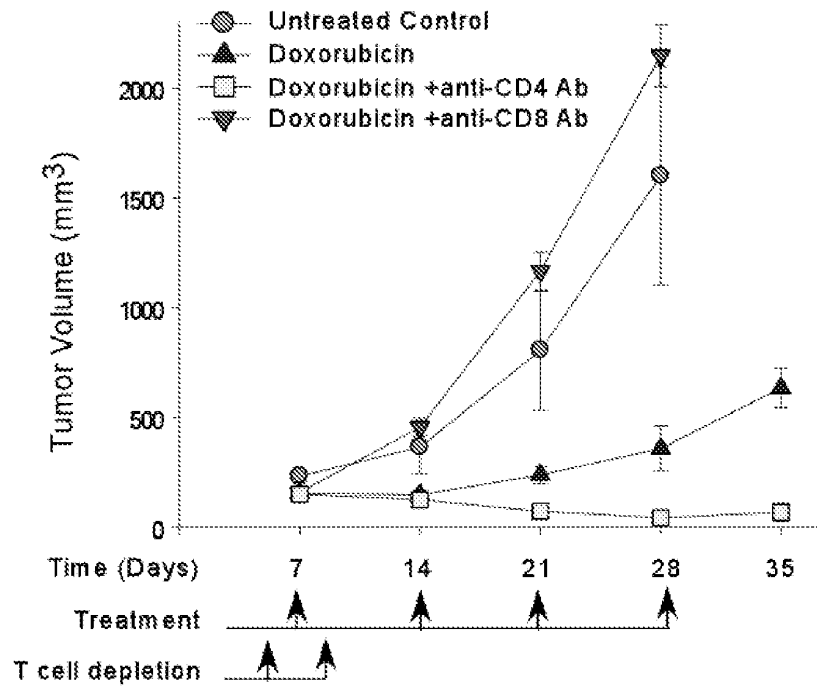


FIG. 55D

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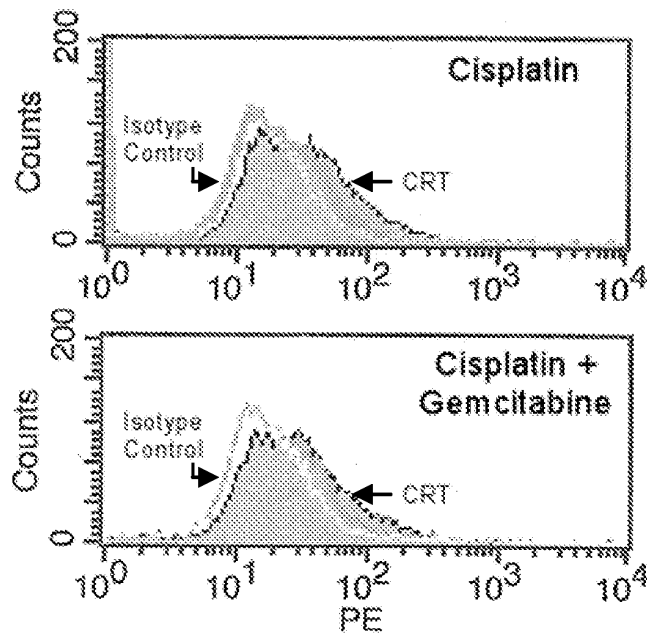


FIG. 56A

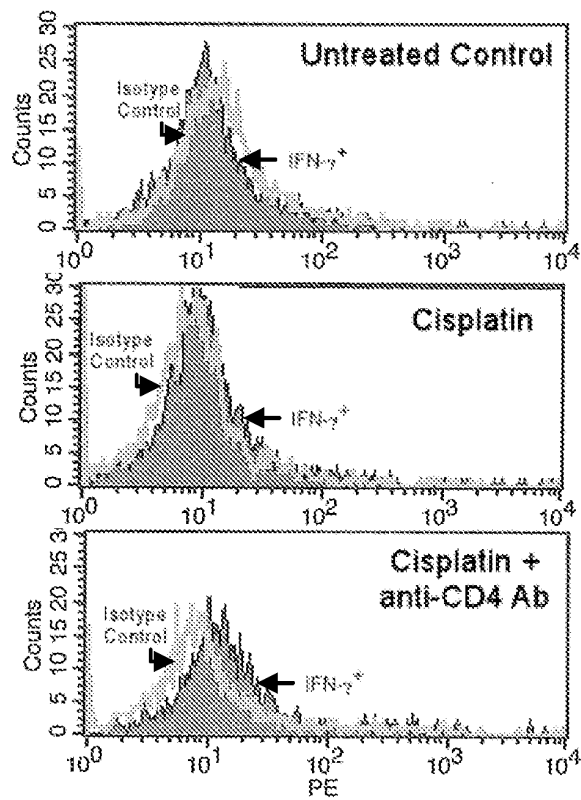


FIG. 56B

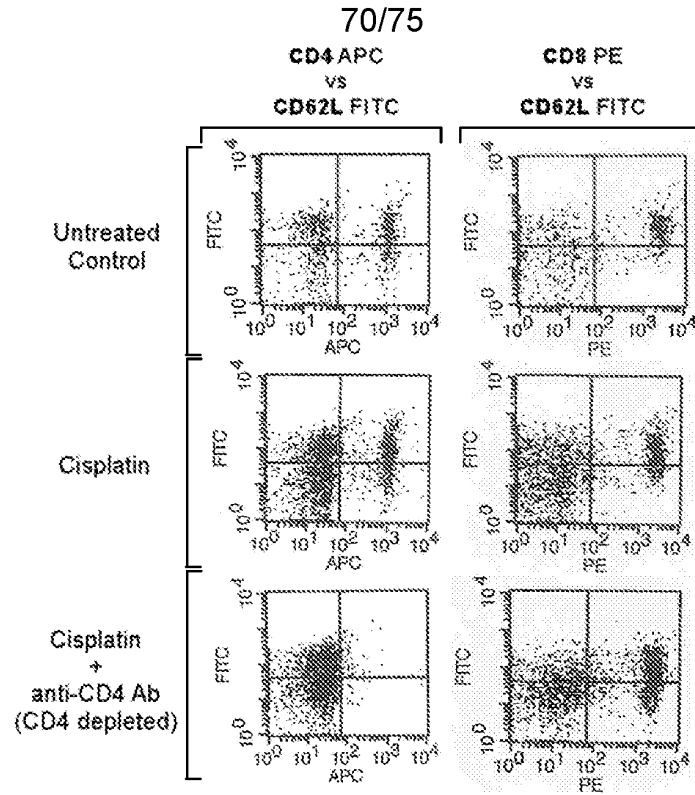


FIG. 56C

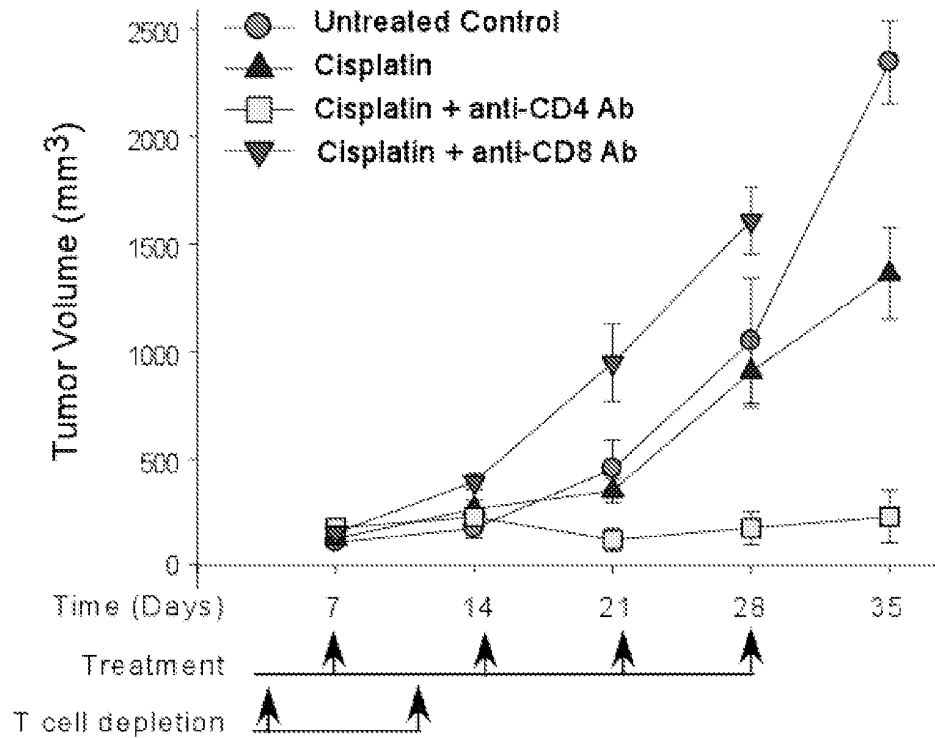


FIG. 56D

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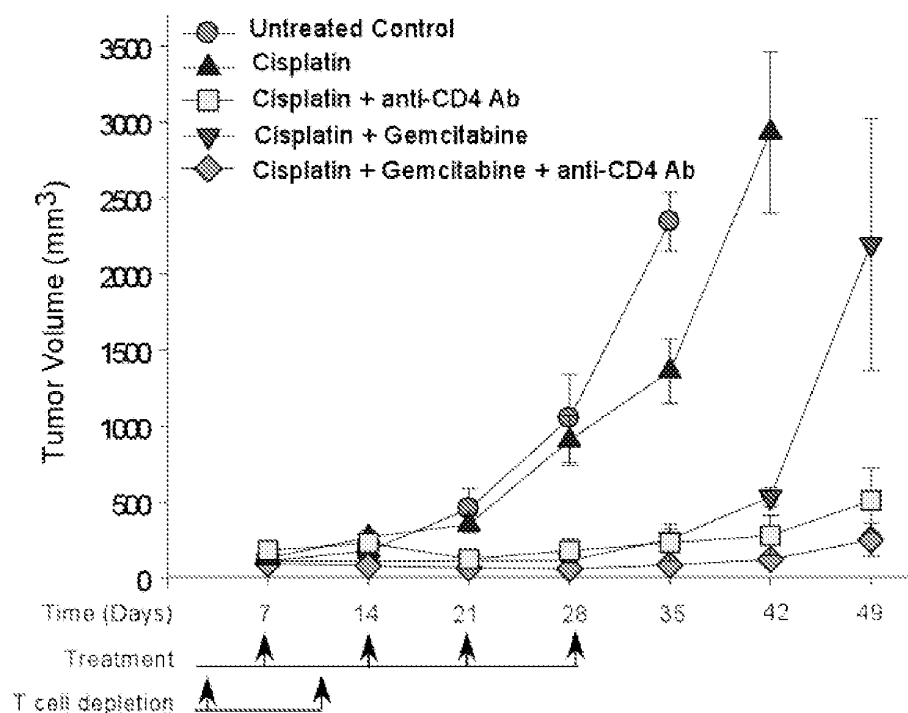


FIG. 56E

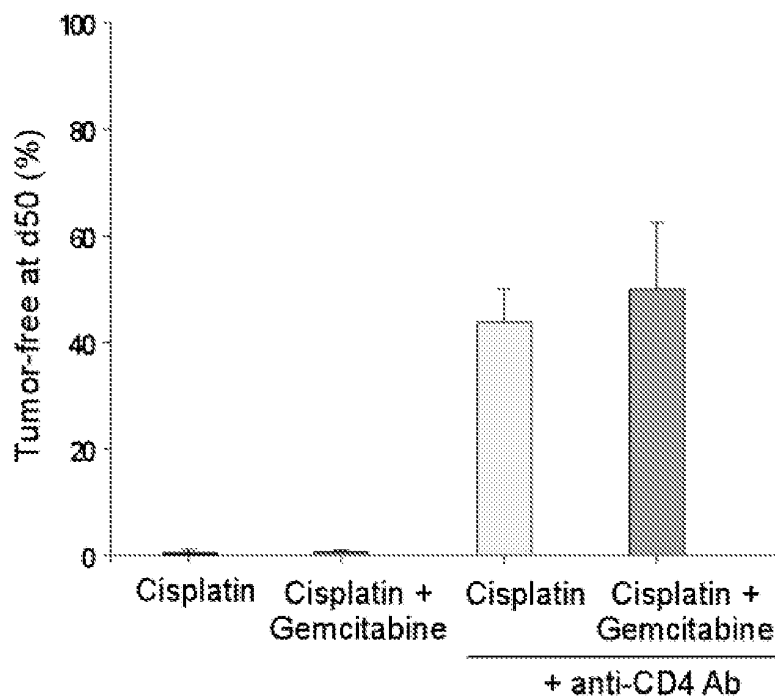


FIG. 56F

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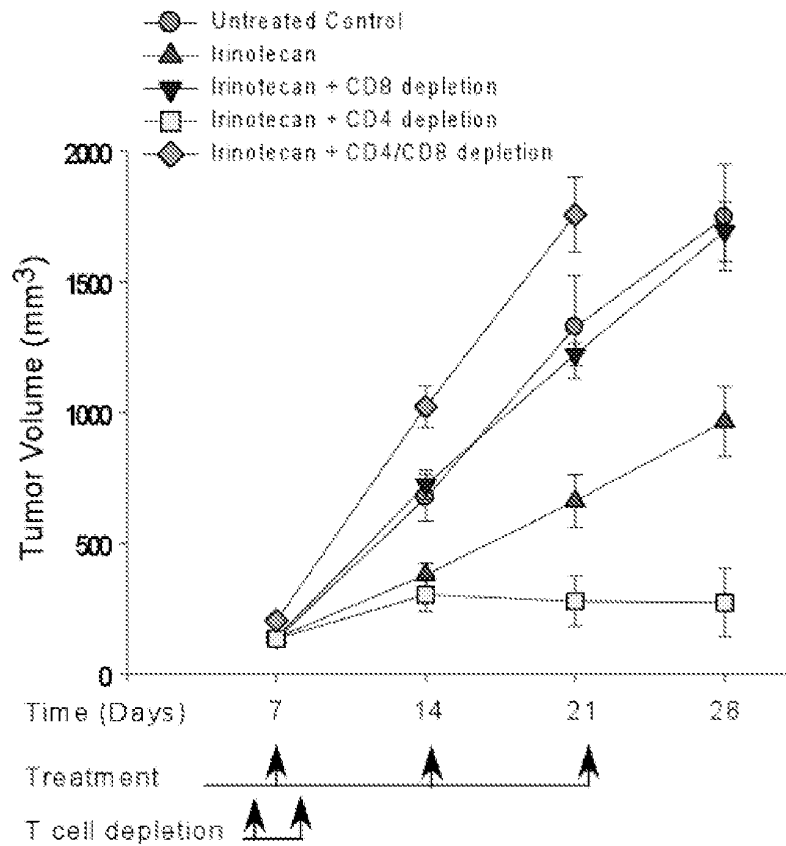


FIG. 57A

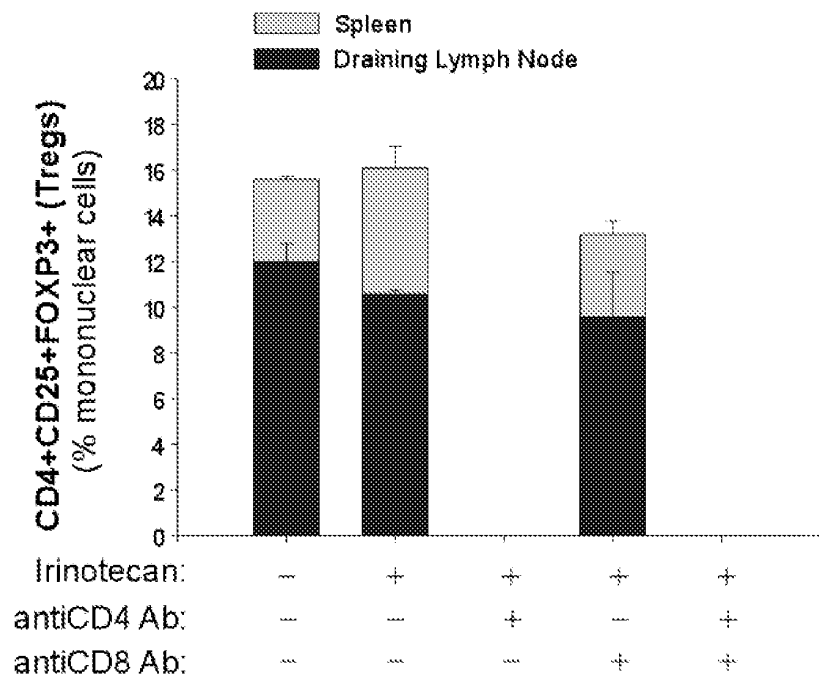


FIG. 57B

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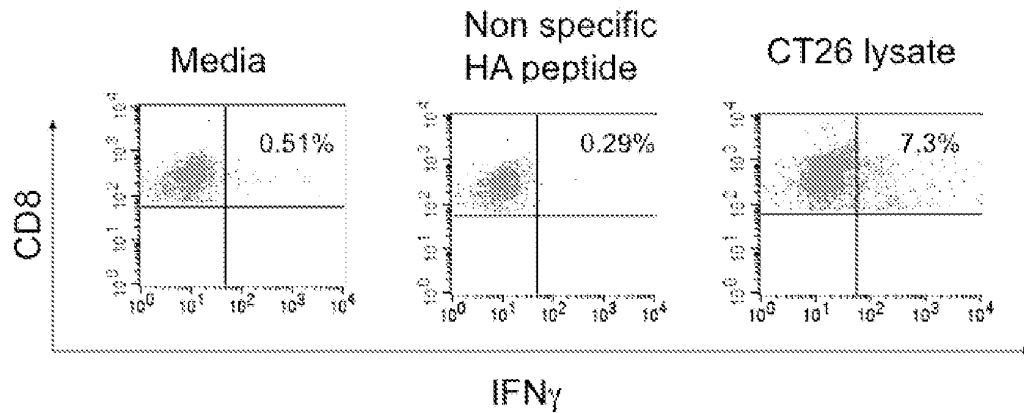


FIG. 57C

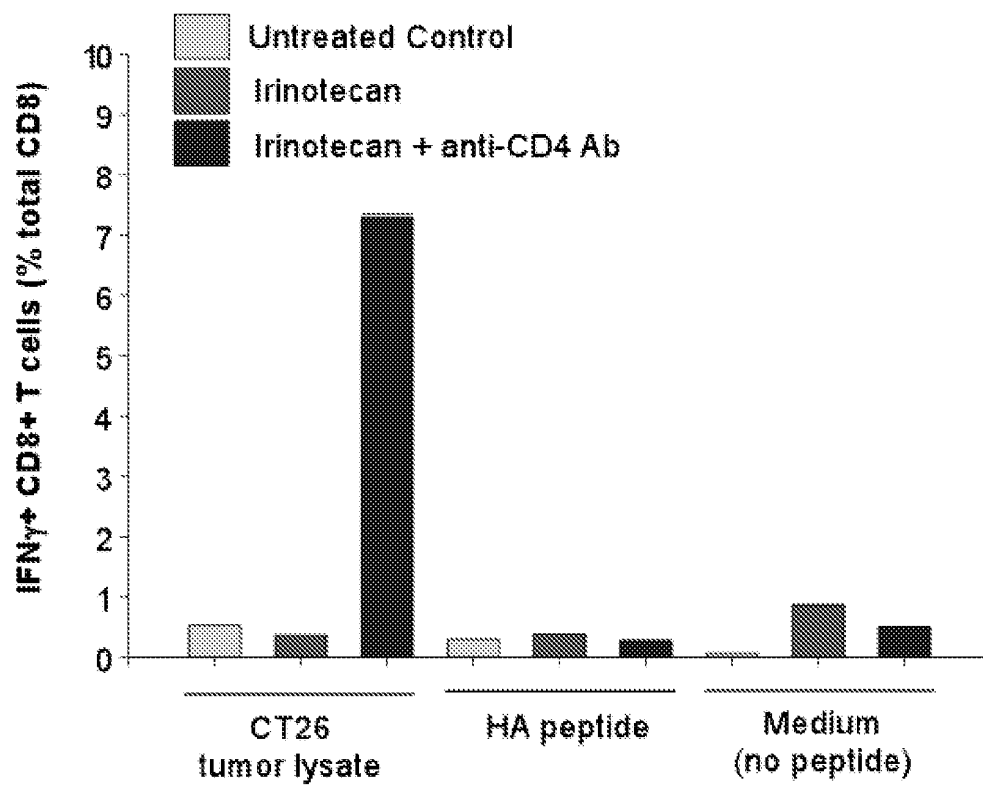


FIG. 57D

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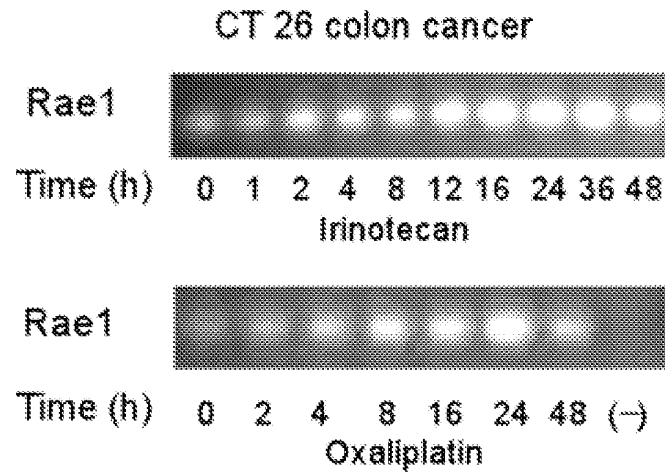


FIG. 57E

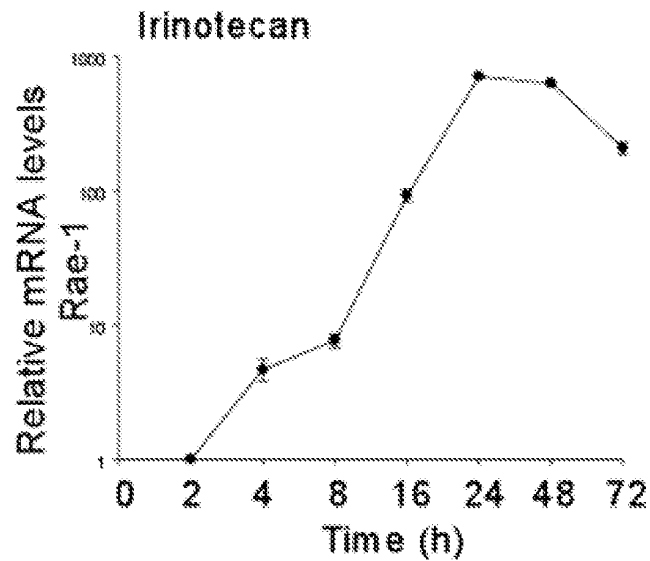


FIG. 57F

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; Expression of MICA/B on HCT116 cells

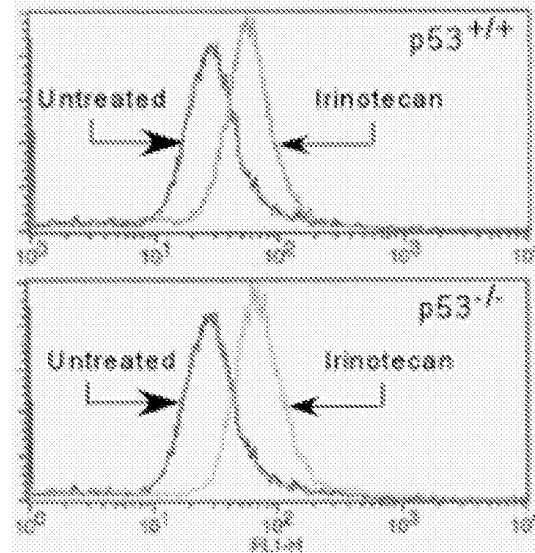


FIG. 57G

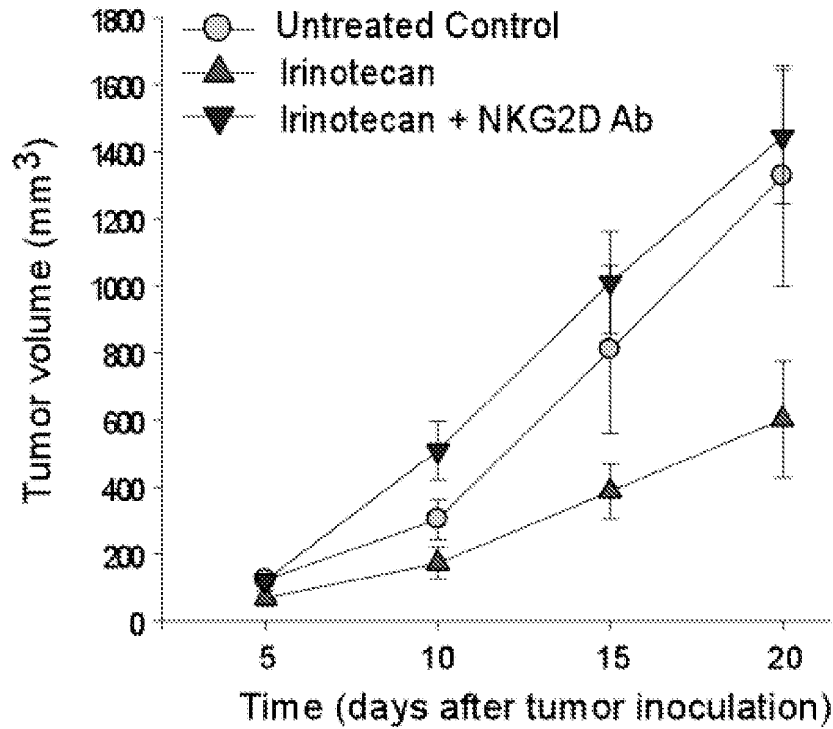


FIG. 57H

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	Not Yet Assigned
Application No.:	Not Yet Assigned	Examiner:	Not Yet Assigned
Filed:	March 14, 2022	Conf. No.:	Not Yet Assigned
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

Dear Sir:

In accordance with 37 C.F.R. § 1.97, enclosed is a Form PTO/SB/08 relating to the above-identified application. Since the present application is a continuing application of a prior application under 37 CFR § 1.98(d), the listed references are not required to be filed with the attached Form PTO/SB/08.

Applicant respectfully submits that the Information was previously submitted to the Office in a prior application number 16/601,347 filed on October 14, 2019, and is relied on earlier filing date under 35 U.S.C. § 120. The Prosecution history documents for the application number 16/601,347 are of record with the U.S. Patent and Trademark Office and are stored in the IFW. In addition, the IDS submitted in the earlier application complies with 37 CFR § 1.98(a)-(c). As such, in accordance with 37 CFR § 1.98(d), the references listed in Form PTO/SB/08 are not provided.

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.

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331323-001301

In re Application of:
Bedi and Ravi
Application No.: Not Yet Assigned
Filed: March 14, 2022
Page 2

PATENT
Attorney Docket No.: JHU3260-9

This Information Disclosure Statement is being submitted before the mailing date of a First Office Action on the Merits for the above-identified application.

No fee is believed 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: March 14, 2022

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DLA Piper LLP (US)
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San Diego, California 92121-2133

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331323-001301

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Substitute for form 1449A/PTO <h2 style="text-align: center; margin: 0;">INFORMATION DISCLOSURE STATEMENT BY APPLICANT</h2> <p style="text-align: center; margin: 0;"><i>(Use as many sheets as necessary)</i></p>		<div style="text-align: center; border: 1px solid black; padding: 2px; margin-bottom: 5px;">Complete if Known</div> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%;">Application Number</td> <td>Not Yet Assigned</td> </tr> <tr> <td>Filing Date</td> <td>March 14, 2022</td> </tr> <tr> <td>First Named Inventor</td> <td>Atul Bedi</td> </tr> <tr> <td>Art Unit</td> <td>Not Yet Assigned</td> </tr> <tr> <td>Examiner Name</td> <td>Not Yet Assigned</td> </tr> <tr> <td>Attorney Docket Number</td> <td>JHU3260-9</td> </tr> </table>		Application Number	Not Yet Assigned	Filing Date	March 14, 2022	First Named Inventor	Atul Bedi	Art Unit	Not Yet Assigned	Examiner Name	Not Yet Assigned	Attorney Docket Number	JHU3260-9
Application Number	Not Yet Assigned														
Filing Date	March 14, 2022														
First Named Inventor	Atul Bedi														
Art Unit	Not Yet Assigned														
Examiner Name	Not Yet Assigned														
Attorney Docket Number	JHU3260-9														
Sheet	1	of	4												

U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			
	1	7,317,091	01-08-2008	Lazar et al.	
	2	7,527,791	05-05-2009	Adams et al.	
	3	7,795,389	09-14-2010	Sun et al.	
	4	7,803,378	09-28-2010	Tranchanf-Bunel	
	5	7,820,165	10-26-2010	McKenna et al.	
	6	8,101,720	01-24-2012	Lazar et al.	
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	8	8,399,618	03-19-2013	Lazar et al.	
	9	8,815,247	08-26-2014	Govindappa et al.	
	10	2005/0054832	03-10-2005	Lazar et al.	
	11	2006/0135459	06-22-2006	Epstein et al.	
	12	2006/0193849	08-31-2006	Krauss et al.	
	13	2006/0263368	11-23-2006	Rosenblum et al.	
	14	2007/0212337	09-13-2007	Bedi et al.	
	15	2007/0244042	10-18-2007	Sun et al.	
	16	2008/0075717	03-27-2008	Tranchand-Bunel	
	17	2009/0053240	02-26-2009	Lazar et al.	
	18	2009/0214533	08-27-2017	Clynes	
	19	2009/0226435	09-10-2009	Sanjay	
	20	2011/0052585	03-03-2011	Scaria et al.	
	21	2013/0017199	01-17-2013	Langermann	

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document	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
		Country Code ³ "Number" ⁴ "Kind Code" ⁵ (if known)				
	22	WO 2009/152610 A1	12-23-2009	The Royal Institution For The Advancement Of Learning/McGill University		
	23	WO 2010/003118 A1	01-07-2010	Trubion Pharmaceuticals, Inc.		

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. ¹Applicant's unique citation designation number (optional). ²See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. ³Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). ⁴For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁵Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. ⁶Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 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.**

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Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>		Complete if Known			
		Application Number	Not Yet Assigned		
		Filing Date	March 14, 2022		
		First Named Inventor	Atul Bedi		
		Art Unit	Not Yet Assigned		
		Examiner Name	Not Yet Assigned		
Sheet	2	of	4	Attorney Docket Number	JHU3260-9

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
	24	Alignment of SEQ ID No. 10 of D3 with SEQ ID No. 87 of B1	
	25	Alignment of SEQ ID No. 744 of D2 with SEQ ID No. 87 of B1	
	26	Canadian Office Action dated Mar. 28, 2018, regarding CA 2,791,383	
	27	Coffelt et al.: "Angiopoietin 2 Stimulates TIE2-Expressing Monocytes to Suppress T Cell Activation and to Promote Regulatory T Cell Expression"; The Journal of Immunology 2011, 186 (7), p. 4183-4190	
	28	Contardi et al, Int. J. Cancer: 117, 538-550; 2005	
	29	EP Communication dated Sep. 5, 2018, pursuant to Rule 114(2) EPC regarding EP 2542590	
	30	European Search Report and Search Opinion Received for EP Application No. 17168491.3, mailed on November 24, 2017, 5 pages	
	31	Fernandez-Botran et al.: "Soluble cytokine receptors in biological therapy"; Expert Opinion on Biological Therapy, 2002, vol. 2, Issue 6, p. 585-605	
	32	Fonseca et al.: "Capitalizing on the immunogenicity of Dying Tumor Cells"; Clinical Cancer Research 2008, 14 (6), p. 1603-1608	
	33	International Preliminary Report on Patentability received for PCT Patent Application No. PCT/US2011/027317, mailed on September 20, 2012, 9 pages	
	34	International Search Report and Written Opinion received for PCT Patent Application No. PCT/US2011/027317, mailed on February 08, 2012, 11 pages	

Examiner Signature		Date Considered	
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*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.**

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		Application Number	Not Yet Assigned		
		Filing Date	March 14, 2022		
		First Named Inventor	Atul Bedi		
		Art Unit	Not Yet Assigned		
		Examiner Name	Not Yet Assigned		
Sheet	3	of	4	Attorney Docket Number	JHU3260-9

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
	35	Japanese Office Action issued on May 27, 2020, regarding JP 2018-140050	
	36	Jones et al., "Degree of CD25 expression in T-Cell lymphoma is dependent on tissue site: implications for targeted therapy", Clinical Cancer Research, 2004, 10:5587-5594	
	37	Jung et al.: "Double anti-angiogenic and anti-inflammatory protein valpha targeting VEGF-A and TNF-alpha. in retinopathy and psoriasis"; JBC, vol. 286, No. 16, Feb. 23, 2011, pp. 14410-14418	
	38	Koh et al.: "Double anti-angiogenic protein, DAAP, targeting VEGF-A and angiopoietins in tumor angiogenesis, metastasis, and vascular leakage"; Cancer Cell, vol. 18, Issue 2, Aug. 16, 2010, p. 171-184. Supplementary data, 28 pages	
	39	Kwiatkowska et al.: "Expression of soluble recombinant TGF-beta. type II receptor fused with the Fc portion of human IgG1 (sT.beta.11-Fc) in NSO cells"; Acta Biochimica Polonica, 2006, 53(2), pp. 361-369	
	40	Letourneau et al.: "IL-2- and CD25-dependent immunoregulatory mechanisms in the homeostasis of T-cell subsets"; J Allergy Clin Immunol. 2009, 123(4), pp. 758-762	
	41	Malek et al.: "Interleukin-2 receptor signaling: at the interface between tolerance and immunity"; Immunity 2010, vol. 33, Issue 2, p. 153-165	
	42	Office Action received for European Application No. 11751481.0, mailed on January 22, 2016, 3 pages	
	43	Podar et al.: "Inhibition of the TGF-beta. signaling pathway in tumor cells"; Recent Results in Cancer Research, 2007, vol. 172, p. 77-97	
	44	Regnault et al.: "Fc.gamma. Receptor-mediated Induction of Dendritic Cell Maturation and Major Histocompatibility Complex Class I-restricted Antigen Presentation after Immune Complex Internalization"; J. Exp. Med., 1999, vol. 189, No. 2, p. 371-380	
	45	Rose-John et al. 2007. Expert Opinion in Therapeutic Targets. 11:613-624	

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		Application Number	Not Yet Assigned		
		Filing Date	March 14, 2022		
		First Named Inventor	Atul Bedi		
		Art Unit	Not Yet Assigned		
		Examiner Name	Not Yet Assigned		
Sheet	4	of	4	Attorney Docket Number	JHU3260-9

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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
	46	Schrama et al.: "Antibody targeted drugs as cancer therapeutics"; Nature Reviews Drug Discovery, Jan. 20, 2006, vol. 5, p. 147-159	
	47	Supplementary European Search Report received for EP Patent Application No. 11751481.0, mailed on June 27, 2013, 16 pages	
	48	Trail et al.: "Monoclonal antibody drug conjugates in the treatment of cancer"; Current Opinion in Immunology, 1999, vol. 11, Issue 5, p. 584-588	
	49	U.S. Appl. No. 61/311;255, Mar. 5, 2010, Atul Bedi	
	50	U.S. Appl. No. 61/435,671, Jan. 24, 2011, Atul Bedi	
	51	Wang et al.: "Tumor necrosis factor and cancer, buddies or foes?"; Acta Pharmacologica Sinica, 2008, 29 (11), p. 1275-1288	
	52	Yang et al.: "The role of T.sub.reg in the cancer immunological response"; American Journal of Immunology, 2009, 5 (1), p. 17-28	
	53	Yao and Chen: "Contribution of B7-H1/PD-1 Co-inhibitory Pathway to T-Ce11 Dysfunction in Cancer"; D.I. Gabrilovich, A.A. Hurwitz (eds.), Tumor-Induced Immune Suppression. pp. 29-40 .COPYRGT. Springer 2008	
	54	Zhang et al., "Design and Optimization of a Linker for Fusion Protein Construction," Prog. Natural Sci. (2009), 19:1197-1200, Elsevier	
	55	Zhang et al: "Expression of a Soluble TGF-.beta. Receptor by Tumor Cells Enhances Dendritic Cell/Tumor FusionVaccine Efficacy.sup.1"; J. Immunology, 2008, 181, pp. 3690-3697	

Examiner Signature		Date Considered	
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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.**

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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 15/362,632 filed on November 28, 2016.

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

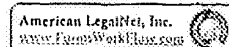
Inventor: Atul Bedi Date (Optional) : _____

Signature: [Signature]

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. 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 1.11 and 1.14. This collection is estimated to take 1 minute 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.

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WEST274425147.1
331323-001171

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 ☐ The attached application, or
is directed to: ☒ United States application or PCT International application number 15/362,632
filed on November 28, 2016.

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 Ravil Date (Optional) : _____

Signature: Rajani Ravil

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

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331323-001171

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	Not Yet Assigned
Application No.:	16/601,347	Examiner:	Not Yet Assigned
Filed:	October 14, 2019	Conf. No.:	9807
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
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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 041196, Frame 0155.

In re Application of:
Bedi And Ravi
Application No.: 16/601,347
Filed: October 14, 2019
Page 2

PATENT
Attorney Docket No.: JHU3260-8

Direct all telephone calls to:

LISA A. HAILE, J.D., Ph.D.
Telephone: (858) 677-1456
Facsimile: (858) 638-5040

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 : 
(Signature)
Name : Steven Kousouris
(Typed or Printed Name)
Title : Executive Director, Johns Hopkins Technology Ventures
Date : 17 October, 2019

287948162.1
331323-001301

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:		Aldon Griffis		
Attorney Docket Number:		JHU3260-9		
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	80	80
UTILITY SEARCH FEE	2111	1	350	350
UTILITY EXAMINATION FEE	2311	1	400	400
Pages:				
UTILITY APPL SIZE FEE PER 50 SHEETS >100	2081	1	210	210
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 (\$)				1040

Electronic Acknowledgement Receipt

EFS ID:	45224950
Application Number:	17694541
International Application Number:	
Confirmation Number:	4182
Title of Invention:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Aldon Griffis
Filer Authorized By:	
Attorney Docket Number:	JHU3260-9
Receipt Date:	14-MAR-2022
Filing Date:	
Time Stamp:	20:30:15
Application Type:	Utility under 35 USC 111(a)

Payment information:

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RAM confirmation Number	E20223DK31532732
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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	C11123- P11123-42_20220314_APP_DL AP_ADS_EFS.pdf	1829816	no	9
			be9384e2f384a8747f76f0a4f5b8c240b0d4b046		
Warnings:					
Information:					
2	Transmittal of New Application	C11123- P11123-42_20220314_APP_DL AP_ContinuationTxEFS.pdf	201940	no	3
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Information:					
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	Multipart Description/PDF files in .zip description				
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	Specification		1	74	
	Claims		75	76	
	Abstract		77	77	
Warnings:					
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4	Drawings-only black and white line drawings	C11123- P11123-42_20220314_APP_DL AP_DrawingsEFS.pdf	2085872	no	75
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New Applications Under 35 U.S.C. 111

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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	JHU3260-9
		Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		
<p>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.</p> <p>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.</p>			

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.)
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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	Timonium	State/Province	MD	Country of Residence	US	
Mailing Address of Inventor:						
Address 1	2211 Dalewood Road					
Address 2						
City	Timonium	State/Province	MD			
Postal Code	21093	Country i	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	Ruxton	State/Province	MD	Country of Residence	US	
Mailing Address of Inventor:						
Address 1	7810 Ballston Road					
Address 2						
City	Ruxton	State/Province	MD			
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	JHU3260-9
		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		<input type="button" value="Add Email"/>	<input type="button" value="Remove Email"/>

Application Information:

Title of the Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		
Attorney Docket Number	JHU3260-9	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		

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	JHU3260-9
		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	16/601347	2019-10-14		
Prior Application Status	Patented		Remove		
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
16/601347	Continuation of	15/362632	2016-11-28	10442860	2019-10-15
Prior Application Status	Patented		Remove		
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
15/362632	Division of	15/231309	2016-08-08	9850306	2017-12-26
Prior Application Status	Patented		Remove		
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
15/231309	Continuation of	14/645282	2015-03-11	9441044	2016-09-13
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		
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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		
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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					

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	JHU3260-9
		Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

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).

Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Access Code ^j (if applicable)	Remove

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

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.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	JHU3260-9
		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.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	JHU3260-9
		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		<input type="button" value="Remove"/>
<p>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.</p>			
		<input type="button" value="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:			
<div style="border: 1px solid black; height: 20px; width: 100%;"></div>			
Name of the Deceased or Legally Incapacitated Inventor: <div style="border: 1px solid black; height: 20px; width: 100%;"></div>			
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.			<input type="button" value="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.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	JHU3260-9
		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.				
				<input type="button" value="Remove"/>
If the Assignee or Non-Applicant Assignee is an Organization check here. ☐				
Prefix	Given Name	Middle Name	Family Name	Suffix
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Mailing Address Information For Assignee including Non-Applicant Assignee:				
Address 1		<input type="text"/>		
Address 2		<input type="text"/>		
City	<input type="text"/>	State/Province	<input type="text"/>	
Country	<input type="text"/>	Postal Code	<input type="text"/>	
Phone Number	<input type="text"/>	Fax Number	<input type="text"/>	
Email Address	<input type="text"/>			
Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.				<input type="button" value="Add"/>

Signature:

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	/Pamela E. Hwang/		Date (YYYY-MM-DD)	2022-03-14
First Name	Pamela	Last Name	Hwang	Registration Number
				54,984
Additional Signature may be generated within this form by selecting the Add button.				<input type="button" value="Add"/>

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	JHU3260-9
		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 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 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: 17694541

Document Date: 03/14/2022

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.

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.

- Sequence Listing

At the time of document entry (noted above):

- USPTO employees may access SCORE content via DAV or via the SCORE web page.
- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: March 1, 2019

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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: Anjum, Durreshwar

Timestamp: [year=2022; month=3; day=15; hr=7; min=3; sec=38; ms=20;]

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Finished: 2022-03-14 20:33:59.617

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RAVI, Rajani

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Asn



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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY DOCKET NO	TOT CLAIMS	IND CLAIMS
17/694,541	03/14/2022		1040	JHU3260-9	11	1

CONFIRMATION NO. 4182

FILING RECEIPT



CC000000132644983

100899

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

Date Mailed: 03/23/2022

Receipt is acknowledged of this non-provisional utility 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 FIRST INVENTOR, 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 corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction 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 provided that the request is grantable.

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 16/601,347 10/14/2019 PAT 11274156
which is a CON of 15/362,632 11/28/2016 PAT 10442860
which is a DIV of 15/231,309 08/08/2016 PAT 9850306
which 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 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: 03/22/2022

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 17/694,541**

Projected Publication Date: 06/30/2022

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Preliminary Class

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).

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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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.

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NOT GRANTED

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875						Application or Docket Number 17/694,541			
APPLICATION AS FILED - PART I									
(Column 1)		(Column 2)		SMALL ENTITY		OTHER THAN SMALL ENTITY			
FOR	NUMBER FILED	NUMBER EXTRA	RATE(\$)	FEE(\$)		RATE(\$)	FEE(\$)		
BASIC FEE <small>(37 CFR 1.16(a), (b), or (c))</small>	N/A	N/A	N/A	80		N/A			
SEARCH FEE <small>(37 CFR 1.16(k), (l), or (m))</small>	N/A	N/A	N/A	350		N/A			
EXAMINATION FEE <small>(37 CFR 1.16(o), (p), or (q))</small>	N/A	N/A	N/A	400		N/A			
TOTAL CLAIMS <small>(37 CFR 1.16(i))</small>	11	minus 20 = *	x 50 =	0.00	OR				
INDEPENDENT CLAIMS <small>(37 CFR 1.16(h))</small>	1	minus 3 = *	x 240 =	0.00					
APPLICATION SIZE FEE <small>(37 CFR 1.16(s))</small>	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).			210					
MULTIPLE DEPENDENT CLAIM PRESENT <small>(37 CFR 1.16(j))</small>				0.00					
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1040		TOTAL			
APPLICATION AS AMENDED - PART II									
(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY		OTHER THAN SMALL ENTITY	
AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)	
	Total <small>(37 CFR 1.16(i))</small>	*	Minus	**	=	OR	x	=	
	Independent <small>(37 CFR 1.16(h))</small>	*	Minus	***	=	OR	x	=	
	Application Size Fee <small>(37 CFR 1.16(s))</small>					OR			
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM <small>(37 CFR 1.16(j))</small>					OR			
			TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE			
(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY		OTHER THAN SMALL ENTITY	
AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)	
	Total <small>(37 CFR 1.16(i))</small>	*	Minus	**	=	OR	x	=	
	Independent <small>(37 CFR 1.16(h))</small>	*	Minus	***	=	OR	x	=	
	Application Size Fee <small>(37 CFR 1.16(s))</small>					OR			
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM <small>(37 CFR 1.16(j))</small>					OR			
			TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE			
<div style="font-size: x-small;"> * 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. </div>									

Under the Paperwork Reduction Act 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 <i>(Use as many sheets as necessary)</i>		Complete if Known			
		Application Number	17/694,541		
		Filing Date	March 14, 2022		
		First Named Inventor	Atul Bedi		
		Art Unit	1647		
		Examiner Name	To Be Assigned		
Sheet	1	of	1	Attorney Docket Number	JHU3260-9

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
	1	Carter et al, "Identification and validation of cell surface antigens for antibody targeting in oncology", Endocr Relat Cancer, December 2004, 11(4):659-687	
	2	EP Third Party Observation in European Application No. EP20200205164, dated on April 5, 2022, 10 pages	
	3	Francisco et al., "PD-L1 regulates the development, maintenance, and function of induced regulatory T cells", J Exp Med, December 2009, 206(13):3015-3029	
	4	Haier et al., "Cell Surface Molecules and Their Prognostic Values in Assessing Colorectal Carcinomas", Annal of Surgery, January 2000, 231(1):11-24	
	5	IN Examination Report in Indian Application No. 201948021756, dated January 24, 2022, 6 pages	

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:	45846226
Application Number:	17694541
International Application Number:	
Confirmation Number:	4182
Title of Invention:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Pamela Elaine Bechtel/Kerstin Kinzer
Filer Authorized By:	Pamela Elaine Bechtel
Attorney Docket Number:	JHU3260-9
Receipt Date:	01-JUN-2022
Filing Date:	14-MAR-2022
Time Stamp:	12:08:12
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	JHU3260-9_IDS_Transmittal_Letter.pdf	101648 575d61468d10d5b48811a3c5e6825c2f197a7355	no	2

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	JHU3260-9_SB08.pdf	222588	no	1
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Total Files Size (in bytes):			26948847		

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.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Bedi & Ravi	Art Unit:	1647
Application No.:	17/694,541	Examiner:	To Be Assigned
Filed:	March 14, 2022	Conf. No.:	4182
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/ Madam:

In accordance with 37 C.F.R. § 1.97, enclosed are references relating to the above-identified application. For the convenience of the Examiner, the cited references are listed on the attached Form PTO/SB/08.

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 before the mailing date of a First Office Action on the Merits for the above-identified application.

In re Application of:
Bedi & Ravi
Application No.: 17/694,541
Filed: March 14, 2022
Page 2

PATENT
Attorney Docket No.: JHU3260-9

No fee is believed to be due in connection with this submission. However, the Commissioner is hereby authorized to charge any 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: June 1, 2022

/Pamela Hwang/
Pamela Hwang, J.D., Ph.D.
Registration No. 54,984
Telephone: (858) 677-1473
Facsimile: (858) 638-5040

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



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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
17/694,541	03/14/2022	Atul Bedi	JHU3260-9

CONFIRMATION NO. 4182

PUBLICATION NOTICE



OC000000134868437

100899

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

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

Publication No.US-2022-0204628-A1

Publication Date:06/30/2022

NOTICE OF PUBLICATION OF APPLICATION

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(57) Abstract: Provided is a novel peptide ligand (Leu-Ala-Arg-Leu-Leu-Thr) for binding to an EGFR surface pocket based on its 3D crystal structure. When conjugated to the distal end of liposome surface PEG moieties, the peptide ligand directs liposome binding and uptake by EGFR high expressing cancer cells (H1299 and SPCA1) specifically and efficiently. The targeted delivery of liposomal anticancer drug doxorubicin results in better therapeutic efficacy towards cells in vitro. In vivo, the targeted liposomes are injected via tail vein and the time course of their distribution and accumulation in xenograft tumor tissues are studied using a live animal fluorescence imaging system. The LARLLT targeted liposomes were seen to gradually concentrate at the tumor site and be preferentially retained more than 80 hours after injection.



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PEPTIDE LIGAND DIRECTED DRUG DELIVERY

BACKGROUND OF THE INVENTION

- [0001] In cancer therapy, chemotherapeutics are widely used but their efficacies are often undermined by serious side effects resulting from drug toxicities to normal tissues. In order to improve the therapeutic indexes of chemotherapeutic agents, many studies have been focusing on strategies to specifically deliver drugs to the tumor tissue while avoiding normal tissue.
- [0002] Several liposome based anticancer drug formulations have been developed and are already being used in patients with better efficacies and less side effects than the nonliposomal formulations. One of the most successful products, Doxil (also known as Caelyx) (1), contains PEG coated liposomes (stealth liposomes) with extended serum half-life and the ability to gradually extravasate through the leaky vasculatures to accumulate in tumors. In addition to such a passive targeting mechanism, active targeting strategies were also proposed, in which antibodies or targeting ligands were used to direct the liposomes to further encourage the interaction between drug loaded liposomes and tumors. Antibody targeted immunoliposomes have been studied vigorously and look promising in both animal and clinical studies (2-6). Meanwhile, small molecule ligands (7, 8) and peptides (9, 10) are also being examined to construct active targeting liposomes for cancer treatment.
- [0003] Compared to *in silico* or *in vitro* binding studies, the *in vivo* environment for binding is much more complex with many anatomical barriers and interference from natural clearance mechanisms such as the reticuloendothelial system (RES) and other nonspecific interactions. Many variables have been identified using immunoliposome and small molecule targeted liposomes (33, 34). Several factors have been identified that influence the pharmacokinetic properties and extravasation behavior of various active targeted liposomes (33, 34). Immune clearance against the surface conjugated ligands was a major concern (35, 36). IgG containing immunoliposomes were found to interact with Fc receptors and cleared quickly (37, 38, 39). Even small molecule ligand folate conjugation caused enhanced RES clearance of liposomes *in vivo* (8). In addition, both surface ligand densities (40) and the length of PEG linkers (8, 19) were also found to be important.

[0004] The epidermal growth factor receptor (EGFR) is over-expressed in a wide variety of human cancers, including, but not limited to: lung, breast, bladder, and ovarian cancers. It has also been found to associate with various features of advanced disease and poor prognosis (11). Various EGFR targeting vectors and conjugates have been reported to enable increased delivery of cytotoxic drugs, toxins, or radionuclides to cancer cells and inhibit tumor growth in animal models (12-16). Mamot and colleagues developed an EGFR specific immunoliposome and showed in a series of studies that it has better delivery properties and antitumor efficacies in vitro and in vivo (17-19).

[0005] There have been several successful drugs developed targeting EGFR, including the tyrosine kinase inhibitors Tarceva® and Iressa®, and its blocking antibody Erbitux®. In addition to designing drugs directed against EGFR, another strategy is to develop systems that can direct existing therapeutic agents towards it. Many studies have exploited the use of its natural ligand, epidermal growth factor (EGF), for targeted delivery of cytotoxic drugs, toxins, liposomes and other drug/gene delivery systems, and radionuclide systems (12-15). But the endogenous pro-proliferation effect of EGF on cancer cells is always a concern. Two other strategies were proposed. One is to use antibodies or antibody fragments to direct binding to EGFR (17, 18, 27), and the other is to use smaller peptides or EGF fragments (12, 28, 29). Small peptides that can strongly and specifically bind to EGFR but with minimal immunogenicity and pro-proliferation effects are quite desirable.

[0006] There remains a need in the art for materials and methods to effectively deliver therapeutic agents to target cells, for example, those overexpressing the EGFR. This need and others are met by the present invention.

SUMMARY OF THE INVENTION

[0007] The present invention provides peptides that can be used to deliver agents, for example, therapeutic agents and/or imaging agents to a desired target cell. In one embodiment, the present invention provides a peptide comprising a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8. In one embodiment, the peptide may comprise the sequence LARLLT (SEQ ID NO:1). Peptides of the invention can be of any length, for example, from about 6 amino acids to about 15

amino acids or from about 6 amino acids to about 10 amino acids in length. Peptides of the invention may be six, seven, eight, nine, ten or more amino acids in length. In some embodiments, peptides of the invention are six amino acids in length and consist of a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8. In some embodiments a peptide of the invention may consist of the amino acid sequence LARLLT. The present inventions also provides peptidomimetics that mimic the EGFR binding activity of the peptides of the invention as well as substitutions of amino acids in the peptide that conserve its function.

[0008] In another embodiment, peptides and peptidomimetics of the invention may be attached, directly or indirectly, to one or more compounds. In some embodiments, a peptide of the invention is attached to a therapeutic and/or an imaging agent. In one embodiment, the present invention provides a molecule comprising a lipid and a peptide comprising a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8. In some embodiments the peptide may comprise the sequence LARLLT. Optionally, the peptide may be attached to the lipid indirectly through the use of a linker. Any linker known to those skilled in the art may be used. Examples of suitable lipids to which the peptides and peptidomimetics of the invention may be attached include, but are not limited to, 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine (DSPE). In some embodiments, a linker may comprise polyethylene glycol (PEG). An example of a lipid attached to a linker is 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (DSPE-PEG2000). Such a molecule may be further attached to one or more peptides and peptidomimetics of the invention.

[0009] Peptides and peptidomimetics of the invention may be attached to an active agent, for example, a therapeutic agent and/or an imaging agent. The attachment may be direct or indirect. Peptides and peptidomimetics of the invention may be attached to active agents by directly reacting the peptide or peptidomimetic with a functional group on the active agent. Peptides and peptidomimetics of the invention may be indirectly attached to an active agent by reacting the peptide and the active agent with a linker. Typically, a linker will be a bifunctional molecule capable of forming a covalent attachment with both the peptide and the active agent.

[0010] In another embodiment, the present invention comprises liposomes. An example of a liposome of the invention is a liposome comprising a molecule comprising a lipid and a peptide of the invention, for example, a peptide comprising a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8. In some embodiments the peptide may comprise the sequence LARLLT. As discussed above, molecules for use in the liposomes of the invention may comprise a linker between a lipid moiety and a peptide moiety. In one embodiment, the lipid may be of 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine (DSPE). In one embodiment, the linker may be polyethylene glycol (PEG). In one embodiment, a lipid-linker-peptide combination may be 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (DSPE-PEG2000)-LARLLT. Liposomes of the invention may be prepared using any lipid or combination of lipids known to those skilled in the art. Examples of suitable lipids that may be used to prepare liposomes include, but are not limited to, phospholipids (e.g., phosphatidyl cholines, phosphatidyl glycerols, and phosphatidyl ethanolamines), lysolipids and PEGylated phospholipids. Suitable lipids may be combined in any ratio. Optionally, a liposome for use in the invention may be a thermosensitive liposome comprised of lipids that have a gel to liquid phase transition temperature of from about 39.0°C to about 45°C. One example of a suitable liposome may comprise DPPC:MSPC at a ratio of 99:1, 98:2, 97:3, 96:4, 95:5, 90:10, to about 80:20, 75:25, 70:30, 65:35, 60:40, or even 51:49 on a mole percentage basis. One or more additional lipids (e.g., the peptide-linker-lipid and/or PEGylated lipids) may be incorporated into thermosensitive liposomes.

[0011] Liposomes of the invention may further comprise one or more compounds selected from the group consisting of therapeutic compounds and imaging compounds. Typically, a liposome of the invention comprises a bilayer and defines an interior space and the compound is in the interior space of the liposome, in the bilayer of the liposome, or is in both the interior space and bilayer. In some embodiments, a compound may be associated with the exterior of the liposome. One example of a suitable type of compound for inclusion in the liposomes of the invention is an anticancer compound. Examples of suitable anticancer compounds include, but are not limited to, alkylating agents, antimetabolites, spindle poison plant alkaloids,

cytotoxic antitumor antibiotics, topoisomerase inhibitors, monoclonal antibodies or fragments thereof, photosensitizers, kinase inhibitors, antitumor enzymes and inhibitors of enzymes, apoptosis-inducers, biological response modifiers, anti-hormones, retinoids and platinum containing compounds.. In some embodiments, a liposome of the invention may comprise docetaxel. In some embodiments, a liposome of the invention may comprise doxorubicin. In some embodiments, a liposome of the invention may comprise a platinum containing compound, for example, carboplatin or cisplatin.

[0012] The present invention also provides a method of administering a compound to a subject in need of the compound. Such methods typically comprise associating the compound with a peptide comprising a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8, and contacting the subject with the peptide-associated compound. In some embodiments the peptide may comprise the sequence LARLLT. Association may be covalent or non-covalent. In some embodiments, the peptide may be directly or indirectly attached to an active agent. In some embodiments, the peptide is attached to a lipid and the peptide-attached lipid is in the form of a liposome. The compound to be delivered may be part of the liposome, for example, in the lipid bilayer or may be contained in the interior space of the liposome. Alternatively, the peptide may be attached to the compound and the compound may be included in the liposome, for example, in the lipid bilayer or in the interior space. When the peptide is attached to a lipid, the peptide-attached lipid may comprise a linker between the lipid and the peptide. An example of a suitable lipid for use in the methods of the invention is 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine (DSPE). A suitable linker may comprise polyethylene glycol (PEG). Thus, one example of a lipid attached to a peptide of the invention through a linker is 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (DSPE-PEG2000)-LARLLT, which may be incorporated into liposomes and used in the methods of the invention. Any other lipids known to those skilled in the art may be included in the liposomes comprising a lipid-linker-peptide of the invention. In some methods of the invention, a compound may be associated with a liposome comprising a peptide-linker-lipid as described above and the liposome may have a gel to liquid phase transition temperature of from about 39.0°C to about 45°C.

Such a liposome may be administered to a subject in need thereof and a portion of the subject may be heated to a temperature above the gel to liquid phase transition temperature of the liposome.

[0013] Any suitable means of heating the target tissue may be used, for example, application of radiofrequency radiation, application of ultrasound which may be high intensity focused ultrasound, application of microwave radiation, sources that generate infrared radiation such as a warm water bath, light, as well as other forms of externally and internally applied radiation such as that generated by radioisotopes, or electrical and magnetic fields.

[0014] Any compound may be administered using the methods of the invention. Suitable examples of compounds that may be administered include, but are not limited to, therapeutic compounds and imaging compounds, which may be directly or indirectly attached to a peptide of the invention or may be included in a liposome comprising a lipid linked to a peptide of the invention. In one method of the invention, the liposome may comprise a bilayer and define an interior space and the compound may be in the interior space of the liposome, in the bilayer of the liposome, or in both the interior space and bilayer. Methods of the invention may be used to deliver therapeutic compounds, for example, anticancer compounds. Anticancer compounds include, but are not limited to, alkylating agents, antimetabolites, spindle poison plant alkaloids, cytotoxic antitumor antibiotics, topoisomerase inhibitors, monoclonal antibodies or fragments thereof, photosensitizers, kinase inhibitors, antitumor enzymes and inhibitors of enzymes, apoptosis-inducers, biological response modifiers, anti-hormones, retinoids and platinum containing compounds. In some embodiments, methods of the invention may be used to deliver docetaxel. In some embodiments, methods of the invention may be used to deliver doxorubicin. In some embodiments, methods of the invention may be used to deliver a platinum containing compound, for example, carboplatin or cisplatin.

[0015] In one embodiment, the present invention provides a method of treating cancer. Such a method may comprise administering to a subject in need thereof an anticancer compound in association with a peptide of the invention. One example of association is to attach a peptide of the invention directly or indirectly to the anticancer compound. Another example is to include the anticancer compound in a liposome wherein the liposome comprises a lipid associated with a peptide comprising a sequence selected

from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8. In some embodiments the peptide may comprise the sequence LARLLT. Typically, the peptide is attached to the lipid directly or through a linker. A suitable lipid is 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine (DSPE). A suitable linker is polyethylene glycol (PEG). Thus, one example of a peptide-linker-lipid that may be incorporated into a liposome and the liposome used to deliver an anticancer compound is 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (DSPE-PEG2000)-LARLLT. Examples of suitable lipids that may be used to prepare liposomes include, but are not limited to, phospholipids (e.g., phosphatidyl cholines, phosphatidyl glycerols, and phosphatidyl ethanolamines), lysolipids and PEGylated phospholipids. Optionally, a liposome for use in methods of treating cancer of the invention may have a gel to liquid phase transition temperature of from about 39.0°C to about 45°C. One example of a suitable liposome may comprise DPPC:MSPC at a ratio of 99:1, 98:2, 97:3, 96:4, 95:5, 90:10, to about 80:20, 75:25, 70:30, 65:35, 60:40, or even 51:49 on a mole percentage basis. Into such liposomes the peptide-linker-lipid may be incorporated. Any known anticancer agent may be used including, but not limited to, alkylating agents, antimetabolites, spindle poison plant alkaloids, cytotoxic antitumor antibiotics, topoisomerase inhibitors, monoclonal antibodies or fragments thereof, photosensitizers, kinase inhibitors, antitumor enzymes and inhibitors of enzymes, apoptosis-inducers, biological response modifiers, anti-hormones, retinoids and platinum containing compounds. In some embodiments, the anticancer compound may be docetaxel. In some embodiments, the anticancer compound may be doxorubicin. In some embodiments, the anticancer compound may be a platinum containing compound, for example, carboplatin. In some methods of treating cancer of the invention, an anticancer compound may be associated with a liposome comprising a peptide-linker-lipid as described above and the liposome may have a gel to liquid phase transition temperature of from about 39.0°C to about 45°C. Such methods may further comprise heating a portion of the subject. Any method of heating the subject may be used, for example, application of microwave energy, other electromagnetic wave energy, radio frequency ablation, high intensity focused ultrasound, ultrasound, application of heated water and the like.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Figure 1. Docked structures of LARLLT and control peptide on EGFR. A. EGFR structure model from PDB. The asterisk-filled area is the EGF binding site. The area circled is the binding pocket we selected for docking. B. The lowest energy docked conformation of LARLLT inside the EGFR pocket. The peptide is shown in the ball-stick mode. Potential hydrogen bonds are indicated as cylinders. C. the lowest energy docked conformation of control peptide inside the EGFR pocket.

[0017] Figure 2. Fluorescence microscopy studies of ligand directed liposome binding to EGFR high expressing cells. Panel A: Binding of LARLLT, control peptide and EGF targeted liposomes to H1299 cells. (i) LARLLT liposomes, (iii) EGF liposomes, (v) control peptide liposomes. (ii), (iv), (vi) are the phase contrast micrograph of the same fields in (i), (ii), (v). Panel B: Binding of LARLLT targeted liposomes to H1299 at 4°C or 37°C in the presence of 50x mole excess free ligands. (i) binding at 37°C with excess free LARLLT, (ii) binding at 37°C without free LARLLT, (iii) binding at 4°C with excess free LARLLT. (iv) binding at 4°C without free LARLLT, (v) binding at 37°C with excess free EGF, and (vi) binding at 37°C without free EGF. Panel C: Binding of LARLLT, control peptide liposomes to SPC-A1 cells. (i) LARLLT liposomes, (iii) control peptide liposomes. (ii) and (iv) are the phase contrast micrograph of the same fields in (i) and (iii).

[0018] Figure 3. Internalization of LARLLT-conjugated liposome by H1299 cells. A. Overlay of phase contrast and fluorescence images of endocytosed LARLLT liposomes. B. 11 slices from the top to the bottom of the cells using the Z stack scan mode of confocal fluorescence microscope.

[0019] Figure 4. Cell killing effects of targeted liposomes containing doxorubicin. A. H1299 cells. The calculated IC₅₀ for with LARLLT liposomes (filled diamond) was ~13 µg/ml, for control peptide liposomes (open square) was 85 µg/ml, and for free doxorubicin alone (filled triangle) was 5.7 µg/ml. B. SPCA1 cells. The calculated IC₅₀ for with LARLLT liposomes (filled diamond) was ~5 µg/ml, for control peptide liposomes (open square) was 15 µg/ml, and for free doxorubicin alone (filled triangle) was 2 µg/ml.

[0020] Figure 5. The fluorescence image of Cy5.5 and Cy5.5 labeled peptides in tumor bearing mice. Images shown were taken at 1 hour and 6 hours after injection of free Cy5.5 dye, LARLLT-Cy5.5 and control peptide-Cy5.5.

[0021] Figure 6. The fluorescence images of peptide directed liposome distribution and accumulation in tumor tissues. Images shown (from left to right) were the light picture of the mouse, fluorescence images taken at 1 hour, 6 hours, 12 hours, 24 hours, 48 hours, 80 hours after the injection of LARLLT liposomes (top row) and control peptide liposomes (bottom row).

[0022] Figure 7 is a bar graph showing the accumulated doxorubicin concentration in xenograft H460 tumor mass in nude mice at various time points after drug injection. The solid black bars represented the tumor doxorubicin concentration after injection of doxorubicin solution. The black bars with dashed lines represented the tumor doxorubicin concentration after injection of regular doxorubicin liposomes, the black bars with wavy lines represented the tumor doxorubicin concentration after injection of LARLLT modified doxorubicin liposomes.

[0023] Figure 8 is line graph showing observed fluorescence of H460 cells treated with various doxorubicin-containing preparations at 0.01 to 10 µg/ml doxorubicin. x = free doxorubicin, ◇ = D4* liposomes, ▲ = D4 4+4 liposomes, ○ = D4 2+2 liposomes, ● = ThermoDox liposomes. D4 is a liposome comprising SEQ ID NO:1 and D4* is a liposome comprising SEQ ID NO:2. D4-ThermoDox2+2, is comprised DPPC:MSPC: DSPE-MPEG2000:D4-DSPE-MPEG2000 in a 90:10:2:2 molar ratio and D4-ThermoDox4+4, is comprised DPPC:MSPC: DSPE-MPEG2000:D4-DSPE-MPEG2000 in a 90:10:4:4 molar ratio. D4*-ThermoDox comprised DPPC:MSPC: DSPE-MPEG2000:D4*-DSPE-MPEG2000 in a 90:10:2:2 molar ratio.

DETAILED DESCRIPTION OF THE INVENTION

[0024] Peptides

[0025] The present invention provides small peptides that can strongly and specifically bind to EGFR. Typically, peptides and peptidomimetics of the invention can bind to the EGFR without inducing immunogenic and/or pro-proliferative effects.

[0026] An exemplary peptide of the invention is a peptide that comprises the amino acid sequence Leu Ala Arg Leu Leu Thr (SEQ ID NO:1). Additional examples of peptides of the invention include, but are not limited to, peptides wherein one or more amino acids of SEQ ID NO:1 have been substituted with a different amino acid. In some embodiments, only one position of SEQ ID NO:1 will be substituted. In some embodiments, more than one position of SEQ ID NO:1 will be substituted.

Substitutions may be made at any position of SEQ ID NO:1. In some embodiments, one or more positions of SEQ ID NO:1 will be substituted with one or more naturally occurring amino acids. In some embodiments, one or more positions SEQ ID NO:1 will be substituted with one or more non-naturally occurring amino acids. In some embodiments, a peptide of the invention may comprise one or more D-amino acids.

[0027] Typically, substitutions of amino acids in the peptides of the invention may be conservative substitutions. As used herein, a conservative substitution is the replacement of one amino acid with another amino acid of similar physical and/or chemical characteristics. The standard genetically coded amino acids can be grouped according to their characteristics, particularly of polarity and charge. One convenient grouping is as follows: glycine and alanine; serine, threonine, asparagine, glutamine and cysteine; lysine, arginine and histidine; aspartic acid and glutamic acid; valine, leucine, isoleucine, and methionine; and phenylalanine, tryptophan and tyrosine. Replacement of an amino acid from one group with another amino acid in the same group is referred to as a "conservative substitution". Such substitutions do not generally materially affect the properties of the peptides of the invention, for example, the ability to bind to EGFR. Peptides of the invention include peptides that differ from SEQ ID NO:1 only by one or more conservative substitutions.

[0028] Examples of peptides of the invention include, but are not limited to:

[0029] Leu Ala Arg Leu Leu Thr (SEQ ID NO:1)

[0030] Xaa₁ Ala Arg Leu Leu Thr (SEQ ID NO:3)

[0031] Leu Xaa₂ Arg Leu Leu Thr (SEQ ID NO:4)

[0032] Leu Ala Xaa₃ Leu Leu Thr (SEQ ID NO:5)

[0033] Leu Ala Arg Xaa₄ Leu Thr (SEQ ID NO:6)

[0034] Leu Ala Arg Leu Xaa₅ Thr (SEQ ID NO:7)

[0035] Leu Ala Arg Leu Leu Xaa₆ (SEQ ID NO:8)

[0036] where Xaa₁ is selected from the group consisting of valine, isoleucine, and methionine; Xaa₂ is glycine; Xaa₃ is selected from the group consisting of lysine and histidine; Xaa₄ is selected from the group consisting of valine, isoleucine, and methionine; Xaa₅ is selected from the group consisting of valine, isoleucine, and methionine; and Xaa₆ is selected from the group consisting of serine, asparagine,

glutamine and cysteine. The present invention also contemplates peptides comprising more than one of the above substitutions.

[0037] In addition, the present invention relates to non-peptide compounds showing the same ability to bind to EGFR as the peptides of the invention. Such peptidomimetics or "small molecules" capable of mimicking the activity of the peptides of the invention may be used in the practice of the invention. Those skilled in the art are able to design peptidomimetics using well known techniques such as those described in Fauchere J., *Adv. Drug Res.* 15: 29 (1986); and Evans et al., *J. Med. Chem.* 30: 1229 (1987).

[0038] Peptidomimetics that have the ability to bind the EGFR may be used analogously as the peptides of the invention. Typically, such peptidomimetics have one or more peptide linkages optionally replaced by a linkage which may convert desirable properties such as resistance to chemical breakdown in vivo. Such linkages include, but are not limited to, --CH₂NH--, --CH₂S--, --CH₂CH₂ --, --CH=CH--, --COCH₂ --, --CH(OH)CH₂--, and --CH₂SO--.

[0039] Peptides and peptidomimetics of the invention may be associated with any desired material to deliver the material to a desired target cell. Examples of suitable materials include, but are not limited to, compounds (e.g., active agents, therapeutic agents, imaging agents etc.), liposomes (e.g., thermosensitive liposomes and stealth liposomes) and nanoparticles (e.g., protein nanoparticles, metal nanoparticles, and nanoparticles of crystallized small molecules).

[0040] Association of the peptides and peptidomimetics of the invention with material to be delivered may be covalent or non-covalent and direct or indirect. In some embodiments, a peptide of the invention may be covalently attached to a material to be delivered, for example, through a linker.

[0041] Examples of linkers are cross linking agents capable of covalently attaching to a peptide of the invention and to a material to be delivered. Typically crosslinkers react with functional moieties (e.g., -OH, NH₂, COOH and, -SH groups) present on the peptide of the invention and the material to be linked. Different crosslinkers may be chosen because of their different ability to react with different functional moieties.

[0042] The techniques of polypeptide conjugation or coupling through activated functional groups presently known in the art are particularly applicable. See, for

example, Aurameas, et al., *Scand. J. Immunol.*, Vol. 8, Suppl. 7:7-23 (1978) and U.S. Pat. Nos. 4,493,795, 3,791,932 and 3,839,153 which are specifically incorporated herein for the disclosure of coupling of peptides. In addition, a site-directed coupling reaction can be carried out so that any loss of activity due to polypeptide orientation after coupling can be minimized. See, for example, Rodwell et al., *Biotech.*, 3:889-894 (1985), and U.S. Pat. No. 4,671,958. Exemplary additional linking procedures include the use of Michael addition reaction products, di-aldehydes such as glutaraldehyde, Klipstein, et al., *J. Infect. Dis.*, 147:318-326 (1983) and the like, or the use of carbodiimide technology as in the use of a water-soluble carbodiimide to form amide links. Alternatively, the heterobifunctional cross-linker SPDP (N-succinimidyl-3-(2-pyridyldithio) propionate)) can be used to conjugate peptides, in which an N-or C-terminal cysteine has been introduced.

[0043] The conjugation of the peptide to an active agent as set forth herein, can be effected by chemical conjugation procedures well known in the art, such as by creating peptide linkages, use of condensation agents, and by employing well known bifunctional cross-linking reagents. The conjugation may be direct, which includes linkages not involving any intervening group, e.g., direct peptide linkages, or indirect, wherein the linkage contains an intervening moiety, such as a protein or peptide, e.g., plasma albumin, or other spacer molecule. For example, the linkage may be via a heterobifunctional or homobifunctional cross-linker, e.g., carbodiimide, glutaraldehyde, N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) and derivatives, bis-maleimide, 4-(N-maleimidomethyl) cyclohexane-1-carboxylate, and the like. Cross-linking may also be accomplished without exogenous cross-linkers by utilizing reactive groups on the molecules being conjugated. Methods for chemically cross-linking peptide molecules are generally known in the art, and a number of hetero- and homobifunctional agents are described in, e.g., U.S. Pat. Nos. 4,355,023, 4,657,853, 4,676,980, 4,925,921, and 4,970,156, and additional cross linking reagents are commercially available, for example, from Pierce Chemical Co., Rockford, IL. 61105, each of which is incorporated herein by reference. Cleavable cross-linkers, particularly those that form cleavable disulfide bonds, may be employed to allow cleavage of the conjugate to free the therapeutic agent under physiological conditions. An example of such a cleavable cross-linker is 4-succinimidylloxycarbonyl-a-(2-pyridyldithio)-toluene. Such conjugation, including cross-linking, should be

performed so as not to substantially affect the desired function of the peptide or active agent conjugated thereto.

[0044] In one embodiment of the invention, peptides and peptidomimetics of the invention may be attached to nanoparticles. Many varieties of nanoparticles are available, such as different polymeric and metal nanoparticles, liposomes, niosomes, solid lipid particles, micelles, quantum dots, dendrimers, microcapsules, cells, cell ghosts, lipoproteins, and different nanoassemblies. Protein nanoparticles may be prepared using any technique known in the art, for example, using a modified desolvation-crosslinking method. A protein in aqueous solution (2%, w/v) can be incubated with an active agent in buffer and then added dropwise into ethanol to form protein nanoparticles incorporating an active agent. These may be crosslinked to the peptide of the invention, for example, using glutaraldehyde. Gold nanoparticles can be synthesized according to standard wet chemical methods using sodium borohydride as a reducing agent.

[0045] In one embodiment, peptides and peptidomimetics of the invention may be attached to a lipid including, but not limited to, attached directly to the headgroup region of the lipid, or attached via a linker, such as a polymer (e.g., PEG), to the headgroup region, and incorporated into a liposome. The liposome (which may be a thermosensitive liposome, i.e., a liposome with a gel to liquid phase transition temperature of from about 39.0°C to about 45°C) will typically comprise an active agent (e.g., therapeutic agent and/or imaging agent.)

[0046] Liposomes of the invention typically comprise one or more phosphatidylcholines. Suitable examples of phosphatidylcholines that can be used in the practice of the invention include, but are not limited to, 1,2-Dilauroyl-sn-glycero-3-phosphocholine (DLPC), 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), and 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC).

[0047] Liposomes of the invention typically comprise one or more phosphatidylglycerols. Suitable examples of phosphatidylglycerols include, but are not limited to, 1,2-Dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG), 1,2-Dipalmitoyl-sn-glycero-3-phosphoglycerol (DPPG), 1,2-Distearoyl-sn-glycero-3-

phosphoglycerol (DSPG), and 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG).

- [0048] Liposomes of the invention typically comprise one or more lysolipids. As used herein “lysolipid” refers to any derivative of phosphatidic acid (1,2-diacyl-*sn*-glycero-3-phosphate) that contains only one acyl chain covalently linked to the glycerol moiety. Derivatives of phosphatidic acid include, but are not limited to, phosphatidylcholine, phosphatidylglycerol, and phosphatidylethanolamine. Any lysolipid known to those skilled in the art may be used in the practice of the invention. Lysolipids include, but are not limited to, monopalmitoylphosphatidylcholine (MPPC), monolaurylphosphatidylcholine (MLPC), monomyristoylphosphatidylcholine (MMPC), monostearoylphosphatidylcholine (MSPC), and mixtures thereof.
- [0049] Liposomes of the invention may also comprise a lipid to which a hydrophilic polymer has been attached, for example, a lipid covalently attached to PEG.
- [0050] Active agents
- [0051] Peptides and peptidomimetics of the invention may be used to deliver active agents. As used herein, “active agent” includes any compound desired to be delivered to a specific site in a subject. Any active agent may be used in the practice of the invention.
- [0052] Anticancer agents may be used as the active agents in the thermosensitive liposomes of the invention. Suitable examples of anticancer agents include:
- [0053] alkylating agents, for example, nitrogen mustards (e.g., Chlorambucil, Chlormethine, Cyclophosphamide, Ifosfamide, Melphalan, nitrosoureas (e.g., Carmustine, Fotemustine, Lomustine, Streptozocin), platinum containing compounds (e.g., Carboplatin, Cisplatin, Oxaliplatin, BBR3464), Busulfan, Dacarbazine, Mechlorethamine, Procarbazine, Temozolomide, ThioTEPA, and Uramustine;
- [0054] antimetabolites that target, for example, folic acid (e.g., aminopterin, methotrexate, pemetrexed, raltitrexed), purine metabolism (e.g., cladribine, clofarabine, fludarabine, mercaptopurine, pentostatin, thioguanine), pyrimidine metabolism (e.g., capecitabine, cytarabine, fluorouracil, floxuridine, gemcitabine);
- [0055] spindle poison plant alkaloids, for example, taxanes (e.g., docetaxel, paclitaxel) and vinca (e.g., vinblastine, vincristine, vindesine, vinorelbine);

- [0056] cytotoxic/antitumor antibiotics, for example, anthracycline antibiotics (e.g., daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, valrubicin, carinomycin, nacetyladrriamycin, rubidazone, 5-imidodaunomycin, N30 acetyl-daunomycin, and epirubicin), bleomycin, mitomycin, and actinomycin;
- [0057] topoisomerase inhibitors, for example, camptothecines (e.g., camptothecin, topotecan, irinotecan), podophyllum (e.g., etoposide, teniposide).
- [0058] monoclonal antibodies or fragments thereof, for example, Alemtuzumab, Bevacizumab, Cetuximab, Gemtuzumab, Panitumumab, Rituximab, Tositumomab, and Trastuzumab;
- [0059] photosensitizers, for example, aminolevulinic acid, methyl aminolevulinate, porfimer sodium, and verteporfin;
- [0060] kinase inhibitors, for example, Dasatinib, Erlotinib, Gefitinib, Imatinib, Lapatinib, Nilotinib, Sorafenib, Sunitinib, and Vandetanib.
- [0061] Enzymes, for example, asparaginase, pegaspargase and inhibitors of enzymes, for example hydroxyurea;
- [0062] Apoptosis-inducers, for example, arsenic trioxide, Velcade and Genasense;
- [0063] Biological response modifiers, for example, Denileukin Diftitox;
- [0064] Anti-hormones, for example, Goserelin acetate, leuprolide acetate, triptorelin pamoate, Megestrol acetate, Tamoxifen, toremifene, Fulvestrant, testolactone, anastrozole, exemestane and letrozole;
- [0065] Retinoids, for example, 9-*cis*-retinoic acid and all-*trans*-retinoic acid
- [0066] In additional embodiments, the thermosensitive liposomes of the invention can comprise more than one antineoplastic agent, or more than one thermosensitive liposome can be used in the methods of the invention, each of which comprises different active agents, for example, different anticancer agents.
- [0067] Additional active agents that can be used in the practice of the present invention include, but are not limited to antibiotics, antifungals, anti-inflammatory agents, immunosuppressive agents, anti-infective agents, antivirals, antihelminthic, and antiparasitic compounds.

[0068] An active agent may be an imaging agent. Imaging agents suitable for use in the present liposome preparations include ultrasound contrast agents, X-Ray contrast agents (for example: gold and barium-based agents, Iohexol, Visipaque and other iodine-based agents, as well as other particulate agents), magnetic resonance contrast agents (for example, paramagnetic materials containing: manganese, iron, gadolinium and other lanthanides), nuclear medicine agents (such as radioisotopes or compounds containing radioisotopes).

[0069] Methods of use

[0070] Active agents associated with peptide of the invention can be administered to a subject using any suitable route, for example, intravenous intraarterial, intramuscular intraperitoneal, subcutaneous, intradermal, intraarticular, intrathecal intracerebroventricular as well as other routes such as by inhalation, orally or directly on to mucous membranes (sublingually). Any tissue can be treated using the materials and methods of the invention. Examples of tissues which can be treated using the materials and methods of the present invention include, but are not limited to, liver, kidney, bone, soft tissue, head and neck tissue, muscle, adrenal tissue, lung, breast, thyroid, pancreas, uterine tissues including endometrial and cervical tissue, ovary, and prostate. Tissues that can be treated include both cancerous tissue, otherwise diseased or compromised tissue, as well as healthy tissue if so desired.

[0071] The dose of active agent administered to the subject using the methods of the invention can be determined by those of skill in the art, and suitably is administered intravenously over an extended time period, for example, from about 1 minutes to about 24 hours.

[0072] The dose of active agent may be adjusted as is known in the art depending upon the active agent comprised in the carrier.

[0073] When an active agent is incorporated into a thermosensitive liposome comprising a peptide of the invention, the target tissue of the subject may be heated before and/or during and/or after administration of the thermosensitive liposomes. In one embodiment, the target tissue is heated first (for example, for 10 to 30 minutes) and the thermosensitive liposomes are delivered into the subject as soon after heating as practicable. In another embodiment, thermosensitive liposomes are delivered to

the subject and the target tissue is heated as soon as practicable after the administration. This heating may continue for up to 3 hours.

[0074] Any suitable means of heating the target tissue may be used, for example, application of radiofrequency radiation, application of ultrasound which may be high intensity focused ultrasound, application of microwave radiation, any source that generates infrared radiation such as a warm water bath, light, as well as other forms of externally and internally applied radiation such as that generated by radioisotopes, electrical and magnetic fields.

[0075] It will be readily apparent to one of ordinary skill in the relevant arts that other suitable modifications and adaptations to the methods and applications described herein can be made without departing from the scope of the invention or any embodiment thereof. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for purposes of illustration only and are not intended to be limiting of the invention.

EXAMPLES

EXAMPLE 1

[0076] Egg Phosphatidylcholine, 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine (DSPE), 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (DSPE-PEG2000), 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Maleimide(Polyethylene Glycol)2000] (DSPE-PEG2000-Mal) and Cholesterol were all purchased from Avanti Polar Lipids (AL, USA). Lissamine™ rhodamine B 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (rhodamine DHPE) and N-(fluorescein-5-thiocarbamoyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (fluorescein DHPE) were purchased from Invitrogen Corporation. N-Succinimidyl 3-(2-pyridyldithio) propionate (SPDP) and tris(2-carboxyethyl) phosphine (TCEP) were purchased from Pierce Biotechnology, Inc. Cy5.5 Mono NHS Ester was supplied by GE Healthcare. All other chemicals in analytical grade were obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

[0077] The crystal structure of EGFR in inactive state was downloaded from PDB (<http://www.rcsb.org/pdb/>, code:1NQL). A 3D structure model was built and scanned for candidate binding pocket using the PSCAN 2.2.2 program provided by Dr. Sheng-Hung Wang (<http://home.pchome.com.tw/team/gentamicin/mol/mol.htm>). A pocket on the surface near the top was selected and shown in Figure 1. The amino acid residue structure inside the pocket was carefully analyzed and a focused library containing 132 hexapeptides was designed based on the Mekler-Idlis amino acid pairing theory (20).

[0078] The library was screened and ranked for binding to the EGFR surface pocket using Autodock3.0. In order to speed up the calculation and run the program on multiple processors, we modified the software to enable parallel computing. All the computing was done on the SGI Onyx3800 machine in SJTU supercomputing center. The 132 peptides were at first roughly screened to select 50 peptides with lower binding energies. For each peptide, 100 separate docking attempts were performed and each docking consisted of 250,000 energy evaluations using the Lamarckian genetic algorithm local search (GALS) algorithm (21). Other docking parameters were all selected based on those described by Csaba and David (22). The selected 50 peptides were further evaluated in a refined search phase with 10 million energy evaluations per docking using the same algorithm. The peptides' lowest docking energies were compared and the 20 lowest ones were selected and sent for synthesis and wet screening. BIACore binding studies indicated that two of the peptides bound to EGFR in a concentration dependant fashion. Especially the peptide with the sequence LARLLT (SEQ ID NO:1) (ranked no. 4 in docking studies) was clearly a potential binder.

[0079] The binding pocket selected with the aid of the PSCAN program is shown in Figure 1A (circled). The binding pocket is on a surface easy to be accessed from the outside but not too close to the EGF binding site. We reasoned that the spatial structures in this pocket are less like to vary with or without EGF binding and EGFR dimerization. The pocket has some hydrophobic residues at the bottom and hydrophilic residues around the opening (charged residues: Asp51, Glu73, Glu110, Arg48, Lys56, Arg74; polar residues: Thr106, Ser53, Ser62).

[0080] The two peptides we selected to use in this study (LARLLT and control peptide) were docked into the pocket and searched for the best docked structures. The

lowest docking energy and free binding energy for LARLLT were -17.9 kcal/mol and -8.54 kcal/mol respectively and those for control peptide were -12.52 kcal/mol and -3.85 kcal/mol.

[0081] As shown in Figure 1B, the peptide LARLLT sat nicely inside the pocket, and almost all the residues interacted with residues on EGFR. There were potentially six hydrogen bonds, involving residues at both ends (LEU1, ALA2 and THR6) and in the center (ARG3). At the N-terminal end, the amino group hydrogen of LEU1 interacted with the carboxyl oxygen of GLU73, and the main-chain oxygen of ALA2 interacted with the main-chain amide hydrogen of GLU73. At the carboxyl end, THR6 was both hydrogen bond donor and receptor using its carboxyl-end oxygen to interact with the hydroxyl hydrogen of SER53 and its side-chain hydroxyl hydrogen to interact with the carboxyl oxygen of ASP51. In the middle, ARG3 interacted with EGFR through both electrostatic interaction (with GLU73 and GLU110) and hydrogen bonding. After these three nicely spaced key residues (LEU1, THR6, ARG3) fixing into the EGFR pocket like anchors, they also facilitated the interaction between LARLLT's more hydrophobic residues ALA2, LEU4 and LEU5 with the more hydrophobic bottom in the pocket. The detailed interactions between the residues on LARLLT and EGFR as shown in the docking structure (Figure 1) were in nice agreement with the M-I pairing theory (20).

[0082] In contrast, as shown in Figure 1C, the control peptide SEQ ID NO:2, with the same amino acid composition but scrambled sequence, could not even fit inside the pocket. The charged ARG1 interacted with ASP51, but other interactions were just too weak to hold down control peptide.

EXAMPLE 2

[0083] The peptide LARLLT was synthesized and purified by GL Biochem Ltd. (Shanghai), and the structure and purity were all confirmed by HPLC and MS. For comparison, a peptide designated D4* with the same amino acids composition as LARLLT but scrambled sequences (RTALLL, control peptide (SEQ ID NO:2)) was also synthesized. To fluorescently label the two peptides, Cy5.5-NHS was mixed at a 1:2 molar ratio with either LARLLT or the control peptide, and were incubated at 25°C over night, protected from light. These fluorescent peptides were used without further purification.

EXAMPLE 3

[0084] EGF or LARLLT/control peptides were dissolved in PBS-EDTA and mixed with N-Succinimidyl 3-(2-pyridyldithio) propionate (SPDP) dissolved in DMSO at 1:1.2 molar ratio for 1 hour in room temperature and then lyophilized. In the mean time, DSPE-PEG2000-Mal lipids in chloroform were dried into a thin film and hydrated in HEPES buffer (pH 7.4) to about 0.4 mM concentration. For conjugation, the thioated protein or peptides were added to tris(2-carboxyethyl) phosphine (TCEP) solutions, incubated for 1 hour at room temperature under nitrogen, quickly mixed with the MAL-PEG2000-DSPE micelle solution, and reacted while maintaining stirring under nitrogen at 10°C overnight at 5:1 molar ratio. By HPLC analysis, almost all the Mal-PEG-DSPEs were modified after such reactions. The excess protein/peptides may be removed using standard techniques, for example, by gel filtration column, dialysis etc.

EXAMPLE 4

[0085] Liposomes were all prepared using the dry film hydration method, followed by extrusion several times through 100nm membranes. Most liposomes used in this study started with the lipid composition of EPC:CHOL:DSPE-PEG =10:5:0.5. Except for rhodamine or fluorescein labeled liposomes, either rhodamine-DHPE or fluorescein-DHPE was added to the lipid composition at about 0.6% mol. total lipids.

[0086] For Cy5.5 labeled liposomes, Cy5.5-DSPE was synthesized first by mixing Cy5.5-NHS, DSPE and triethylamine at 3:1:3.5 molar ratio in chloroform and incubating (protected from light) at 25°C over night. The resulting Cy5.5-DSPE was incorporated into the lipid composition at about 0.5% mol. total lipids. The excess Cy5.5 was removed after liposome formation by dialysis.

EXAMPLE 5

[0087] Liposomes encapsulating doxorubicin were made following the transmembrane gradient procedure developed (23, 24). Briefly, lipids were hydrated in 125 mM ammonium sulfate at pH 4.0, and extruded to obtain ~100nm liposomes. They were then dialyzed in HEPES buffer (pH 7.5) and diluted to the required concentration with 20mM HEPES, 150mM NaCl (pH 7.5). Doxorubicin was added and incubated with the liposomes at 60°C for 10 min.

[0088] The ligand conjugated lipids were inserted into the preformed liposomes based on the procedure developed by Ishida et al. with minor modifications (25). Briefly, DSPE-PEG2000-ligand micelle solutions were added to the preformed liposome solutions at 9:100 mol. lipid ratios and incubated at 60°C for 1 hour (55°C and 30min. for doxorubicin containing liposomes). The solutions were then dialyzed against PBS using SnakeSkin™ Pleated Dialysis Tubing 10,000 MWCO (Pierce Chemical Company) for 4 hours. As control, mPEG2000-DSPE micelles were also mixed and incubated at the same ratio to make non-targeted liposomes.

[0089] The peptide ligands were conjugated to DSPE-PEG2000 by a sulfhydryl-maleimide coupling reaction. The reactions were monitored and confirmed using Reverse Phase HPLC analysis. The resulted peptide-PEG-DSPE or the mPEG-DSPE controls were incorporated into preformed liposomes using widely used incubation methods (25). We tested different peptide-PEG-lipid (or mPEG-lipid) to lipid ratios (3:100, 6:100, and 9:100 mol.) during the incubation and selected the 9:100 ratio to deter nonspecific binding of the liposomes to most cells. We confirmed that such a procedure was not disturbing to the integrity of the liposomes. No more than 5% of the encapsulated doxorubicin leaked during and after the procedure. The final size distribution of the liposomes were determined by photon correlation spectroscopy (PCS) using a Zetasizer3000H (Malvern Instruments). Compared to the liposomes made right after the extrusion (~110nm), the particle sizes after the peptide lipid incorporation were slightly bigger (130~150nm) but still stable.

EXAMPLE 6

[0090] The human non-small cell lung carcinoma cell line H1299 and the human lung adenocarcinoma cell line SPCA1, both have high EGFR expressing levels, were used in this study. The cells were cultured in RPMI1640 culture medium containing 10% fetal bovine serum at 37°C in humidified atmosphere containing 5% CO₂.

[0091] Both H1299 and SPCA1 xenograft mouse models were prepared by the animal experimental center of Shanghai Cancer Institute. They were used in the in vivo imaging experiments about 3~4 weeks after tumor cell inoculation (tumor size about 5-7mm diameter), and humanely sacrificed afterwards. The animal study protocols were approved by the Animal Study Committee of Shanghai Jiaotong University, School of Pharmacy.

EXAMPLE 7

[0092] EGFR high-expressing H1299 or SPCA1 cells were plated in 35mm-diameter culture dish (1×10^6 cells per dish) and cultured in RPMI1640 medium for 24~48 hours. After the cell culture reached about 80% confluence, ligand-conjugated rhodamine liposomes were diluted in RPMI1640 and added into the culture dish at either 4°C or 37°C. In the competitive binding experiments, 50 fold molar excess of free LARLLT or EGF were added into the medium 2 hours before the addition of LARLLT incorporated liposomes. The cells were incubated at the specific temperatures for 4 hours, and then washed six times with PBS (pH 7.4). The remaining bound and internalized fluorescence lipid were visualized using a Confocal Laser scanning Microscope (CLSM, Zeiss LSM, Germany). For the flow cytometric studies, H1299 or SPCA1 cells were plated in 35mm-diameter well until cells grew up to about 80% confluence. After incubation with ligand-modified fluorescein labeled liposomes for 3 hours at 37°C, cells were washed with PBS, treated with trypsin for suspension, and analyzed by a flow cytometer (BD FACSCalibur, Becton Dickinson).

[0093] The EGF, LARLLT, or control peptide modified rhodamine labeled liposomes were tested for their binding ability to EGFR high-expressing cells in vitro. As shown in Figure 2A, both EGF and LARLLT modified liposomes bound extensively to the H1299 cells. The fluorescence is a bit stronger with EGF modified liposomes than LARLLT modified liposomes, suggesting tighter binding by EGF. But with control peptide, there was almost no fluorescence shown. Similar situations were observed with another EGFR expressing cell line SPC-A1 (Figure 2C)

[0094] The detailed binding characteristics were further evaluated in H1299 cells at different temperatures. At 4°, the fluorescence was mostly seen on cell surfaces, and could be largely competed off by excess unlabeled free LARLLT (Figure 2B-iii & iv). At 37°, however, the competition effect of free LARLLT was less obvious (Figure. 2B-I & ii), suggesting there might be active endocytosis and receptor turnaround after the binding of LARLLT to EGFR. It is also interesting to note that the presence of free EGF at 37° actually affected the LARLLT liposome fluorescence to a certain degree, although not completely (Figure. 2B-v & vi). It is possible that EGF interfered with the endocytic pathway of LARLLT after binding.

[0095] Figure 3 showed the LARLLT modified liposome binding and uptake by H1299 cells in higher magnification (Figure 3A) and in Z stack serial scan images (Figure 3B). Lipid fluorescence was seen in numerous endocytic vesicles inside cells. It is noteworthy that the binding of LARLLT to a trivial surface pocket on EGFR far away from the EGF binding site can initiate such active endocytic activities.

[0096] In order to compare the different binding efficiencies of LARLLT and control peptide incorporated liposomes in larger cell populations, we used flow cytometry. Liposomes were labeled with fluorescein-DSPE and added to H1299 and SPCA1 cells respectively. After 3 hours of incubation, 75% of H1299 cells were highly fluorescent (101~103 FL1 value) with LARLLT liposome binding, but only 27% were fluorescent after control peptide liposomes binding. For SPCA1 cells, 68% were fluorescent (35~103 FL1 value gate) with LARLLT liposome and 17% with control peptide liposomes.

EXAMPLE 8

[0097] H1299 and SPCA1 cells were plated at the density of 10^4 cells/well in 96-well plates and grown overnight. Free doxorubicin, LARLLT modified doxorubicin liposomes and control peptide modified doxorubicin liposomes were added into the medium and incubated for 2 hours. The cells were then washed with fresh medium, and let grown for another 48 hours. The cell viabilities after different treatments were determined using a MTT assay (26).

[0098] Doxorubicin loaded liposomes were modified by LARLLT or control peptide conjugated lipids and added to EGFR high expressing cells. The cell killing effects were assayed using MTT. In both H1299 and SPCA1 cells, the LARLLT modified liposomes were better in delivering drugs than control peptide modified liposomes (Figure 4A&B). The calculated IC_{50} s for LARLLT liposomes were about 3-5 times cytotoxic than those for control peptide liposomes in both cases.

EXAMPLE 9

[0099] Cy5.5, LARLLT-Cy5.5, control peptide-Cy5.5, and LARLLT and control peptide incorporated Cy5.5 labeled liposomes were injected through the tail vein to H1299 or SPCA1 xenograft tumor bearing mice. At various time points afterwards, the mice were anesthetized and imaged with an Optix in vivo fluorescence imaging

system (GE Healthcare). Excepted for the Cy5.5 dye only control, all the other groups contained at least 3 mice. The representative images from the same most representative mouse in each group were shown. The images had been processed using the fluorescence lifetime gating for Cy5.5 (0.8-1.1ns) to remove most autofluorescent interferences.

[0100] LARLLT and control peptide were labeled using the near infrared dye Cy5.5 and injected via tail vein to H1299 tumor bearing mice. Cy5.5 fluorescence distributions inside the mice were imaged at various time points after injection. In all groups including the free dye control, fluorescences were first seen all over the body, and then quickly afterwards in the kidney and bladder. To avoid the interference from the strong fluorescence in kidney and bladder, we only showed the focused imaging scan at the subcutaneous tumor bearing region (Figure 5). In Cy5.5 dye injected mice, the fluorescence signal in the tumor region was the same as background. But in LARLLT-Cy5.5 injected mice, between 1 to 6 hours after injection, fluorescence signals in the tumor tissue were much stronger, as compared to the surrounding tissues. In contrast, Cy5.5-control peptide injected mice didn't show any fluorescence accumulation in the tumor region either.

[0101] LARLLT and control peptide modified Cy5.5 labeled liposomes were injected via the tail vein into H1299 tumor bearing mice. The entire bodies of the mice were scanned at various times after the injection and the fluorescence intensity from the tumor bearing region at different time points were quantified. As shown in Figure 6, LARLLT modified liposomes accumulated gradually inside the tumor region from 6 hours and on. Even after 80 hours, the signals and the contrast were still significant. But for control peptide modified liposomes, the fluorescence intensities in the tumor region were always as background. We calculated the relative ratios of fluorescence intensities inside the tumor region to the whole body signals (Table 1). Fluorescence intensities(NC) of tumor region and the whole body were calculated using eXplore OptiView 1.0.0.0 software. The percentage of the fluorescence inside the tumor region were also listed.

[0102] Table1 Fluorescence intensities in tumor region at various time points after fluorescent liposome injection.

	Body		Tumor		Tumor / Body	
	LARLLT-L	control peptide-L	LARLLT-L	control peptide-L	LARLLT-L	control peptide-L
1hour	4.68E+04	4.15E+04	6.00E+03	4.16E+03	13%	10%
6hour	2.75E+04	2.58E+04	4.37E+03	3.45E+03	16%	13%
12hour	2.34E+04	1.91E+04	3.92E+03	1.99E+03	17%	10%
24hour	1.84E+04	1.89E+04	3.38E+03	2.02E+03	18%	11%
48hour	1.34E+04	1.30E+04	2.69E+03	1.35E+03	20%	10%
80hour	1.07E+04	9.92E+03	1.99E+03	1.14E+03	19%	11%

[0103] The ratios actually got better and better from 1 hour after injection all the way to 76-80 hours, when it seemed to plateau, suggesting a gradual accumulation and the targeted liposomes inside the tumors and strong retention afterwards.

[0104] In some embodiments, peptides and peptidomimetics of the invention may be designed to bind to a surface pocket away from the EGF binding site. This may allow peptides and peptidomimetics of the invention (and therapeutics attached to the peptides) to bind EGFR expressing cells even if the binding affinities of the peptides may be less than the binding affinity of EGF since there will be no direct competition for binding between the peptides and EGF.

[0105] When peptides and peptidomimetics of the invention are conjugated to various compounds, for example, to the distal end of PEG2000-DSPE and incorporated into liposomes, peptides and peptidomimetics of the invention retain their EGFR binding ability and mediate specific attachment and uptake of the compounds (e.g., liposomes) by EGFR high-expressing cells. As shown above, when compounds to which the peptides and peptidomimetics of the invention are attached (e.g., the peptide incorporated liposomes shown above) were injected in vivo, the compounds are directed to the target cells (e.g., tumor cells) and cause the peptide conjugated compound (e.g., peptide conjugated liposomes) to accumulate at the target (e.g., in the tumor).

[0106] In our studies, we used only PEG2000 linker, and in vitro binding to cells was found most significant (with minimal background nonspecific binding) when the ligand containing lipids were incorporated under the condition of 2-4:100 lipid ratio. Any other linker known to those skilled in the art may be used. Likewise other ratios of lipids may be used.

[0107] The same liposome formulations were used in vivo. The liposomes had relative high surface ligand-PEG densities and slowly accumulated in tumors. In fact, several earlier studies have indicated that during circulation and extravasation, active

targeting liposomes would at the best behave similar as Stealth liposomes (4, 6, 41). Their targeting advantages were only significant after extravasation into the tumor tissue, when they could bind tightly with cancer cells and facilitate cell uptake (8, 42). Our observations using the in vivo fluorescence imaging system (Figure 6) agreed with such a mechanism. The ligand incorporated LARLLT liposomes were found to stay in the tumor tissue for a very long time (>80 hours). The control group, however, didn't show significant accumulation in the tumor tissue around 12-24 hour points, as we would expect for non-targeting stealth liposomes.

[0108] Small animal in vivo fluorescence imaging is a newly developed tool for monitoring the biodistribution of labeled drugs and delivery systems. Cy5.5 is the most commonly used dye because of its superior tissue penetration and low background. The main advantage of the in vivo imaging technique is that it's noninvasive and allows continues monitoring of the same live animals throughout the course of the study. Many earlier studies had used radio labeled drugs or lipids to follow their distribution in vivo, it was necessary to sacrifice numbers of animals at different time points for obtain averaged readouts. In our experiences using in vivo imaging system, different animals in a study group may still have variation in their general health, physiology, metabolism, tumor size and location etc. The time-sequenced distribution profile of active targeting liposomes also agreed well with what had been reported using other methods (8, 41).

EXAMPLE 10

[0109] The ability of liposomes targeted using the peptides of the invention to enhance accumulation of drug in the target tumor was assessed. 5mg/kg doxorubicin was injected intravenously into H460 tumor bearing nude mice. Free doxorubicin (Figure 7, Dox) was compared to doxorubicin incorporated into a PEGylated liposome (Figure 7, PEG) and doxorubicin incorporated into a liposome comprising a peptide of the invention (Figure 7, D4). Mice were sacrificed at 0.5h, 6h and 48h post-injection and the tumors were excised and assayed for doxorubicin content. N=3 animals were used per experimental group.

[0110] As shown in Figure 7, liposomes comprising the peptides invention resulted in significantly more doxorubicin being accumulated in the tumor as compared to free doxorubicin and doxorubicin in a PEGylated liposome.

EXAMPLE 11

[0111] FACS analysis of binding of liposomes comprising peptides of the invention

[0112] H-460 cells were grown in cell culture plate under sterile conditions and harvested by scraping. The liposome samples (empty ThermoDox liposome, ThermoDox, D4-ThermoDox, D4*-ThermoDox where D4 ThermoDox comprises SEQ ID NO:1 and D4*ThermoDox comprises SEQ ID NO:2) and doxorubicin hydrochloride were added and incubated at 37°C with H-460 cells for 4 hours. Two different liposomes comprising different amounts of the peptide of the invention (D4-ThermoDox) were tested. The two liposomes were designated D4-ThermoDox2+2, which comprised DPPC:MSPC: DSPE-MPEG2000:D4-DSPE-MPEG2000 in a 90:10:2:2 molar ratio and D4-ThermoDox4+4, which comprised DPPC:MSPC: DSPE-MPEG2000:D4-DSPE-MPEG2000 in a 90:10:4:4 molar ratio. The D4*-ThermoDox comprised DPPC:MSPC: DSPE-MPEG2000:D4*-DSPE-MPEG2000 in a 90:10:2:2 molar ratio. After incubation, the cells were washed 4 times with PBS, and counted by FACS using doxorubicin fluorescence (excitation at 488nm, and emission at 575nm).

[0113] The following groups were examined: a. Non-treatment control; b. ThermoDox at approx. 0.01-10 µg (doxorubicin)/mL; c. D4-ThermoDox2+2 at approx. 0.01-10 µg (doxorubicin)/mL; d. D4-ThermoDox4+4 at approx. 0.01-10 µg (doxorubicin)/mL; e. D4*-ThermoDox at approx. 0.01-10 µg (doxorubicin)/mL; and f. doxorubicin hydrochloride 0.01-10 µg.

[0114] The experiments were repeated at least three times, and the representative histograms from the same experiments are shown. The detailed numbers may shift due to the variations in cell number and FACS parameters, but the general pattern and formulation differences were always consistent.

[0115] At the 10 µg/ml dose, the doxorubicin fluorescence intensities of D4-ThermoDox(2+2) in vitro are stronger than ThermoDox and D4*-ThermoDox, only weaker than free doxorubicin hydrochloride. Given that the free drug is relatively more permeable through cell membrane, the D4-liposome is confirmed to bind more to H460 cells than the control liposomes. At the 1µg/ml dose, the D4-ThermoDox (2+2, 4+4) treated cells generated more doxorubicin fluorescence signal than ThermoDox

and D4*-ThermoDox. At both the 0.1 µg/ml and 0.01 µg/ml doses, there were no significant differences between liposomes of different formulations

[0116] Figure 8 is a line graph of the mean of each of the FACS histograms plotted against the amount of doxorubicin administered for each formulations tested. The doxorubicin uptake (mean fluorescence intensity) was the highest using the drug solution because it can permeate cell membrane quite efficiently. Comparing the different liposome formulations, the two D4 liposome preparations were much better than ThermoDox controls and D4* liposome controls.

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- [0160] All publications, patents and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains, and 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.

What is claimed is:

1. A peptide comprising a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8 wherein the peptide is from about 6 to about 15 amino acids in length.
2. A peptide according to claim 1, comprising SEQ ID NO:1.
3. A peptide according to claim 1, consisting of SEQ ID NO:1.
4. A molecule comprising a lipid associated with a peptide comprising a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.
5. A molecule according to claim 4, comprising a linker between the lipid and the peptide.
6. A molecule according to claim 5, wherein the lipid is 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine (DSPE).
7. A molecule according to claim 5, wherein the linker comprises polyethylene glycol (PEG).
8. A molecule according to claim 4, comprising 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (DSPE-PEG2000).
9. A molecule according to claim 4, comprising SEQ ID NO:1.
10. A liposome comprising:
a molecule comprising a lipid associated with a peptide,
wherein the peptide comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

11. A liposome according to claim 10, wherein the molecule comprises a linker between the lipid and the peptide.
12. A liposome according to claim 10, wherein the lipid is 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine (DSPE).
13. A liposome according to claim 12, wherein the linker comprises polyethylene glycol (PEG).
14. A liposome according to claim 12, comprising 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (DSPE-PEG2000)-LARLLT.
15. A liposome according to claim 10, wherein the liposome further comprises a lipid selected from the group consisting of phosphatidylcholines, phosphatidylglycerols, and lysolipids.
16. A liposome according to claim 10, wherein the liposome comprises 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and monostearoylphosphatidylcholine (MSPC).
17. A liposome according to claim 10, further comprising a compound selected from the group consisting of therapeutic compounds and imaging compounds.
18. A liposome according to claim 17, wherein the liposome comprises a bilayer and defines an interior space and the compound is in the interior space of the liposome, in the bilayer of the liposome, or is in both the interior space and bilayer.
19. A liposome according to claim 17, wherein the therapeutic compound is an anticancer compound.
20. A liposome according to claim 19, wherein the anticancer compound is selected from a group consisting of alkylating agents, antimetabolites, spindle poison plant alkaloids, cytotoxic antitumor antibiotics, topoisomerase inhibitors, monoclonal antibodies or fragments thereof, photosensitizers, kinase inhibitors, antitumor enzymes and inhibitors of enzymes,

apoptosis-inducers, biological response modifiers, anti-hormones, retinoids and platinum containing compounds.

21. A liposome according to claim 19, wherein the anticancer compound is selected from a group consisting of docetaxel, doxorubicin, and carboplatin.
22. A liposome according to claim 17, comprising an imaging agent.
23. A liposome according to claim 17, comprising 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (DSPE-PEG2000)-LARLLT, wherein the therapeutic agent is carboplatin.
24. A method of administering a compound to a subject in need thereof, comprising:
associating the compound with a peptide comprising a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8; and
contacting the subject with the peptide-associated compound.
25. A method according to claim 24, wherein the peptide is attached to a lipid and the peptide-attached lipid is in the form of a liposome.
26. A method according to claim 24, wherein the peptide-attached lipid comprises a linker between the lipid and the peptide.
27. A method according to claim 25, wherein the lipid is 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine (DSPE).
28. A method according to claim 26, wherein the linker comprises polyethylene glycol (PEG).
29. A method according to claim 25, wherein the peptide-attached lipid comprises 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (DSPE-PEG2000)-LARLLT.

30. A method according to claim 24, wherein the liposome further comprises 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and monostearoylphosphatidylcholine (MSPC) and wherein the liposome has a gel to liquid phase transition temperature of from about 39.0°C to about 45°C.
31. A method according to claim 24, wherein the liposome further comprises a PEGylated lipid.
32. A method according to claim 24, wherein the compound is selected from the group consisting of therapeutic compounds and imaging compounds..
33. A method according to claim 24, wherein the liposome comprises a bilayer and defines an interior space and the compound is in the interior space of the liposome, in the bilayer of the liposome or is in both the interior space and bilayer.
34. A method according to claim 32, wherein the therapeutic compound is an anticancer compound.
35. A method according to claim 34, wherein the anticancer compound is selected from a group consisting of alkylating agents, antimetabolites, spindle poison plant alkaloids, cytotoxic antitumor antibiotics, topoisomerase inhibitors, monoclonal antibodies or fragments thereof, photosensitizers, kinase inhibitors, antitumor enzymes and inhibitors of enzymes, apoptosis-inducers, biological response modifiers, anti-hormones, retinoids and platinum containing compounds.
36. A method according to claim 34, wherein the anticancer compound is selected from a group consisting of docetaxel, doxorubicin, and carboplatin.
37. A method according to claim 32, wherein the compound is an imaging agent.
38. A method according to claim 24, wherein the method further comprises heating a portion of the subject.

39. A method according to claim 38, wherein heating comprises application of electromagnetic energy, infrared radiation, or ultrasound.
40. A method of treating cancer in a subject in need thereof, comprising:
administering to the subject a liposome, wherein the liposome comprises a lipid associated with a peptide comprising a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8, and the liposome comprises an anticancer compound.
41. A method according to claim 40, wherein the peptide is attached to the lipid.
42. A method according to claim 41, wherein the peptide-attached lipid comprises a linker between the lipid and the peptide.
43. A method according to claim 41, wherein the lipid is 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine (DSPE).
44. A method according to claim 41, wherein the linker comprises polyethylene glycol (PEG).
45. A method according to claim 41, wherein the peptide-attached lipid comprises 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (DSPE-PEG2000)-LARLLT.
46. A method according to claim 40, wherein the liposome further comprises 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and monostearoylphosphatidylcholine (MSPC) and wherein the liposome has a gel to liquid phase transition temperature of from about 39.0°C to about 45°C.
47. A method according to claim 40, wherein the liposome comprises a PEGylated lipid.
48. A method according to claim 40, wherein the liposome comprises a bilayer and defines an interior space and the anticancer compound is in the interior space of the liposome, in the bilayer of the liposome or is in both the interior space and bilayer.

49. A method according to claim 40, wherein the anticancer compound is selected from a group consisting of alkylating agents, antimetabolites, spindle poison plant alkaloids, cytotoxic antitumor antibiotics, topoisomerase inhibitors, monoclonal antibodies or fragments thereof, photosensitizers, kinase inhibitors, antitumor enzymes and inhibitors of enzymes, apoptosis-inducers, biological response modifiers, anti-hormones, retinoids and platinum containing compounds.

50. A method according to claim 40, wherein the anticancer compound is selected from a group consisting of docetaxel, doxorubicin, and carboplatin.

51. A method according to claim 40, wherein the method further comprises heating a portion of the subject.

52. A method according to claim 51, wherein heating comprises application of electromagnetic energy, infrared radiation, or ultrasound.

FIGURE 1

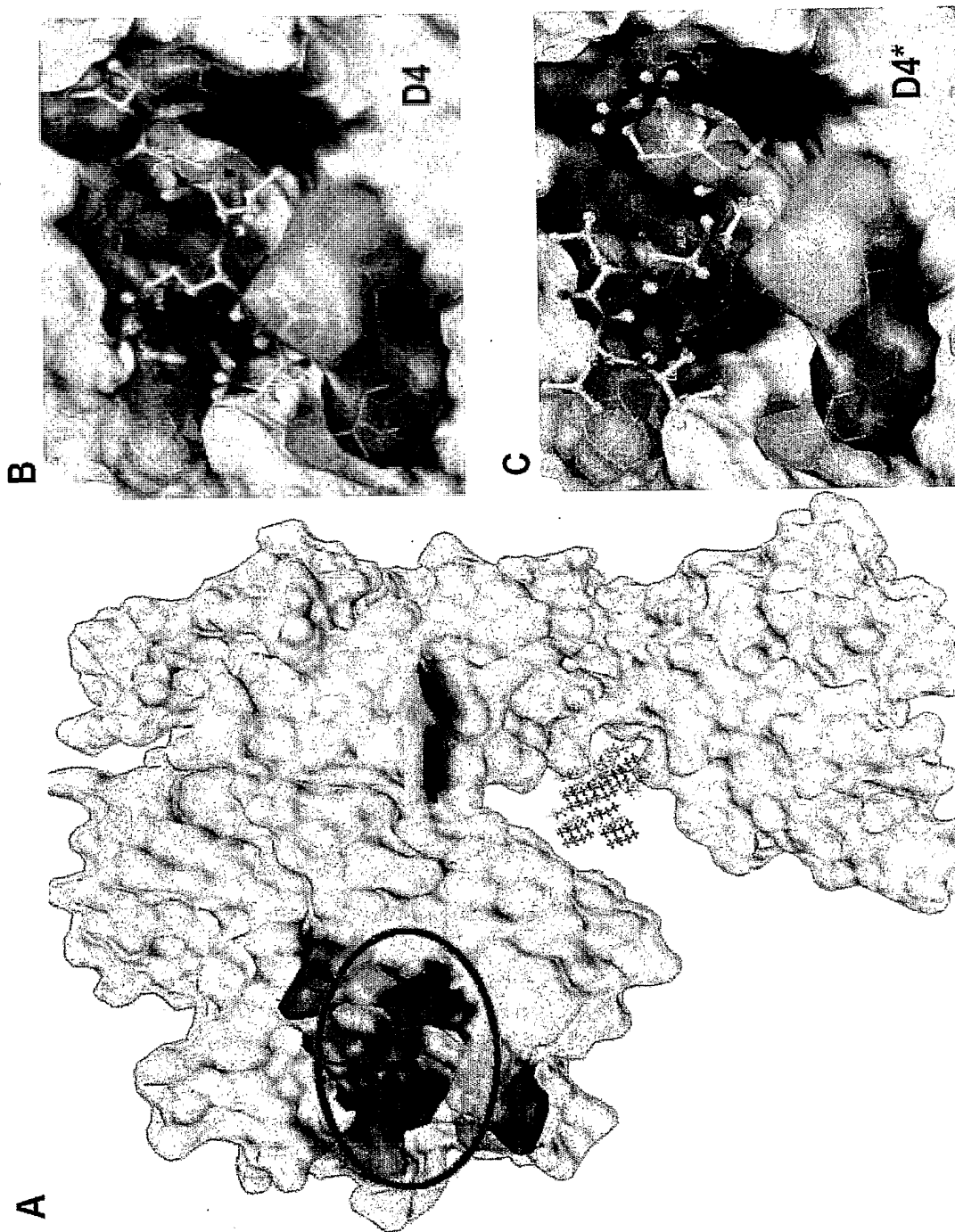


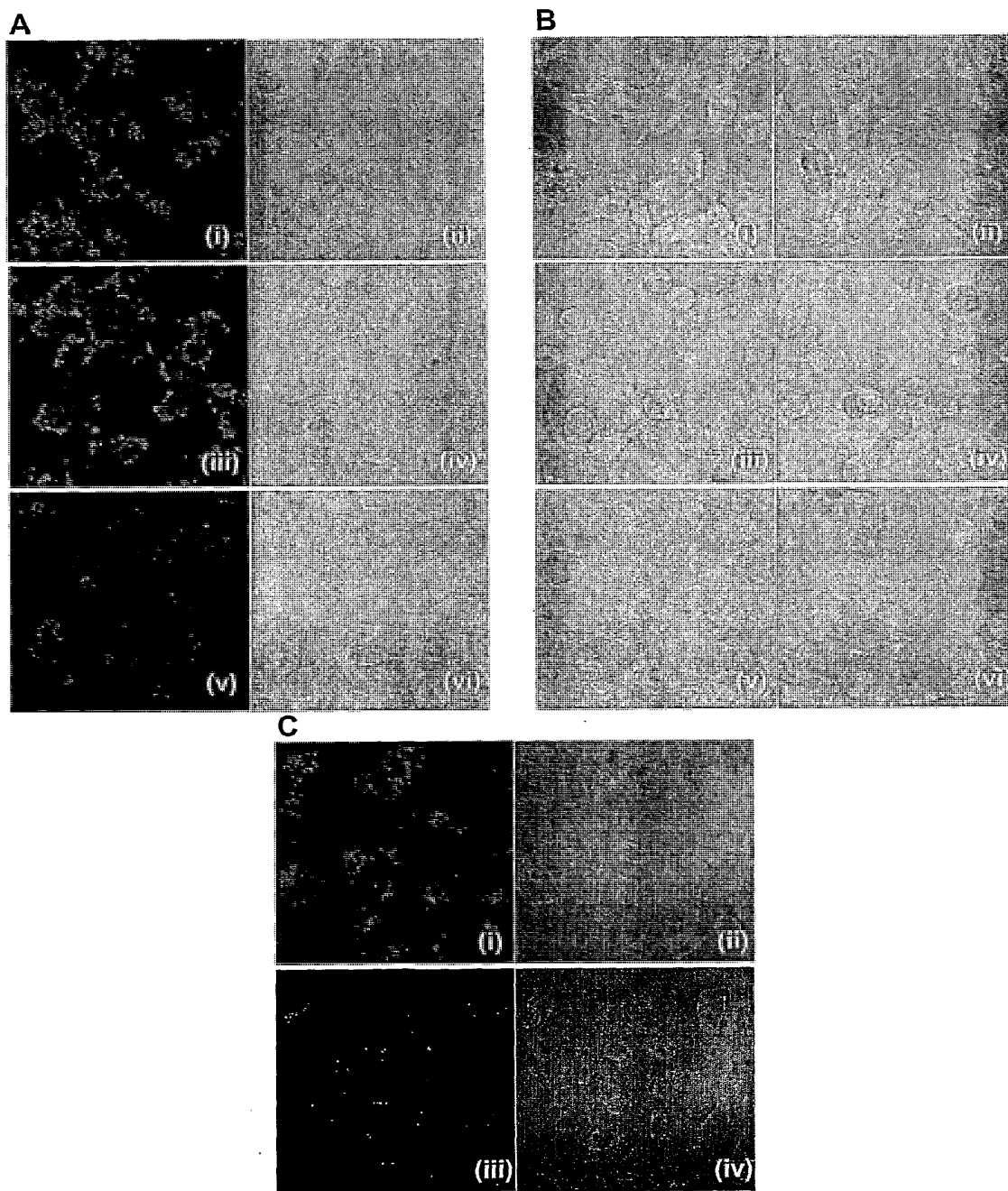
FIGURE 2

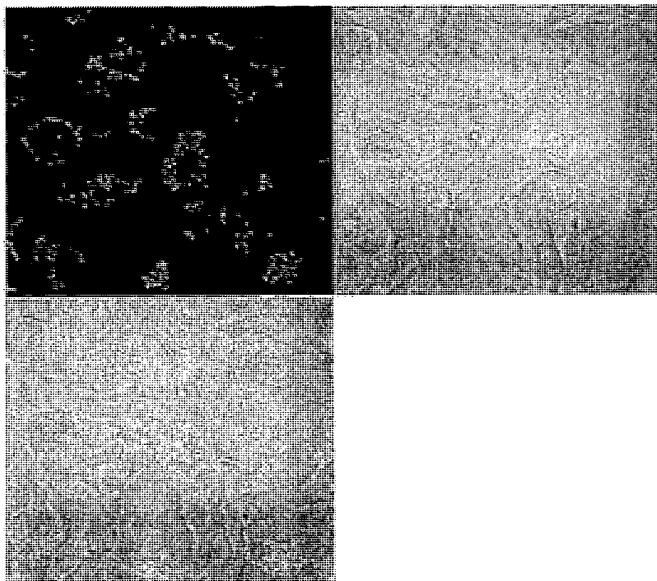
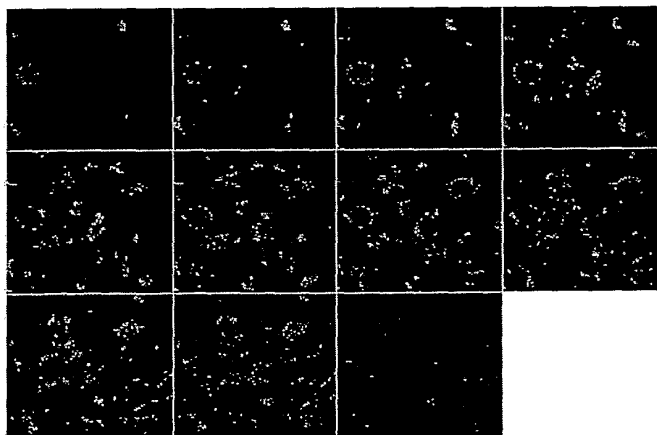
FIGURE 3**A****B**

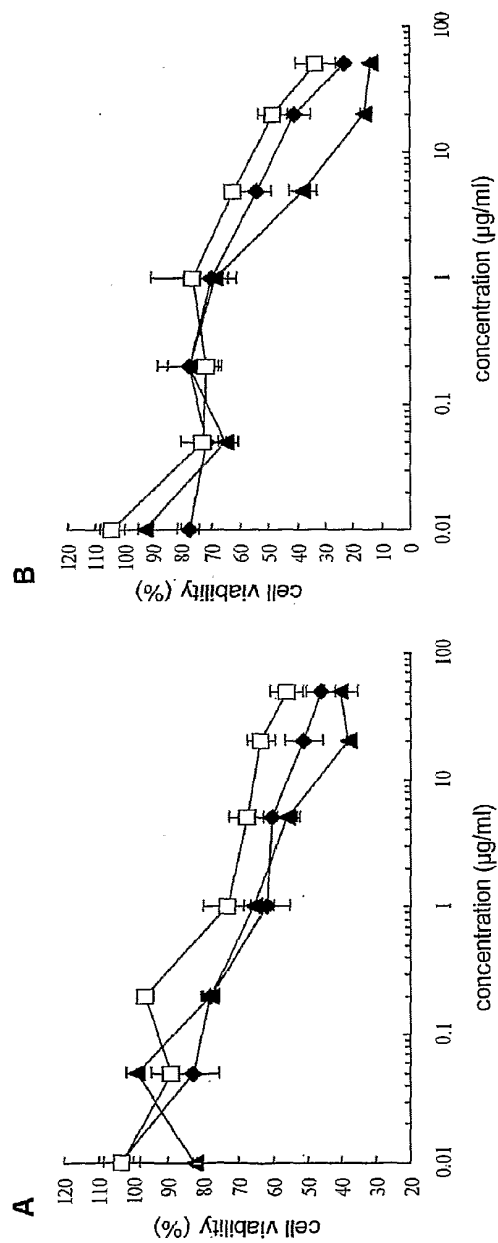
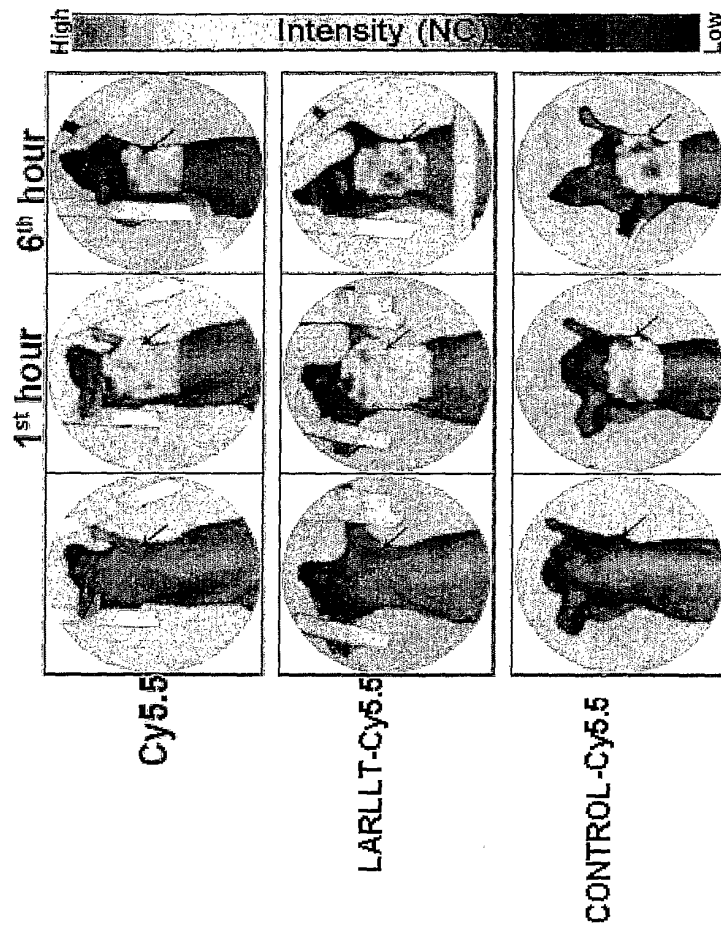
FIGURE 4

FIGURE 5



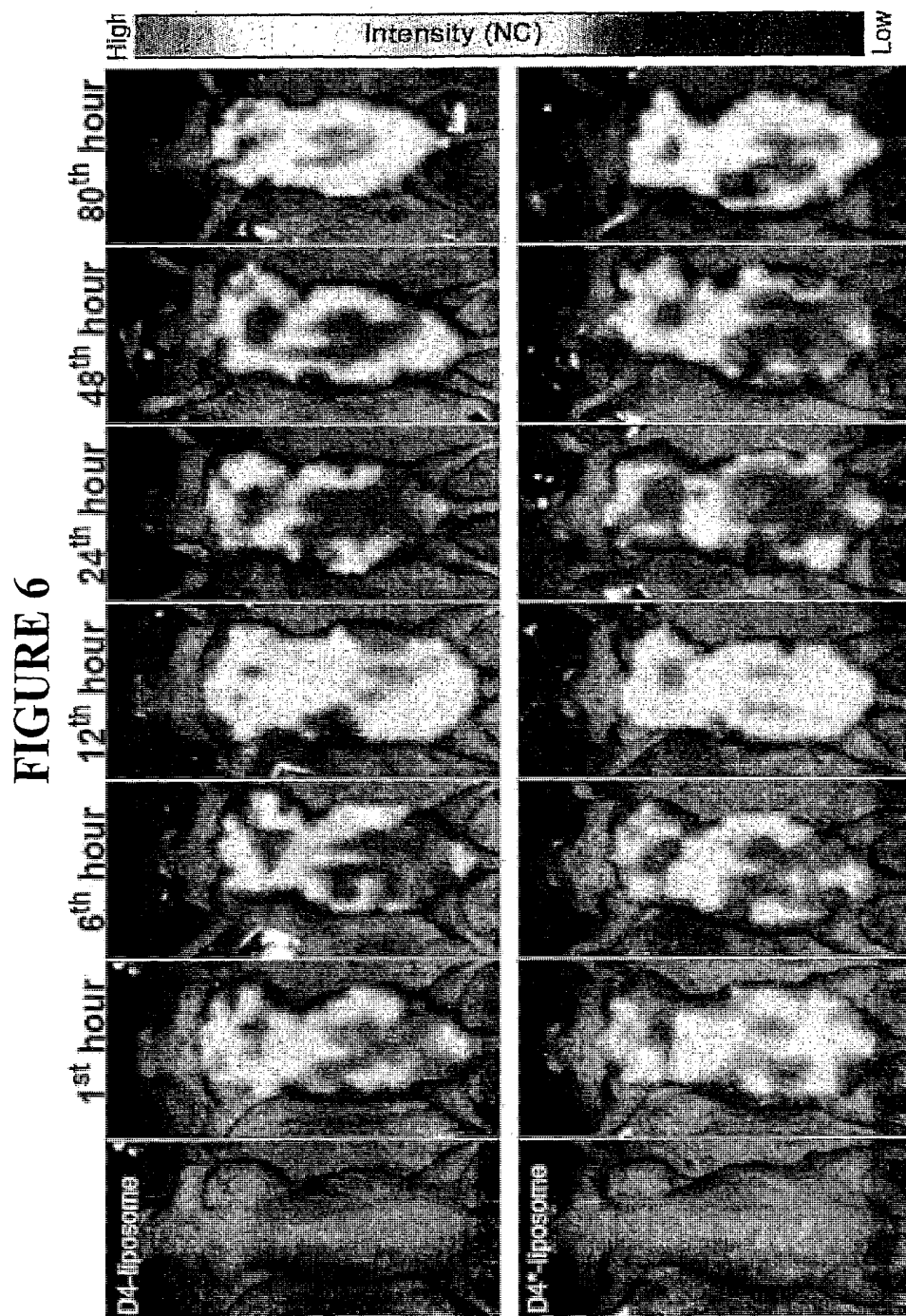


FIGURE 7

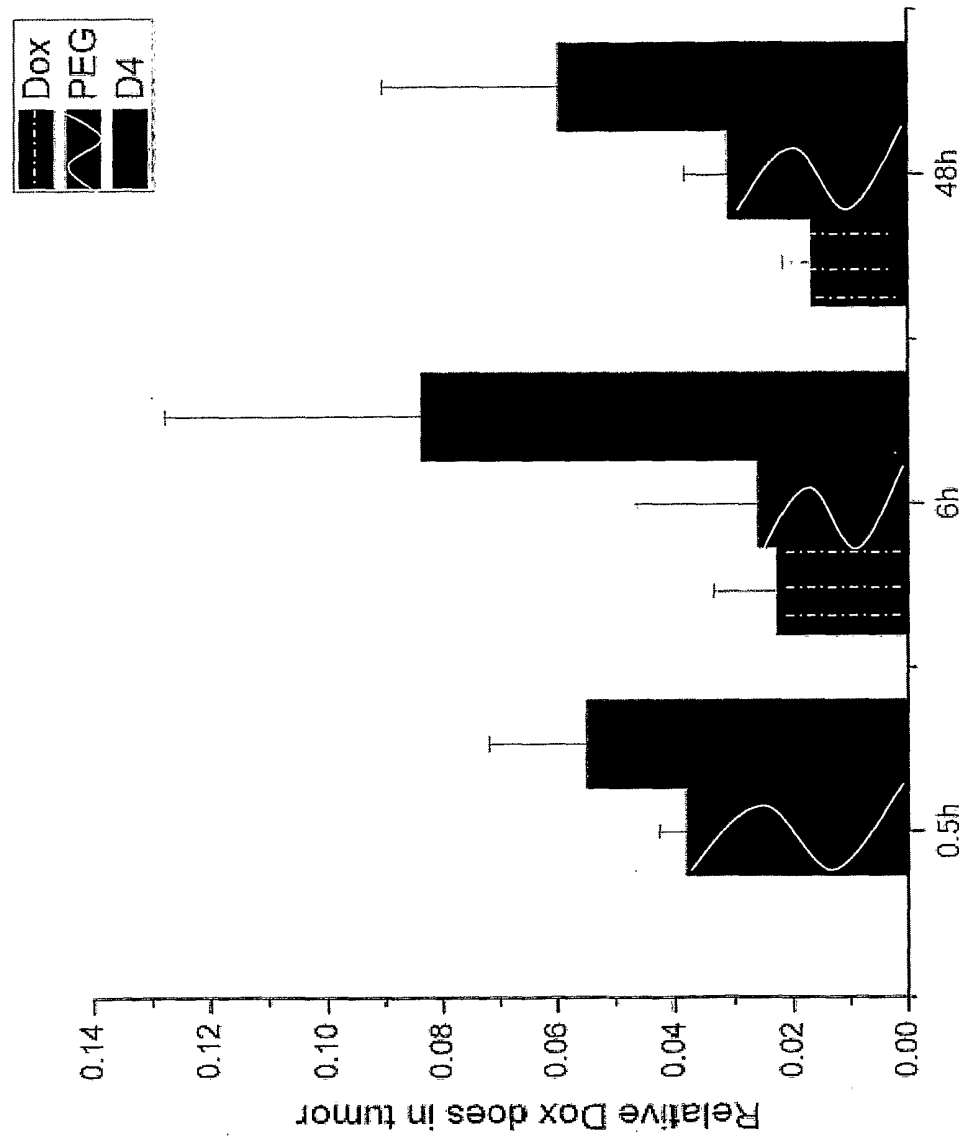
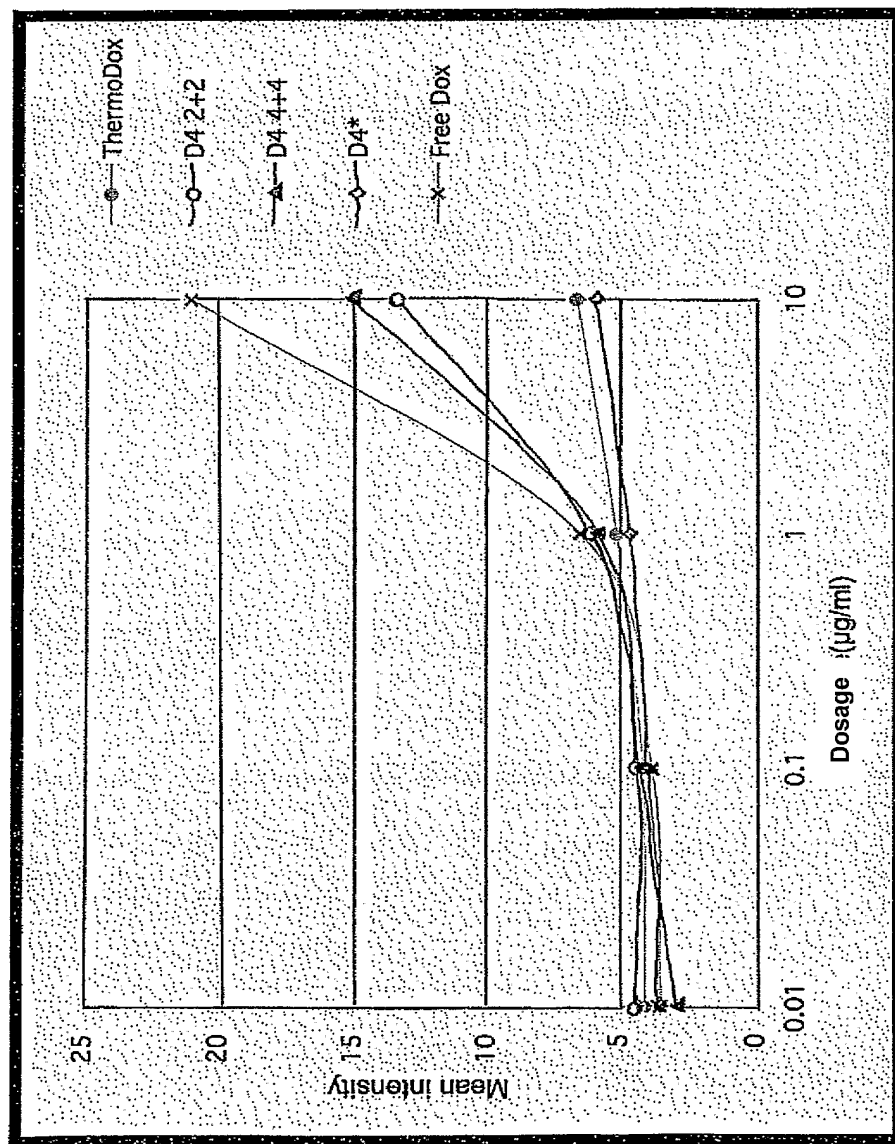


FIGURE 8



INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2008/001847

A. CLASSIFICATION OF SUBJECT MATTER		
See extra sheet		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
IPC: C07K; A61K; A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
CPRS; CNKI; WPI; EPODOC; PAJ; MEDLINE and keywords: EGFR, epidermal growth factor receptor, ligand, peptide, target+, dock+, lipid, liposome etc. GenBank; EMBL: SEQ ID NOS: 1, 3-8		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5726023 A (UNIV WASHINGTON) 10 Mar. 1998(10.03.1998)	1
Y	SEQ ID NOS: 47, 66 abstract, columns 3-4 and 11-12 of the description, SEQ ID NOS: 47	4-8, 10-13, 15-16
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 12 Jan. 2009(12.01.2009)		Date of mailing of the international search report 05 Feb. 2009 (05.02.2009)
Name and mailing address of the ISA/CN The State Intellectual Property Office, the P.R.China 6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088 Facsimile No. 86-10-62019451		Authorized officer LIN, Junkai Telephone No. (86-10)62411095

Form PCT/ISA/210 (second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2008/001847

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 24-52
because they relate to subject matter not required to be searched by this Authority, namely:
Methods for treatment of the human or animal body by therapy (see Rules 39.1(iv), 67.1(iv)).
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2008/001847

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CN 1617740 A (CHIRON SPA) 18 May 2005(18.05.2005) SEQ ID NO: 39, claims 1-10	4-13, 15-16
Y	CN 1582900 A (UNIV SHANGHAI COMMUNICATION) 23 Feb. 2005(23.02.2005) claims 1-3	4-9
Y	SONG, S. X. et al. Peptide ligand targeted delivery to EGFR expressing cancer cells in vitro and in vivo. 2006 International Conference on Biomedical and Pharmaceutical Engineering. 2006, vols. 1-2, pages 490-493, ISBN: 978-981-05-7943-2 page 490, right column to page 491, left column	4-13, 15
A	the whole document	1-3, 14, 17-23
Y	CHIU, G. N. C. et al. Encapsulation of doxorubicin into thermosensitive liposomes via complexation with the transition metal manganese. Journal of Controlled Release. 18 May 2005, vol. 104, No. 2, pages 271-288, ISSN: 0168-3659 abstract	16
A	CN 1676166 A (XINSHIJIIE GENE TECHNOLOGY DEV) 05 Oct. 2005(05.10.2005) the whole document	1-23
A	DENG, H. W. et al. A novel small molecule ligand for epidermal growth factor receptor targeting. Prog. Biochem. Biophys. 2005, vol. 32, No. 2, pages 180-186, ISSN: 1000-3282 the whole document	1-23
A	LI, Z. H. et al. Identification and characterization of a novel peptide ligand of epidermal growth factor receptor for targeted delivery of therapeutics. The FASEB Journal. December 2005, vol. 19, No. 14, pages 1978-1985, ISSN: 0892-6638 the whole document	1-23

Form PCT/ISA/210 (continuation of second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2008/001847

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
US 5726023 A	10.03.1998	WO 9630514 A1	03.10.1996
		AU 5522296 A	16.10.1996
		EP 0817846 A1	14.01.1998
		NO 974502 A	27.11.1997
		CZ 9703096 A3	14.01.1998
		US 5801005 A	01.09.1998
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		US 5846538 A	08.12.1998
		US 5876712 A	02.03.1999
		JP 11502710 T	09.03.1999
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		BRPI 9607889 A	10.08.1999
		KR 19987003453 A	05.11.1998
		MX 9707501 A1	01.06.1998
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		NZ 306616 A	30.03.2001
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		US 2004082758 A1	29.04.2004
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		RU 2236461 C2	20.09.2004
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		EP 0817846 B1	06.07.2005
		DE 69634912 E	11.08.2005
		US 2005191285 A1	01.09.2005
		ES 2245783 T3	16.01.2006
		CN 1150318 C	19.05.2004

Form PCT/ISA/210 (patent family annex) (April 2007)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2008/001847

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
		DE 69634912 T2	20.04.2006
		CZ 296617 B6	17.05.2006
		CZ 296618 B6	17.05.2006
		NO 20061723 A	01.10.1996
		US 7247703 B2	24.07.2007
		CN 1951398 A	25.04.2007
		JP 2008056679 A	13.03.2008
		US 2008076904 A1	27.03.2008
		KR 100554186 B1	11.04.2007
CN 1617740 A	18.05.2005	WO 03049762 A2	19.06.2003
		AU 2002366592 A1	23.06.2003
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		KR 20040074085 A	21.08.2004
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		AU 2007231768 A1	29.11.2007
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		NZ 533428 A	28.03.2008
		US 7361353 B2	22.04.2008
		RU 2331435 C2	20.08.2008
		EP 1463520 B1	03.09.2008
CN 1582900 A	23.02.2005	NONE	
CN 1676166 A	05.10.2005	CN 100356981 C	26.12.2007

Form PCT/ISA/210 (patent family annex) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2008/001847

CLASSIFICATION OF SUBJECT MATTER

C07K 7/00 (2006.01) i

C07K 17/08 (2006.01) i

A61K 38/08 (2006.01) i

A61K 9/127 (2006.01) i

A61K 47/48 (2006.01) i

A61P 35/00 (2006.01) i

C07K 14/71 (2006.01) n

Electronic Acknowledgement Receipt

EFS ID:	46891162
Application Number:	17694541
International Application Number:	
Confirmation Number:	4182
Title of Invention:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Pamela Elaine Bechtel/Kerstin Kinzer
Filer Authorized By:	Pamela Elaine Bechtel
Attorney Docket Number:	JHU3260-9
Receipt Date:	25-OCT-2022
Filing Date:	14-MAR-2022
Time Stamp:	18:17:36
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	JHU3260-9_IDS_Transmittal_Letter.pdf	101874 c98a6d722db2a7a0a3e45895cc0977d1e2b9817e	no	2

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	JHU3260-9_SB08.pdf	233833	no	3
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Warnings:					
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3	Foreign Reference	WO2009062399.pdf	2331634	no	53
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Warnings:					
Information:					
4	Non Patent Literature	JPOA_01AUG2022.pdf	920825	no	6
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Information:					
Total Files Size (in bytes):			3588166		
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Bedi & Ravi	Art Unit:	1647
Application No.:	17/694,541	Examiner:	To Be Assigned
Filed:	March 14, 2022	Conf. No.:	4182
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/ Madam:

In accordance with 37 C.F.R. § 1.97, enclosed are references relating to the above-identified application. For the convenience of the Examiner, the cited references are listed on the attached Form PTO/SB/08.

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 filed before the mailing date of a Non-Final Office Action on the merits for the above-referenced application.

300424642.1
331323-001416

In re Application of:
Bedi & Ravi
Application No.: 17/694,541
Filed: March 14, 2022
Page 2

PATENT
Attorney Docket No.: JHU3260-9

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 25, 2022

/Pamela Hwang/
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4365 Executive Drive, Suite 1100
San Diego, California 92121-2133
USPTO Customer No.: 100899

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Bedi and Ravi	Art Unit:	1647
Application No.:	17/694,541	Examiner:	Bridget E. Bunner
Filed:	March 14, 2022	Conf. No.:	4182
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 Madam:

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This Information Disclosure Statement is being filed before the mailing date of a Non-Final Office Action on the merits for the above-referenced application.

1615987680.1
331323.001416

In re Application of:
Bedi and Ravi
Application No.: 17/694,541
Filed: March 14, 2022
Page 2

PATENT
Attorney Docket No.: JHU3260-9

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: January 3, 2025

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Pamela Hwang, J.D., Ph.D.
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USPTO Customer No.: 100899



ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
17/694,541

RECEIPT DATE / TIME
01/03/2025 04:31:59 PM Z ET

ATTORNEY DOCKET #
JHU3260-9

Title of Invention

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	4182	FILED BY	Karen LePari
PATENT CENTER #	68600635	FILING DATE	03/14/2022
CUSTOMER #	100899	FIRST NAMED INVENTOR	Atul Bedi
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Pamela Hwang

Documents

TOTAL DOCUMENTS: 5

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
JHU3260-9 IDS 03JAN2025.pdf	2	Transmittal Letter	102 KB
JHU3260-9 SB08 03JAN2025.pdf	2	Information Disclosure Statement (IDS) Form (SB08)	242 KB
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JHU3260-9 12-3-24 JHU3260-10 NFOA 03JAN2025.pdf	17	Non Patent Literature	677 KB
JHU3260-9 Ref Huang 03JAN2025.pdf	12	Non Patent Literature	1813 KB
JHU3260-9 Ref Schmidt	11	Non Patent Literature	1076 KB

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DOCUMENT	MESSAGE DIGEST(SHA-512)
JHU3260-9 IDS 03JAN2025.pdf	DE760650249A4FCEF076697755A4ED7E57A30058DB1D5889B 977568208AD334976193AB3A8FAF7209F73BA0F21F78982C19 1B62388A6D12F89BF434EE274C2E3
JHU3260-9 SB08 03JAN2025.pdf	B826BE95841AB2F2ED233F90632DA7FFF7FE45A086C890A89 E6B769D2B4961C55B062E2CE40EE983E7EAA43058E3EF391 D0CF54630688884C4BE24C50A22A3B1
JHU3260-9 12-3-24 JHU3260- 10 NFOA 03JAN2025.pdf	4A5F4240A09FA5C7D7E4D02FF70ECE3A38A9F58ABC4DDB09 4BC49399D4306A9592E65E7C3865AE1906542DE37F2895580E 1918AEF3A98EEE1ED1DA7A5D41B6F4
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JHU3260-9 Ref Schmidt 03JAN2025.pdf	76C4D046FD16973D589250CDF158A00D232DDE14D6D382839 9EC4BCDD0FC8889DFA7341C738E8F255D94AF182992D10E9 1BCA5CF1D229413B85A0F3A156C340B

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New Applications Under 35 U.S.C. 111

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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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Substitute for form 1449A/PTO <h2 style="margin: 0;">INFORMATION DISCLOSURE STATEMENT BY APPLICANT</h2> <p style="margin: 0;">(Use as many sheets as necessary)</p>				Complete if Known	
		Application Number	17/694,541		
		Filing Date	March 14, 2022		
		First Named Inventor	Atul Bedi		
		Art Unit	1647		
		Examiner Name	Bridget E. Bunner		
Sheet 1		of 2	Attorney Docket Number JHU3260-9		

U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			

FOREIGN PATENT DOCUMENTS						
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		Country Code ³ "Number" ⁴ "Kind Code" ⁵ (if known)				

Examiner Signature		Date Considered	
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Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>				Complete if Known	
				Application Number	17/694,541
				Filing Date	March 14, 2022
				First Named Inventor	Atul Bedi
				Art Unit	1647
				Examiner Name	Bridget E. Bunner
Sheet		of	2	Attorney Docket Number	JHU3260-9

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
	1	US Office Action in U.S. Application No. 18/883,719, dated December 3, 2024, 17 pages	
	2	Huang et al. A Yeast Platform for the Production of Single-Chain Antibody-Green Fluorescent Protein Fusions. Appl Environmental Microbiol 72(12): 7748-7759, 2006	
	3	Schmidt et al. Suppression of metastasis formation by a recombinant single chain antibody-toxin targeted to full-length and oncogenic variant EGF receptors. Oncogene 18: 1711-1721, 1999	

Examiner Signature		Date Considered	
--------------------	--	-----------------	--

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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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Notice of References Cited	Application/Control No. 17/694,541		Applicant(s)/Patent Under Reexamination Bedi et al.	
	Examiner BRIDGET E BUNNER		Art Unit 1647	Page 1 of 5

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	N	WO-03066002-A2	2003-08-14	WO	Kotenko et al.	--
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<i>Search Notes</i> 	Application/Control No. 17/694,541	Applicant(s)/Patent Under Reexamination Bedi et al.
	Examiner BRIDGET E BUNNER	Art Unit 1647

CPC - Searched*		
Symbol	Date	Examiner
C07K14/5428; C07K16/30; C07K19/00; C07K2319/00; C07K2319/32 ; C07K2319/74; C07K2319/33; C07K16/2866; C07K16/2863; A61K38/2066; A61K39/395; A61K39/3955; A61K39/39558	02/06/2025	BEB

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US Classification - Searched*			
Class	Subclass	Date	Examiner

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Search Notes		
Search Notes	Date	Examiner
PE2E (JPO, EPO, USPAT, DERWENT); US-PGPUB; DAV (inventors ' names); ProQuest Dialog; see search history printout	02/06/2025	BEB
STIC search SEQ ID NO: 93 (see search results in DAV/SCV)	02/06/2025	BEB

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		Application Number	Not Yet Assigned		
		Filing Date	March 14, 2022		
		First Named Inventor	Atul Bedi		
		Art Unit	Not Yet Assigned		
		Examiner Name	Not Yet Assigned		
Sheet	1	of	4	Attorney Docket Number	JHU3260-9

U. S. PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	
		Number-Kind Code ² (if known)				
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	2	7,527,791	05-05-2009	Adams et al.		
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		Country Code ³ "Number" ⁴ "Kind Code" ⁵ (if known)				
	22	WO 2009/152610 A1	12-23-2009	The Royal Institution For The Advancement Of Learning/McGill University		
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Sheet	2	of	4	Attorney Docket Number	JHU3260-9

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	24	Alignment of SEQ ID No. 10 of D3 with SEQ ID No. 87 of B1 no date provided	
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Substitute for form 1449A/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>		Complete if Known			
		Application Number	17/694,541		
		Filing Date	March 14, 2022		
		First Named Inventor	Atul Bedi		
		Art Unit	1647		
		Examiner Name	To Be Assigned		
Sheet	1	of	2	Attorney Docket Number	JHU3260-9

U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document	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
		Country Code ³ "Number" ⁴ "Kind Code" ⁵ (if known)				
	1	WO 2009/062399 A1	05-22-2009	Shanghai Jiaotong University		

Examiner Signature		Date Considered	
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		Application Number	17/694,541		
		Filing Date	March 14, 2022		
		First Named Inventor	Atul Bedi		
		Art Unit	1647		
		Examiner Name	To Be Assigned		
Sheet	2	of	2	Attorney Docket Number	JHU3260-9

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
	2	JP Office Action in Japanese Application No. 2021-094042, dated August 1, 2022, 6 pages (with English translation)	

Examiner Signature	/BRIDGET E BUNNER/	Date Considered	02/05/2025
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Bicara v. JHU - PGR2026-00025

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BIB DATA SHEET

CONFIRMATION NO. 4182

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.	
17/694,541	03/14/2022	530	1647	JHU3260-9	
APPLICANTS The Johns Hopkins University, Baltimore, MD;					
INVENTORS Atul Bedi, Timonium, MD; Rajani Ravi, Ruxton, MD;					
** CONTINUING DATA ***** This application is a CON of 16/601,347 10/14/2019 PAT 11274156 which is a CON of 15/362,632 11/28/2016 PAT 10442860 which is a DIV of 15/231,309 08/08/2016 PAT 9850306 which 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 ** 03/22/2022					
Foreign Priority claimed ☐ Yes ☒ No 35 USC 119(a-d) conditions met ☐ Yes ☒ No Verified and /BRIDGET E BUNNER/ Acknowledged Examiner's Signature	☐ Met after Allowance BB Initials	STATE OR COUNTRY MD	SHEETS DRAWINGS 75	TOTAL CLAIMS 11	INDEPENDENT CLAIMS 1
ADDRESS The John Hopkins University c/o DLA Piper LLP (US) 4365 Executive Drive, Suite 1100 San Diego, CA 92121-2133 UNITED STATES					
TITLE COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS					
FILING FEE RECEIVED 0.00	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:		☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____		

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>		Complete if Known			
		Application Number	17/694,541		
		Filing Date	March 14, 2022		
		First Named Inventor	Atul Bedi		
		Art Unit	1647		
		Examiner Name	Bridget E. Bunner		
Sheet	1	of	2	Attorney Docket Number	JHU3260-9

U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document	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
		Country Code ³ "Number" ⁴ "Kind Code" ⁵ (if known)				

Examiner Signature		Date Considered	
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				Application Number	17/694,541
				Filing Date	March 14, 2022
				First Named Inventor	Atul Bedi
				Art Unit	1647
				Examiner Name	Bridget E. Bunner
Sheet		of	2	Attorney Docket Number	JHU3260-9

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
	1	US Office Action in U.S. Application No. 18/883,719, dated December 3, 2024, 17 pages	
	2	Huang et al. A Yeast Platform for the Production of Single-Chain Antibody-Green Fluorescent Protein Fusions. Appl Environmental Microbiol 72(12): 7748-7759, 2006	
	3	Schmidt et al. Suppression of metastasis formation by a recombinant single chain antibody-toxin targeted to full-length and oncogenic variant EGF receptors. Oncogene 18: 1711-1721, 1999	

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Bicara Ex. 1005, Page 290 of 651

Bicara v. JHU - PGR2026-00025

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	1	Carter et al, "Identification and validation of cell surface antigens for antibody targeting in oncology", Endocr Relat Cancer, December 2004, 11(4):659-687	
	2	EP Third Party Observation in European Application No. EP20200205164, dated on April 5, 2022, 10 pages	
	3	Francisco et al., "PD-L1 regulates the development, maintenance, and function of induced regulatory T cells", J Exp Med, December 2009, 206(13):3015-3029	
	4	Haier et al., "Cell Surface Molecules and Their Prognostic Values in Assessing Colorectal Carcinomas", Annal of Surgery, January 2000, 231(1):11-24	
	5	IN Examination Report in Indian Application No. 201948021756, dated January 24, 2022, 6 pages	

Examiner Signature	/BRIDGET E BUNNER/	Date Considered	02/05/2025
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PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L1	23	((("BEDI") near3 ("Atul")) OR ((("RAVI") near3 ("Rajani")))).INV.	(US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 12:45 PM
L2	6880	((("THE") near3 ("JOHNS") near3 ("HOPKINS") near3 ("UNIVERSITY"))).AS,A ANM.	(US-PGPUB; USPAT)	OR	ON	ON	2025/02/06 12:46 PM
L4	6817	IL-10R OR IL10R OR CD210 OR IL-10R1 OR IL-10RA OR IL-10R2 OR IL-10RB OR IL10R1 OR IL10RA OR IL10R2 OR IL-10RB OR (interleukin near2 "10" near2 receptor)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 01:36 PM
L5	13268	IL-10R OR IL10R OR CD210 OR IL-10R1 OR IL-10RA OR IL-10R2 OR IL-10RB OR IL10R1 OR IL10RA OR IL10R2 OR IL-10RB OR (interleukin near2 "10" near2 receptor)	(FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN))	OR	ON	ON	2025/02/06 01:37 PM
L6	15	L1 AND L5	(FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN))	OR	ON	ON	2025/02/06 01:45 PM
L7	13	L1 AND L4	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 01:45 PM
L8	45	L2 AND L4	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 01:45 PM
L9	1236	L4 SAME (fusion OR fuse OR fused OR join	(US-PGPUB; USPAT; EPO; JPO;	OR	ON	ON	2025/02/06 02:14 PM

L10	2314	OR joined OR link OR linked OR linkage) L5 SAME (fusion OR fuse OR fused OR join OR joined OR link OR linked OR linkage)	DERWENT) (FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN))	OR	ON	ON	2025/02/06 02:14 PM
L11	383	L4 SAME (fusion OR fuse OR fused OR join OR joined OR link OR linked OR linkage) SAME (extracellular OR ectodomain)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 02:17 PM
L12	633	L5 SAME (fusion OR fuse OR fused OR join OR joined OR link OR linked OR linkage) SAME (extracellular OR ectodomain)	(FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN))	OR	ON	ON	2025/02/06 02:17 PM
L13	407	L4 SAME (fusion OR fuse OR fused OR join OR joined OR link OR linked OR linkage) SAME soluble	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 02:31 PM
L14	7740	(fusion OR fuse OR fused OR join OR joined OR link OR linked OR linkage) SAME (soluble OR extracellular OR ectodomain) SAME ((cancer OR tumor) adj8 (target OR targeting OR antigen OR epitope OR moiety))	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 02:33 PM
L15	179	L14 same5 L4	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 02:34 PM
L16	141	L14 SAME L4	(US-PGPUB; USPAT; EPO; JPO;	OR	ON	ON	2025/02/06 02:34 PM

L17	135969	(C07K14/5428 OR C07K16/30 OR C07K19/00 OR C07K2319/00 OR C07K2319/32 OR C07K2319/74 OR C07K2319/33 OR C07K16/2866 OR C07K16/2863 OR A61K38/2066 OR A61K39/395 OR A61K39/3955 OR A61K39/39558).cpc.	DERWENT) (US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 02:48 PM
L18	507	L9 AND L17	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 02:49 PM
L19	68	L4 WITH decoy	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 02:49 PM
L20	12	L4 WITH decoy WITH (fusion OR fused OR fuse OR join OR joined OR link OR linked OR linkage)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 02:53 PM
L21	36	L4 SAME decoy SAME (fusion OR fused OR fuse OR join OR joined OR link OR linked OR linkage)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 02:54 PM
L22	330	L12 AND L17	(FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN))	OR	ON	ON	2025/02/06 02:55 PM
L23	176	L11 AND L17	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 02:57 PM
L24	434	L4 SAME (fusion OR fuse OR fused OR join OR joined) SAME (CD19 OR EGFR OR EGFR1 OR Erbb1 OR CD20 OR HER2 OR VEGF OR IGF1R OR IGF1R OR IGF-1R OR IGF-1R OR TRAIL OR	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 04:32 PM

L25	23	mucin OR CD30 OR CD33 OR CD40 OR PDL1 OR IL2 OR IL-2 OR CTLA4 OR CTLA-4 OR CD25 OR PD-1 OR RANKL OR OX40) L4 near25 (fusion OR fuse OR fused OR join OR joined) near25 (CD19 OR EGFR OR EGFR1 OR Erbb1 OR CD20 OR HER2 OR VEGF OR IGFIR OR IGF1R OR IGF-IR OR IGF-1R OR TRAIL OR mucin OR CD30 OR CD33 OR CD40 OR PDL1 OR IL2 OR IL-2 OR CTLA4 OR CTLA-4 OR CD25 OR PD-1 OR RANKL OR OX40)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 04:36 PM
L26	67	L4 near15 (fusion OR fuse OR fused OR join OR joined) same4 (CD19 OR EGFR OR EGFR1 OR Erbb1 OR CD20 OR HER2 OR VEGF OR IGFIR OR IGF1R OR IGF-IR OR IGF-1R OR TRAIL OR mucin OR CD30 OR CD33 OR CD40 OR PDL1 OR IL2 OR IL-2 OR CTLA4 OR CTLA-4 OR CD25 OR PD-1 OR RANKL OR OX40)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 04:37 PM
L27	195	L4 near15 (fusion OR fuse OR fused OR join OR joined)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 04:42 PM
L28	214	L4 SAME (trap OR traps)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 05:08 PM
L29	1345	cytokine near5 receptor near5 fusion near5 (protein OR proteins)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 05:15 PM
L30	188	L29 AND L4	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 05:15 PM
L31	151	L30 AND L17	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 05:15 PM
L32	1	WO-2016174575-\$.did.	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:20 PM
L33	7	"20090175819"	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06

L34	2	WO-2009154995-\$.did.	EPO; JPO; DERWENT) (US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	06:23 PM 2025/02/06 06:25 PM
L35	2	WO-2006084327-\$.did.	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:26 PM
L36	1	WO-2003066002-\$.did.	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:26 PM
L37	13037	interleukin SAME (fusion OR fuse OR fused OR join OR joined) SAME (CD19 OR EGFR OR EGFR1 OR Erbb1 OR CD20 OR HER2 OR VEGF OR IGFIR OR IGF1R OR IGF-IR OR IGF-1R OR TRAIL OR mucin OR CD30 OR CD33 OR CD40 OR PDL1 OR IL2 OR IL-2 OR CTLA4 OR CTLA-4 OR CD25 OR PD-1 OR RANKL OR OX40)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:35 PM
L38	6362	interleukin WITH (fusion OR fuse OR fused OR join OR joined) WITH (CD19 OR EGFR OR EGFR1 OR Erbb1 OR CD20 OR HER2 OR VEGF OR IGFIR OR IGF1R OR IGF-IR OR IGF-1R OR TRAIL OR mucin OR CD30 OR CD33 OR CD40 OR PDL1 OR IL2 OR IL-2 OR CTLA4 OR CTLA-4 OR CD25 OR PD-1 OR RANKL OR OX40)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:35 PM
L39	589	interleukin near5(fusion OR fuse OR fused OR join OR joined) near5(CD19 OR EGFR OR EGFR1 OR Erbb1 OR CD20 OR HER2 OR VEGF OR IGFIR OR IGF1R OR IGF-IR OR IGF-1R OR TRAIL OR mucin OR CD30 OR CD33 OR CD40 OR PDL1 OR IL2 OR IL-2 OR CTLA4 OR CTLA-4 OR CD25 OR PD-1 OR	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:36 PM

L40	21	RANKL OR OX40) interleukin near5 (extracellular OR ectodomain) near5(fusion OR fuse OR fused OR join OR joined) near5(CD19 OR EGFR OR EGFR1 OR ErbB1 OR CD20 OR HER2 OR VEGF OR IGFIR OR IGF1R OR IGF-IR OR IGF-1R OR TRAIL OR mucin OR CD30 OR CD33 OR CD40 OR PDL1 OR IL2 OR IL-2 OR CTLA4 OR CTLA-4 OR CD25 OR PD-1 OR RANKL OR OX40)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:36 PM
L41	207	(interleukin OR IL-1 OR IL-2 OR IL4 OR IL-15 OR IL-12) near10 (extracellular OR ectodomain) near5(fusion OR fuse OR fused OR join OR joined) near5(CD19 OR EGFR OR EGFR1 OR ErbB1 OR CD20 OR HER2 OR VEGF OR IGFIR OR IGF1R OR IGF-IR OR IGF-1R OR TRAIL OR mucin OR CD30 OR CD33 OR CD40 OR PDL1 OR IL2 OR IL-2 OR CTLA4 OR CTLA-4 OR CD25 OR PD-1 OR RANKL OR OX40)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:37 PM
L42	117722	(antibody OR antibodies) near3(CD19 OR EGFR OR EGFR1 OR ErbB1 OR CD20 OR HER2 OR VEGF OR IGFIR OR IGF1R OR IGF-IR OR IGF-1R OR TRAIL OR mucin OR CD30 OR CD33 OR CD40 OR PDL1 OR IL2 OR IL-2 OR CTLA4 OR CTLA-4 OR CD25 OR PD-1 OR RANKL OR OX40)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:41 PM
L43	15	(interleukin OR IL-1 OR IL-2 OR IL4 OR IL-15 OR IL-12) near10	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:42 PM

L44	112832	(extracellular OR ectodomain) near15(fusion OR fuse OR fused OR join OR joined) near15 L42	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:43 PM
L45	614	(antibody OR antibodies) near3(CD19 OR EGFR OR EGFR1 OR ErbB1 OR CD20 OR HER2 OR VEGF OR IGF1R OR IGF1R OR IGF-1R OR TRAIL OR mucin OR CD30 OR CD33 OR CD40 OR PDL1 OR CTLA4 OR CTLA-4 OR CD25 OR PD-1 OR RANKL OR OX40)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:44 PM
L46	92	(interleukin OR IL-1 OR IL-2 OR IL4 OR IL-15 OR IL-12) SAME (extracellular OR ectodomain) SAME (fusion OR fuse OR fused OR join OR joined) SAME L44	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:44 PM
L47	331	(interleukin OR IL-1 OR IL-2 OR IL4 OR IL-15 OR IL-12) WITH (extracellular OR ectodomain) WITH (fusion OR fuse OR fused OR join OR joined) WITH L44	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:47 PM
L48	331	(interleukin OR IL-1 OR IL-2 OR IL4 OR IL-15 OR IL-12) WITH (soluble) WITH (fusion OR fuse OR fused OR join OR joined) WITH L44	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:49 PM
L49	4	L4 WITH (soluble) WITH (fusion OR fuse OR fused) WITH L44	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:50 PM
L50	45	L4 SAME soluble SAME (fusion OR fuse OR fused) SAME L44	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/02/06 06:50 PM

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(54) Title: IFN-A/B-INDEPENDENT MECHANISM OF ANTIVIRAL PROTECTION THROUGH A NOVEL LIGAND-RECEPTOR PAIR: IFN-L LIGANDS ENGAGE A NOVEL RECEPTOR IFN-LR1 (CRF2-12) AND IL-10R2 (CRF2-4) FOR SIGNALING AND INDUCTION OF BIOLOGICAL ACTIVITIES

(57) Abstract: A novel IFN- α/β independent ligand receptor system which upon engagement leads, among other things, to the establishment of an anti-viral state is disclosed. Further disclosed are three closely positioned genes on human chromosome 19 that encode distinct but highly homologous proteins, designated IFN- λ 1, IFN- λ 2, IFN- λ 3, based, inter alia, in their ability to induce antiviral protection. Expression of these proteins is induced upon viral infection. A receptor complex utilized by all three IFN- λ proteins for signaling is also disclosed. The receptor complex is generally composed of two subunits, a novel receptor designated IFN- λ R1 or CRF2-12, and a second subunit, IL-10R2 or CRF2-4, which is also a shared receptor component for the IL-10 and IL-22 receptor complexes. The gene encoding IFN- λ R1 is generally widely expressed, including many different cell types and tissues. Expression of these proteins is induced by immune events, including, for example, upon viral infection. Apoptosis may also be induced under effective conditions.

**IFN- α/β -independent mechanism of antiviral protection through a novel
ligand-receptor pair: IFN- λ ligands engage a novel receptor IFN- λ R1 (CRF2-12)
and IL-10R2 (CRF2-4) for signaling and induction of biological activities**

STATEMENT OF GOVERNMENT GRANT

This invention was partially funded by a grant from the United States Government AI51139 from the National Institute of Allergy and Infectious Diseases and therefore the United States Government may have rights in this invention.

This application claims priority to provisional application entitled "New Human Class II Cytokine Receptor" serial number 60/355,196 filed on February 8, 2002 and provisional application serial number 60/418,474, entitled IFN- α/β -independent mechanism of antiviral protection through a novel ligand-receptor pair: IFN- λ ligands engage a novel receptor IFN- λ R1 (CRF2-12) and IL-10R2 (CRF2-4) for signaling and induction of biological activities filed October 15, 2002, both of which are incorporated herein by reference in their entirety, including the figures.

FIELD OF THE INVENTION

The present invention relates to three novel human genes encoding related polypeptides that are members of the interferon family and a novel receptor and receptor complex responsive to these novel interferons. More specifically, isolated nucleic acid molecules are provided encoding human polypeptides named IFN- λ 1, IFN- λ 2 and IFN-

$\lambda 3$ and IFN- λ R1. Polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. Also provided are diagnostic methods for detecting disorders and therapeutic methods for treating disorders. The invention further relates to screening methods for identifying agonists and antagonists of IFN- $\lambda 1$, IFN- $\lambda 2$ and IFN- $\lambda 3$ and IFN- λ R1.

BACKGROUND OF THE INVENTION

Cytokines are small soluble regulatory molecules of the mammalian immune system. They control the growth, function and differentiation of a wide variety of cells and are therefore at the heart of all processes in which the body defends itself from infection of any nature. Banyer, et al., (2000) Rev Immunogenet 2, 359-373. Recently six new ligands with limited homology to IL-10 have been identified. (Reviewed in Kontenko, et al., (2002)). Only a very limited amount is known about the function of these IL-10 homologous ligands. Jiang et al, 1995, reported cloning melanoma differentiation associated gene 7 (mda-7), a IL-10 homolog as a protein whose expression is elevated in terminally-differentiated human melanoma cells. Soo et al, 1999 reported expression of the rat mda-7 paralog was linked to wound healing; Zhang et al, 2000 designated the protein c49a and linked it to ras transformation. The protein has also been designated mob-5. The expression of rat mda-7 (c49a) was localized primarily to fibroblast-like cells at the wound edge and base. Soo, et al, 1999 further reported that during wound healing the level of c49a mRNA was transiently elevated 9 to 12-fold above unwounded controls. In addition, expression of rat mda-7 (mob-5) was demonstrated to be induced by expression of oncogenic ras. Moreover, mob-5 and its

putative receptor are oncogenic ras specific targets; mob-5 binds to the cell surface of ras-transformed cells but not of parental untransformed cells. Zhang et al, 2000.

Saeki, et al., *Oncogene* 21(29):4558-66 (2002), reported that in addition to the overexpression of the melanoma differentiation associated gene-7 (mda-7) in vitro resulting in suppression of lung cancer cell proliferation, overexpression of the mda-7 gene in human non-small cell lung carcinoma cells in vivo has an effect on tumor growth. In particular, Saeki, et al., reported that adenovirus-mediated overexpression of MDA-7 in p53-wild-type A549 and p53-null H1299 subcutaneous tumors resulted in significant tumor growth inhibition through induction of apoptosis, and that decreased CD31/PECAM expression and upregulation of APO2/TRAIL were observed in tumors expressing MDA-7. According to the authors, the data demonstrates that Ad-mda7 functions as a multi-modality anti-cancer agent, possessing both pro-apoptotic and anti-angiogenic properties and thus may potentially serve as a therapeutic agent against human lung cancer.

Knappe, et al., *J Virol* 74(8):3881-7 (2000) reported cloning another IL-10 homolog, designated ak155, as a protein expressed by herpesvirus saimiri-transformed T lymphocytes.

Gallagher, et al., *Genes Immunol* (7):442-50 (2000) reported the identification and cloning of a gene and corresponding cDNAs encoding a homologue of IL-10, which they designated IL-19. According to Gallagher, et al., IL-19 expression was induced in monocytes by Salmonella lipopolysaccharide (LPS) treatment, but IL-19 did not bind or

signal through the canonical IL-10 receptor complex, suggesting existence of an IL-19 specific receptor complex, the identity of which remained to be discovered.

Blumberg, et al., *Cell* 104(1):9-19 (2001), also reported cloning a protein with homology to IL-10, which they designated Zcyto10 (GenBank accession number AF224266) or IL-20. Several lines of evidence demonstrate that IL-20 may play a functional role in epidermal development and psoriasis.

Dumoutier, et al., *J Immunol* 164(4):1814-9 (2000) reported cloning IL-TIF (IL-10 related T cell-derived inducible factor), an IL-10 homolog, expressed by IL-9 treated murine T cells. Dumoutier, et al., *Proc Natl Acad Sci U S A* 97(18):10144-9 (2000) and Xie, et al., *J Biol Chem* 275(40):31335-9 (2000) reported cloning IL-TIF's human orthologue (human IL-22). According to these reports, murine IL-22 expression can be induced by IL-9 in thymic lymphomas, T cells and mast cells in vitro and by LPS in various organs in vivo, and IL-TIF injection induced production of acute phase reactants in mouse liver, suggesting involvement of IL-TIF in the inflammatory response.

With the sequence of the human genome being completed, cytokines and receptors have been discovered "in silico." Currently there are 11 members of the class II cytokine receptor family with assigned functions. Kotenko, S. V. (2002) *Cytokine Growth Factor Rev.* 13, 223-240; Kotenko, et al., (2000) *Oncogene* 19, 2557-2565; Dumoutier, et al., (2002) *Eur Cytokine Netw.* 13, 5-15; Fickenscher, et al., (2002) *Trends Immunol* 23, 89-96. These receptors are primarily utilized for signaling by members of two cytokine families, IFN and IL-10. The IFN family is further divided to type I and type II IFNs. The type II group is represented by a single member, IFN- γ ,

whereas the type I group is comprised of 13 IFN- α species (Data were obtained from the website of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>) with the use of various software tools for analyzing genome and EST databases available at the site.), a single species of IFN- β , IFN- ω and IFN- κ (LaFleur, et al., (2001) J Biol Chem 276, 39765-39771), and a mouse cytokine designated limitin (Oritani, et al., (2000) Nat. Med 6, 659-666) for which a human homolog has not yet been identified (and may not exist).

All known type I IFN genes are clustered on human chromosome 9. There is at least one additional member of this family positioned in the same cluster.

The family of IL-10-related cytokines consists of six members of cellular origin, IL-10, IL-19, IL-20, IL-22, IL-24 and IL-26, as well as several viral cytokines (for review see Kotenko, S. V. (2002) Cytokine Growth Factor Rev. 13, 223-240; Dumoutier, et al., (2002) Eur Cytokine Netw. 13, 5-15; Fickenscher, et al., (2002) Trends Immunol 23, 89-96).

All of these cytokines possess important biological activities. Several members of the IL-10 family are involved in a complex regulation of inflammatory responses. Whereas anti-inflammatory activity of IL-10 (on macrophages) is well characterized (Moore, et al., (2001) Annu. Rev Immunol 19, 683-765), the functions of the other IL-10-related cytokines are not well defined. IL-22 upregulates expression of several acute phase proteins in liver and hepatoma cells, and induces expression of pancreatitis-associated protein (PAP1) in pancreatic acinar cells (Dumoutier, et al., (2000) Proc Natl

Acad Sci U S A 97, 10144-10149; Aggarwal, et al., (2001) J Interferon Cytokine Res 21, 1047-1053).

IL-20 seems to be involved in regulation of normal skin development because IL-20-transgenic mice display skin abnormalities characterized by altered epidermal differentiation with hypoproliferation of keratinocytes (Blumberg, et al., (2001) Cell 104, 9-19).

The activities of IL-19 and IL-24 are not well characterized. However the fact that they share receptors with IL-20 and IL-22 indicates that they may share at least a subset of biological activities as well. No information about the factional activities or the receptor for IL-26 is currently available.

Type I and type II IFNs are important immunomodulators. Type II or immune IFN (IFN- γ) is a Th1-type cytokine which regulates both innate and adaptive immunity. IFN- γ stimulates cell-mediated immune responses which are critical for mediating protection against infection by intracellular parasites infections (many viruses) and is a part of antiviral defense.

Type I IFNs are well known for their ability to induce antiviral protection in wide variety of cells. They also activate host adaptive and innate immune forces to eliminate viral infections.

Antiviral protection is a highly complex process which involves several levels of defense starting with efforts of an infected cell to prevent the replication of a virus and warn other cells about viral presence, and finishing with the engagement of an entire

army of immune protective forces to combat virus propagation in a body. It seems that the main task of a first set of cells undergoing primary infection is to release signals to neighboring cells and immune system in general to alert them about viral presence and to force them to build antiviral protection before a virus infects them. Thus, the next wave of infected cells are better prepared to fight and upon sensing the presence of a virus through various means activate intracellular mechanisms of antiviral protection or even commit suicide (apoptosis) to prevent viral replication (reviewed in Levy, et al., (2001) Cytokine Growth Factor Rev 12, 143-156 and Samuel, C. E. (2001) Clin Microbiol Rev 14, 778-809). These alert signals are also produced by a subset of dendritic cells, which are able to sense the presence of a virus through mechanisms not necessarily involving viral entry into the cells. It is always a race between viral replication and development of cellular and immune defense forces that determines severity, duration and outcome of a viral infection.

Type I IFNs have been known for their ability to induce antiviral protection on both cellular and immune levels. Virus-induced robust production and secretion of IFNs leads to the induction of expression of many proteins with antiviral actions. The most well studied proteins in this respect are double-stranded RNA-activated protein kinase (PKR), 2',5'-oligoadenylate synthetase (OAS) and Mx proteins. These IFN-inducible proteins prevent viral replication through various mechanisms. dsRNA-activated PKR phosphorylates translation-initiation factor eIF-2 α blocking protein synthesis. OAS activates RNase L which cleaves mRNA and rRNA, thus inhibiting viral replication on

both transcriptional and translational levels. Clearly, such drastic measures affect cellular viability; indeed, both enzymes have been implicated in apoptosis.

Mx proteins are GTPases and some of them possess antiviral activity. The mechanism of their action is not completely understood. Mx proteins are found associated with viral ribonucleoprotein complexes and can interfere with their transcriptional functioning and/or trafficking.

Several other IFN-inducible proteins are likely to participate in cellular antiviral protection through mechanisms which remains to be determined. IFNs also modulate function of several type of immune cells (NK, CD-8+ and DC cells) in the direction favorable for clearing viral infection (reviewed in Biron, C. A. (2001) *Immunity* 14, 661-664 and Le Bon, A. & Tough, D. F. (2002) *Curr. Opin. Immunol* 14, 432-436).

There is a certain pattern of a ligand-receptor complex composition within the IFN and IL-10 families (Kotenko, 2002). A specific receptor complex for a particular ligand from these families is composed of two different receptor chains with distinct functions. The receptor chains within a given receptor complex can be divided into two types denoted R1 and R2 based on their functions in signaling. Although both R1 and R2 receptors are associated with Jak tyrosine kinases, only R1 type subunits have a long intracellular domain, are phosphorylated on Tyr residues after receptor engagement, therefore drive recruitment of various signaling molecules and, thus, determine the specificity of cytokine signaling. R2 type subunits possess a short intracellular domain and support signaling by bringing tyrosine kinase to a receptor complex but do not

determine the specificity of signaling. The length of the CRF2-12 intracellular domain indicated that this receptor represents the R1 type subunit.

There are five known receptor chains which can combine to form two-chain receptors for one or more of the six known ligands of the IL-10 family, but these five known receptors do not by themselves allow the full potential repertoire of IL-10 homologue signaling to be realized. That is, there are too many ligands and not enough receptors. Furthermore and most importantly, the wide and well-characterized in vivo and in vitro activities of cytokines and their receptors acting in concert demonstrated a clear need for and clinical potential of novel cytokines, cytokine receptors, cytokine mimics, blockers, agonists and antagonists. The present invention addresses these needs directly.

Type I IFNs are thought to exert their biological activities through binding to the specific cell surface receptor complex composed of two chains IFN- α R1 and IFN- α R2c (reviewed in Domanski, et al., (1996) Cytokine Growth Factor Rev 7, 143-151). IFN- α R2c is a signal-competent splice variant encoded by the IFNAR2 gene. Studies with IFN- α/β receptor knockout mice in which either subunit of the IFN- α receptor complex have been disrupted demonstrate an essential role for IFN- α signaling in the induction of antiviral resistance (Steinhoff, et al., (1995) J Virol 69, 2153-2158; Muller, et al., (1994) Science 264, 1918-1921; Hwang, et al., (1995) Proc Natl Acad Sci U S A 92, 11284-11288). However, the loss of antiviral protection to different viruses is variable in IFN- α receptor knockout mice. For instance, infection with rotavirus proceeds

similarly in mice with disrupted or intact IFN- α receptor system (Angel, et al., (1999) J Interferon Cytokine Res 19, 655-659).

IFNs appear to share a common receptor mechanism, the type I IFN-R composed of IFNAR1 and IFNAR2 subunits. IFNAR2 has membrane bound forms that can be short or long and soluble forms. IFN induced receptor dimerization of the IFNAR1 and IFNAR2c chains initiates a signaling cascade that involves tyrosine phosphorylation of the Tyk2 and Jak1 tyrosine kinases and subsequent phosphorylation of the STAT1 and STAT2 proteins (Stark et al., Ann. Rev. Biochem. 67:227-64 (1998); Science 296:1632-1657 (2002)). Association of the phosphorylated STATs with the p48 DNA binding subunit, forms the ISGF3 multisubunit complex that translocates to the nucleus and binds to interferon-stimulated response elements (ISRE) found upstream of the interferon inducible genes. While the type I IFNs bind the same receptor there appears to be subsequent signaling differences. In contrast to the type I IFNs there is only one member of the type II IFN, namely IFN gamma, which is encoded by a single gene (containing three introns) located on chromosome 12. The protein is produced predominantly by T lymphocytes and NK cells, is 166 amino acids in length and shows no homology to type I interferons.

A range of biological activities are associated with IFNs including antiviral, anti-microbial, tumor anti-proliferative, anti-proliferative, enhancement of NK cell activity, induction of MHC class I expression, and immunoregulatory activities. IFN alpha is marketed by Schering Plough (Intron; IFN alpha 2B) and Hoffman La Roche (Roferon; IFN alpha 2A). Therapeutic uses include the treatment of Hairy Cell leukemia, Chronic

myelogenous leukemia, low grade non-Hodgkin lymphoma, cutaneous T cell lymphoma, carcinoid tumors, renal cell carcinoma, squamous epithelial tumors of the head and neck, multiple myeloma, and malignant melanoma. With regards to viral disease, Interferon alpha has been found to aid the treatment of chronic active hepatitis, caused by either Hepatitis B or C viruses. IFN Beta has been demonstrated to have clinical benefit in the treatment of multiple sclerosis. Clinical trials with Interferon gamma have shown potential in the treatment of cutaneous and also visceral leishmanias.

Both recombinant interferons and interferons isolated from natural sources have been approved in the United States for treatment of auto-immune diseases, condyloma acuminatum, chronic hepatitis C, bladder carcinoma, cervical carcinoma, laryngeal papillomatosis, fungoides mycosis, chronic hepatitis B, Kaposi's sarcoma in patients infected with human immunodeficiency virus, malignant melanoma, hairy cell leukemia and multiple sclerosis.

Members of the type I interferon family have also been shown to influence neural cell activity and growth (see, for example, Dafny et al., Brain Res. 734:269 (1996); Pliopsys and Massimini, Neuroimmunomodulation 2:31 (1995)). In addition, intraventricular injection of neural growth factors has been shown to influence learning in animal models (see, for example, Fischer et al., Nature 329:65 (1987)).

IFNs have been used clinically for anti-viral therapy, for example, in the treatment of AIDS (HIV infection) (Lane, Semin. Oncol. 18:46-52 (1991)), viral hepatitis including chronic hepatitis B, hepatitis C (Woo, M. H. and Brunakis, T. G., Ann. Pharmacother, 31:330-337 (1997); Gibas, A. L., Gastroenterologist, 1:129-142

(1993)), hepatitis D, papilloma viruses (Levine, L. A. et al., *Urology* 47:553-557 (1996)), herpes (Ho, M., *Ann. Rev. Med.* 38:51-59 (1987)), viral encephalitis (Wintergerst et al., *Infection*, 20:207-212 (1992)), respiratory syncytial virus, panencephalitis, and other therapies, for example, mycosis fungoides and in the prophylaxis of rhinitis and respiratory infections (Ho, M., *Annu. Rev. Med.* 38:51-59 (1987)).

IFNs have been suggested for anti-parasite therapy, for example, IFN-gamma for treating *Cryptosporidium parvum* infection (Rehg, J. E., *J. Infect. Dis.* 174:229-232 (1996)).

Anti-bacterial: IFNs have been used clinically for anti-bacterial therapy. For example, IFN-gamma has been used in the treatment of multidrug-resistant pulmonary tuberculosis (Condos, R. et al., *Lancet* 349:1513-1515 (1997)).

Interferon therapy has been used in the treatment of numerous cancers (e.g., hairy cell leukemia (Hoffmann et al., *Cancer Treat. Rev.* 12 (Suppl. B): 33-37 (1985)), acute myeloid leukemia (Stone, R. M. et al. *Am. J. Clin. Oncol.* 16:159-163 (1993)), osteosarcoma (Strander, H. et al., *Acta Oncol.* 34:877-880 (1995)), basal cell carcinoma (Dogan, B. et al., *Cancer Lett.* 91:215-219 (1995)), glioma (Fetell, M. R. et al., *Cancer* 65: 78-83 (1990)), renal cell carcinoma (Aso, Y. et al. *Prog. Clin. Biol. Res.* 303:653-659 (1989)), multiple myeloma (Peest, D. et al., *Br. J. Haematol.* 94:425-432 (1996)), melanoma (Ikic, D. et al., *Int. J. Dermatol.* 34:872-874 (1995)), myelogenous leukemia, colorectal cancer, cutaneous T cell lymphoma, myelodysplastic syndrome, glioma, head and neck cancer, breast cancer, gastric cancer, anti-cancer vaccine therapy, and

Hodgkin's disease (Rybak, M. E. et al., J. Biol. Response Mod. 9:1-4 (1990)).

Synergistic treatment of advanced cancer with a combination of alpha interferon and temozolomide has also been reported (Patent publication WO 9712630 to Dugan, M. H.).

IFNs have been used clinically for immunotherapy or more particularly, for example, to prevent graft vs. host rejection, or to curtail the progression of autoimmune diseases, such as arthritis, multiple sclerosis, or diabetes. IFN-beta is approved of sale in the United States for the treatment (i.e., as an immunosuppressant) of multiple sclerosis. Recently it has been reported that patients with multiple sclerosis have diminished production of type I interferons and interleukin-2 (Wandinger, K. P. et al., J. Neurol. Sci. 149: 87-93 (1997)). In addition, immunotherapy with recombinant IFN-alpha (in combination with recombinant human IL-2) has been used successfully in lymphoma patients following autologous bone marrow or blood stem cell transplantation, that may intensify remission following translation (Nagler, A. et al., Blood 89: 3951-3959 (June 1997)).

The administration of IFN-gamma has been used in the treatment of allergies in mammals (See, International Patent Publication WO 8701288 to Parkin, J. M. and Pinching, A. J.). It has also recently been demonstrated that there is a reduced production of IL-12 and IL-12-dependent IFN-gamma release in patients with allergic asthma (van der Pouw Kraan, T. C. et al., J. Immunol. 158:5560-5565 (1997)). Thus, IFN may be useful in the treatment of allergy by inhibiting the humoral response.

Interferons may be used as an adjuvant or coadjuvant to enhance or simulate the immune response in cases of prophylactic or therapeutic vaccination (Heath, A. W. and Playfair, J. H. L., Vaccine 10:427-434 (1992)), such as in anti-cancer vaccine therapy.

Interferons have been used to treat corneal haze.

SUMMARY OF THE INVENTION

The present invention provides an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence, for example, including: a nucleotide sequence encoding the CRF2-12 polypeptide having the complete amino acid sequence of SEQ ID NO: 2; a nucleotide sequence encoding the CRF2-12 polypeptide having the complete amino acid sequence of SEQ ID NO: 2 excepting the N-terminal methionine (i.e., residues 2-520 of SEQ ID NO: 2); a nucleotide sequence encoding the mature CRF2-12 polypeptide shown as residues P19-520 of SEQ ID NO: 2; a nucleotide sequence encoding the CRF2-12 polypeptide extracellular domain; a nucleotide sequence encoding the extracellular domain of the CRF2-12 protein in plasmid pEF-CRF2-12/ γ R1; a nucleotide sequence encoding the extracellular domain of the CRF2-12 protein in plasmid pEF3-FL γ R2/ α R2c; a nucleotide sequence encoding the intracellular domain of the CRF2-12 protein in plasmid pEF-FLCRF2-12; and a nucleotide sequence complementary to any of these nucleotide sequences.

The present invention also provides an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence, for example, including: a nucleotide sequence encoding a chimeric receptor comprising the extracellular domain of CRF2-12 and the intracellular domain of another membrane bound receptor; a nucleotide sequence encoding a chimeric receptor comprising the extracellular domain of CRF2-12 and the intracellular domain of another membrane bound tyrosine kinase receptor; a nucleotide sequence encoding a chimeric receptor comprising the extracellular domain of CRF2-12 and the intracellular domain of a cytokine receptor; a nucleotide sequence

encoding a chimeric receptor comprising the extracellular domain of CRF2-12 and the intracellular domain of an IFN R1 type receptor; a nucleotide sequence encoding a chimeric receptor comprising the extracellular domain of CRF2-12 and the intracellular domain of IFN γ R1; a nucleotide sequence encoding the chimeric protein in plasmid pEF-CRF2-12/ γ R1; and a nucleotide sequence complementary to any of these nucleotide sequences.

The present invention also provides an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence, for example, including: a nucleotide sequence encoding a chimeric receptor comprising the intracellular domain of CRF2-12 and the extracellular domain of another membrane bound receptor; a nucleotide sequence encoding a chimeric receptor comprising the intracellular domain of CRF2-12 and the extracellular domain of another membrane bound tyrosine kinase receptor; a nucleotide sequence encoding a chimeric receptor comprising the intracellular domain of CRF2-12 and the extracellular domain of a cytokine receptor; a nucleotide sequence encoding a chimeric receptor comprising the intracellular domain of CRF2-12 and the extracellular domain of an IFN R1 type receptor; a nucleotide sequence encoding a chimeric receptor comprising the intracellular domain of CRF2-12 and the extracellular domain of IL-10R1; a nucleotide sequence encoding the chimeric protein in plasmid pEF-IL-10R1/IFN- λ R1; and a nucleotide sequence complementary to any of these nucleotide sequences.

The present invention also provides an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence, for example, including: a tandem vector

having a nucleotide sequence encoding two receptors, wherein the first receptor is an R1 type receptor and the second receptor is an R2 type receptor, and wherein the expression of each receptor is controlled by separate promoters and polyadenylation signals; a tandem vector having a nucleotide sequence encoding two receptors, wherein the first receptor comprises the extracellular domain of CRF2-12 and the second receptor is an R2 type receptor, and wherein the expression of each receptor is controlled by separate promoters and polyadenylation signals; a tandem vector having a nucleotide sequence encoding two receptors, wherein the first receptor comprises the intracellular domain of CRF2-12 and the second receptor is an R2 type receptor, and wherein the expression of each receptor is controlled by separate promoters and polyadenylation signals; a tandem vector having a nucleotide sequence encoding two receptors, wherein the first receptor comprises CRF2-12 and the second receptor is an R2 type receptor, and wherein the expression of each receptor is controlled by separate promoters and polyadenylation signals; any of the above tandem vectors, wherein the R2 type receptor comprises CRF2-4; the tandem vector pEF-CRF2-12/ γ R1+CRF2-4; and a nucleotide sequence complementary to any of these nucleotide sequences.

The present invention also provides isolated nucleic acid molecules comprising a polynucleotide encoding at least a portion of one of the IFN λ polypeptides having the complete amino acid sequence shown of SEQ ID NOS: 8, 10 and 12.

The nucleotide sequence determined by sequencing the IFN- λ 1 clone, which is shown in FIG. 16 (SEQ ID NO: 7), contains an open reading frame encoding a full

length polypeptide of 200 amino acid residues, including an initiation codon encoding an N-terminal methionine.

The nucleotide sequence determined by sequencing the IFN- λ 2 clone, which is shown in FIG. 17 (SEQ ID NO: 9), contains an open reading frame encoding a full length polypeptide of 196 amino acid residues, including an initiation codon encoding an N-terminal methionine.

The nucleotide sequence determined by sequencing the IFN- λ 3 clone, which is shown in FIG. 18 (SEQ ID NO: 11), contains an open reading frame encoding a full length polypeptide of 196 amino acid residues, including an initiation codon encoding an N-terminal methionine.

Nucleic acid molecules of the invention include those encoding the complete amino acid sequence excepting the N-terminal methionine shown in SEQ ID NOS: 8, 10 and 12, which molecules also can encode additional amino acids fused to the N-terminus of the amino acid sequences of IFN- λ 1, IFN- λ 2 and IFN- λ 3.

Nucleic acid molecules of the invention include those encoding the complete amino acid sequence excepting the N-terminal methionine shown in SEQ ID NOS: 8, 10 and 12, which molecules also can encode additional amino acids fused to the N-terminus of the amino acid sequences of IFN- λ 1, IFN- λ 2 and IFN- λ 3.

The encoded polypeptides have a predicted leader sequence of 22 amino acids, shown as the boxed consensus in FIG. 19A.

Thus, one aspect of the invention provides an isolated nucleic acid molecule comprising a polynucleotide comprising a nucleotide sequence, for example, including: a nucleotide sequence encoding the IFN- λ 1 polypeptide having the complete amino acid sequence of SEQ ID NO: 8; a nucleotide sequence encoding the IFN- λ 1 polypeptide having the complete amino acid sequence of SEQ ID NO: 8 excepting the N-terminal methionine (i.e., residues 2-200 of SEQ ID NO: 8); a nucleotide sequence encoding the mature IFN- λ 1 polypeptide shown as residues 23-200 of SEQ ID NO: 8; a nucleotide sequence of the genomic fragment encoding the complete IFN- λ 1 gene contained in plasmid pEF-FL-IFN- λ 1 gene; a nucleotide sequence encoding the complete polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 1; a nucleotide sequence encoding the mature polypeptide encoded by the human cDNA contained in plasmid pEF-FL-IFN- λ 1; a nucleotide sequence encoding the mature polypeptide encoded by the human cDNA contained in plasmid pEF2-FL-IFN- λ 1; and a nucleotide sequence complementary to any of these nucleotide sequences.

Another aspect of the invention provides an isolated nucleic acid molecule comprising a polynucleotide comprising a nucleotide sequence, for example, including: a nucleotide sequence encoding the IFN- λ 2.1 polypeptide having the complete amino acid sequence of SEQ ID NO: 10; a nucleotide sequence encoding the IFN- λ 2.1 polypeptide having the complete amino acid sequence of SEQ ID NO: 10 excepting the N-terminal methionine (i.e., residues 2-196 of SEQ ID NO: 10); a nucleotide sequence encoding the mature IFN- λ 2.1 polypeptide shown as residues 23-196 of SEQ ID NO: 10; a nucleotide sequence encoding IFN- λ 2.2 polypeptide (residues M12-196 of SEQ ID

NO: 10); a nucleotide sequence encoding IFN- λ 2.2 polypeptide residues A13-196 of SEQ ID NO: 10; a nucleotide sequence encoding the mature IFN- λ 2.2 polypeptide shown as residues P31-196 of SEQ ID NO: 10; a nucleotide sequence encoding IFN- λ 2.3 polypeptide (residues M92-196 of SEQ ID NO: 10); a nucleotide sequence encoding IFN- λ 2.3 polypeptide residues A93-196 of SEQ ID NO: 10; a nucleotide sequence encoding the mature IFN- λ 2.3 polypeptide shown as residues P113-196 of SEQ ID NO: 10; the nucleotide sequence of the genomic fragment encoding the complete IFN- λ 2 gene contained in plasmid pEF-FL-IFN- λ 2gene; a nucleotide sequence encoding a polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 2; a nucleotide sequence encoding the mature polypeptide encoded by the cDNA contained in clone pEF-FL-IFN- λ 2; and a nucleotide sequence complementary to any of these nucleotide sequences.

Another aspect of the invention provides an isolated nucleic acid molecule comprising a polynucleotide comprising a nucleotide sequence, for example, including: a nucleotide sequence encoding the IFN- λ 3.1 polypeptide having the complete amino acid sequence of SEQ ID NO: 12; a nucleotide sequence encoding the IFN- λ 3.1 polypeptide having the complete amino acid sequence of SEQ ID NO: 12 excepting the N-terminal methionine (i.e., residues 2-196 of SEQ ID NO: 12); a nucleotide sequence encoding the mature IFN- λ 3.1 polypeptide shown as residues P23-196 of SEQ ID NO: 12; a nucleotide sequence encoding IFN- λ 3.2 polypeptide (residues M6-196 of SEQ ID NO: 12); a nucleotide sequence encoding IFN- λ 3.3 polypeptide (residues M12-196 of SEQ ID NO: 12); a nucleotide sequence encoding IFN- λ 3.2 polypeptide residues P7-

196 of SEQ ID NO: 12; a nucleotide sequence encoding IFN- λ 3.3 polypeptide residues A13-196 of SEQ ID NO: 12; a nucleotide sequence encoding the mature IFN- λ 3.2 polypeptide shown as residues P31-196 of SEQ ID NO: 12; the nucleotide sequence of the genomic fragment encoding the complete IFN- λ 3 gene contained in plasmid pEF-FL-IFN- λ 3gene; a nucleotide sequence encoding a polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 3; a nucleotide sequence encoding a mature polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 3; and a nucleotide sequence complementary to any of these nucleotide sequences.

Further embodiments of the invention include isolated nucleic acid molecules that comprise a polynucleotide having a nucleotide sequence at least 80%, 85%, or 90% identical, more preferably at least 91%, 92%, 93%, and 94% and most preferably at least 95%, 96%, 97%, 98% or to any of the nucleotide sequences, above, or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide of the present invention. This polynucleotide of the present invention, which hybridizes under stringent conditions defined herein does not hybridize to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues. An additional nucleic acid embodiment of the invention relates to an isolated nucleic acid molecule comprising a polynucleotide that encodes the amino acid sequence of an epitope-bearing portion of a polypeptide having an amino acid sequence described above.

A further aspect of the invention is a DNA sequence that represents the complete regulatory region of a gene of the present invention. DNA constructs containing the

regulatory region are also provided. Further, host cells comprising such constructs, which cells are in vitro or in vivo, are also encompassed by the present invention.

The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of polypeptides or peptides of the present invention by recombinant techniques.

The invention further provides isolated polypeptides comprising an amino acid sequence, for example, including: the amino acid sequence of the full-length IFN- λ 1 polypeptide having the complete amino acid sequence of SEQ ID NO: 8; the amino acid sequence of the full-length IFN- λ 1 polypeptide having the complete amino acid sequence of SEQ ID NO: 8 excepting the N-terminal methionine (i.e., residues 2-200 of SEQ ID NO: 8); the amino acid sequence the mature IFN- λ 1 polypeptide shown as residues 23-200 of SEQ ID NO: 8; an IFN- λ 1 polypeptide encoded by the genomic fragment encoding the IFN- λ 1 gene contained in plasmid pEF-FL-IFN- λ 1 gene; an IFN- λ 1 polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 1; a mature IFN- λ 1 polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 1. The polypeptides of the present invention also include polypeptides having an amino acid sequence at least 80% identical, more preferably at least 90% identical, and still more preferably 95%, 96%, 97%, 98% or 99% identical to those described above, as well as polypeptides having an amino acid sequence with at least 90% similarity, and more preferably at least 95% similarity, to those above.

The invention further provides isolated polypeptides comprising an amino acid sequence, for example, including: the IFN- λ 2.1 polypeptide having the complete amino acid sequence of SEQ ID NO: 10; the IFN- λ 2.1 polypeptide having the complete amino acid sequence of SEQ ID NO: 10 excepting the N-terminal methionine (i.e., residues 2-196 of SEQ ID NO: 10); the mature IFN- λ 2.1 polypeptide shown as residues 23-196 of SEQ ID NO: 10; the IFN- λ 2.2 polypeptide shown as residues M12-196 of SEQ ID NO: 10; the IFN- λ 2.2 polypeptide shown as residues A13-196 of SEQ ID NO: 10; the mature IFN- λ 2.2 polypeptide shown as residues P31-196 of SEQ ID NO: 10; the IFN- λ 2.3 polypeptide shown as residues M92-196 of SEQ ID NO: 10; the IFN- λ 2.3 polypeptide shown as residues A93-196 of SEQ ID NO: 10; the mature IFN- λ 2.3 polypeptide shown as residues P113-196 of SEQ ID NO: 10; a IFN- λ 2 polypeptide coded by plasmid pEF-FL-IFN- λ 2gene; a polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 2; a mature polypeptide encoded by the cDNA contained in clone pEF-FL-IFN- λ 2. The polypeptides of the present invention also include polypeptides having an amino acid sequence at least 80% identical, more preferably at least 90% identical, and still more preferably 95%, 96%, 97%, 98% or 99% identical to those described above, as well as polypeptides having an amino acid sequence with at least 90% similarity, and more preferably at least 95% similarity, to those above.

The invention further provides isolated polypeptides comprising an amino acid sequence, for example, including: a IFN- λ 3.1 polypeptide having the complete amino acid sequence of SEQ ID NO: 12; a IFN- λ 3.1 polypeptide having the complete amino acid sequence of SEQ ID NO: 12 excepting the N-terminal methionine (i.e., residues 2-

196 of SEQ ID NO: 12); a mature IFN- λ 3.1 polypeptide shown as residues P23-196 of SEQ ID NO: 12; a IFN- λ 3.2 polypeptide shown as residues M6-196 of SEQ ID NO: 12; a IFN- λ 3.3 polypeptide shown as residues M12-196 of SEQ ID NO: 12; a IFN- λ 3.2 polypeptide shown as residues P7-196 of SEQ ID NO: 12; a IFN- λ 3.3 polypeptide shown as residues A13-196 of SEQ ID NO: 12; a mature IFN- λ 3.2 polypeptide shown as residues P31-196 of SEQ ID NO: 12; a IFN- λ 3 polypeptide coded by plasmid pEF-FL-IFN- λ 3 gene; a polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 3; a mature polypeptide encoded by the cDNA contained in clone pEF-FL-IFN- λ 3. The polypeptides of the present invention also include polypeptides having an amino acid sequence at least 80% identical, more preferably at least 90% identical, and still more preferably 95%, 96%, 97%, 98% or 99% identical to those described above, as well as polypeptides having an amino acid sequence with at least 90% similarity, and more preferably at least 95% similarity, to those above.

An additional embodiment of this aspect of the invention relates to a peptide or polypeptide which comprises the amino acid sequence of an epitope-bearing portion of a polypeptide of the present invention having an amino acid sequence described above. Peptides or polypeptides having the amino acid sequence of an epitope-bearing portion of a polypeptide of the invention include portions of such polypeptides with at least six or seven, preferably at least nine, and more preferably at least about 30 amino acids to about 50 amino acids, although epitope-bearing polypeptides of any length up to and including the entire amino acid sequence of a polypeptide of the invention described above also are included in the invention.

In another embodiment, the invention provides an isolated antibody that binds specifically to a polypeptide having an amino acid sequence described above. The invention further provides methods for isolating antibodies that bind specifically to a polypeptide having an amino acid sequence as described herein. Such antibodies are useful therapeutically as described below.

The invention also provides for pharmaceutical compositions comprising a polypeptide of the present invention. The invention also provides for pharmaceutical compositions comprising combinations of polypeptides of the present invention.

In one embodiment, the invention provides for a composition comprising IFN- λ 1.

In another embodiment, the invention provides for a composition comprising IFN- λ 2.

In another embodiment, the invention provides for a composition comprising IFN- λ 3.

In one embodiment, the invention provides for a composition comprising a combination of polypeptides of the present invention, wherein the ratio of IFN- λ 1:IFN- λ 2:IFN- λ 3 is described by the formula m:n:o, wherein m may be any number between 0 and 1000, n may be any number between 0 and 1000 and o may be any number between 0 and 1000, and wherein at least one of m, n or o is different from 0. The invention also provides for a composition comprising a combination of polypeptides of the present invention, wherein the ratio of IFN- λ 1:IFN- λ 2:IFN- λ 3 is described by the formula

m:n:o, wherein m may be any number between 0 and 1000, n may be any number between 0 and 1000 and o may be any number between 0 and 1000, and wherein at least two of m, n or o are different from 0. The invention further provides for a composition comprising a combination of polypeptides of the present invention, wherein the ratio of IFN- λ 1:IFN- λ 2:IFN- λ 3 is described by the formula m:n:o, wherein m may be any number between 0 and 1000, n may be any number between 0 and 1000 and o may be any number between 0 and 1000, and wherein all three of m, n and o are different from 0. The compositions may be employed, for instance, as a positive control in assays for IFN- λ activity, as a positive control for CRF2-12 receptor and receptor complex activity, or as a pharmaceutical composition to treat immune system-related disorders, and other disorders such as viral infection, parasitic infection, bacterial infection, cancer, autoimmune disease, multiple sclerosis, lymphoma and allergy. Methods of treating individuals in need of polypeptides of the present invention are also provided.

The invention also provides for pharmaceutical compositions comprising an antibody of the present invention. The invention also provides for pharmaceutical compositions comprising combinations of antibodies of the present invention. In one embodiment, the invention provides a composition comprising a combination of anti-IFN- λ 1 specific antibody, anti-IFN- λ 2 specific antibody and anti-IFN- λ 3 specific antibody, wherein the ratio of anti-IFN- λ 1 specific antibody: anti-IFN- λ 2 specific antibody: anti-IFN- λ 3 specific antibody is described by the formula x:y:z, wherein x may be any number between 0 and 1000, y may be any number between 0 and 1000 and z may be any number between 0 and 1000, and wherein at least one of x, y or z is

different from 0. The invention also provides for pharmaceutical compositions comprising a combination of anti-IFN- λ 1 specific antibody, anti-IFN- λ 2 specific antibody and anti-IFN- λ 3 specific antibody, wherein the ratio of anti-IFN- λ 1 specific antibody: anti-IFN- λ 2 specific antibody: anti-IFN- λ 3 specific antibody is described by the formula $x:y:z$, wherein x may be any number between 0 and 1000, y may be any number between 0 and 1000 and z may be any number between 0 and 1000, and wherein at least two of x , y or z are different from 0. The invention also provides for pharmaceutical composition comprising a combination of anti-IFN- λ 1 specific antibody, anti-IFN- λ 2 specific antibody and anti-IFN- λ 3 specific antibody, wherein the ratio of anti-IFN- λ 1 specific antibody: anti-IFN- λ 2 specific antibody: anti-IFN- λ 3 specific antibody is described by the formula $x:y:z$, wherein x may be any number between 0 and 1000, y may be any number between 0 and 1000 and z may be any number between 0 and 1000, and wherein all three of x , y or z are different from 0. The compositions may be employed, for instance, to treat immune system-related disorders, and other disorders such as viral infection, parasitic infection, bacterial infection, cancer, autoimmune disease, multiple sclerosis, lymphoma and allergy. Methods of treating individuals in need of polypeptides of the present invention are also provided.

The invention further provides compositions comprising a polynucleotide or a polypeptide for administration to cells in vitro, to cells ex vivo and to cells in vivo, or to a multicellular organism. In certain particularly preferred embodiments of this aspect of the invention, the compositions comprise a polynucleotide for the expression of a polypeptide of the present invention in a host organism for use to treat a disease.

Particularly preferred in this regard is expression in a human patient for treatment of a dysfunction associated with aberrant endogenous activity of an interferon or a receptor thereof.

The present invention also provides a screening method for identifying compounds capable of enhancing or inhibiting a biological activity of the polypeptides of the present invention, which involves contacting a receptor which is activated by the polypeptides of the present invention with the candidate compound in the presence of a polypeptide of the present invention, assaying, for example, anti-viral activity or MHC expression in the presence of the candidate compound and the polypeptide of the present invention, and comparing the activity to a standard level of activity, the standard being assayed when contact is made between the receptor and a polypeptide of the present invention in the absence of the candidate compound. In this assay, an increase in activity over the standard indicates that the candidate compound is an agonist and a decrease in activity compared to the standard indicates that the compound is an antagonist of the activity of the polypeptide of the present invention.

The present invention also provides a screening method for identifying compounds capable of enhancing or inhibiting a biological activity of the polypeptides of the present invention, which involves contacting a receptor which is activated by an IFN- λ with the candidate compound in the presence and in the absence of the IFN- λ , assaying for receptor activity in the presence of the candidate compound and the IFN- λ , assaying for receptor activity in the presence of the candidate compound and the absence of the IFN- λ , and comparing the activity to a standard level of activity, the standard

being assayed when contact is made between the receptor and the IFN- λ in the absence of the candidate compound.

Expression of the polypeptides of the present invention can be regulated by double stranded RNA as well as other cytokines.

Polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of a number of disorders, particularly of the immune system, significantly higher or lower levels of expression of genes of the present invention may be detected in certain tissues (e.g., cancerous and wounded tissues), cells or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a standard gene expression level, i.e., the expression level of the gene in healthy tissue from an individual not having the disorder. Thus, the invention provides a diagnostic method useful during diagnosis of such a disorder, which involves: (a) assaying the expression level of an mRNA or polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the expression level with a standard expression level, whereby an increase or decrease in the assayed expression level compared to the standard expression level is indicative of disorder.

An additional aspect of the invention is related to a method for treating an individual in need of an increased level of interferon activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an isolated polypeptide of the invention, combinations thereof, or an agonist

thereof, or administration of DNA encoding one or more effective polypeptides of the present invention.

A still further aspect of the invention is related to a method for treating an individual in need of a decreased level of interferon activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of an antagonist. Preferred antagonists for use in the present invention are IFN- λ -specific antibodies. Also preferred are antibodies specific for CRF2-12. Also preferred are antibodies specific for the extracellular domain of CRF2-12. Particularly preferred are monoclonal antibodies.

A still further aspect of the invention is related to a method for treating viral, parasitic, cancerous, allergic and other medical conditions wherein the compositions of the present invention are effective.

A still further aspect of the invention is related to a method for modulating the function of immune cells, including, for example isotype switching.

Another aspect of the present invention is the characterization of genetic disorders defined by polymorphisms in the genes or chromosomal loci of the present invention.

In another embodiment, the compositions of the present invention serve as an adjuvant for vaccination.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows the nucleotide sequence (SEQ ID NO: 1) of human CRF2-12 cDNA, with capital letters representing a predicted coding sequence.

FIG. 2 shows a deduced amino acid sequence (SEQ ID NO: 2) for human CRF2-12.

FIG. 3 shows the CRF2-12 gene is located on human chromosome 1, between 20.2 million bases and 20.4 million bases, adjacent to and telomeric of the gene for IL22R1 receptor chain, in the cytogenic region 1p36.11-1p36.12.

FIG. 4 shows an alignment between the amino acid sequence of the extracellular portion of CRF2-12 and that of IL22R1.

FIG. 5 shows an alignment of the CRF2-12 polypeptide (SEQ ID NO: 2) of the present invention with several other members of the IL-10 receptor polypeptide family. Shown are (labeled IL10Ra), (labeled IL10Rb), (labeled IL20Ra), (labeled IL20Rb), (labeled IL22Ra), (labeled IL22BP). Amino acids identical to the CRF2-12 polypeptide are boxed. By examining the regions of the boxed amino acids, the skilled artisan can readily identify conserved domains between the polypeptides. These conserved domains are preferred embodiments of the present invention.

FIG. 6 shows an alignment of the CRF2-12 polypeptide (SEQ ID NO:2) of the present invention with several other members of the class-II cytokine receptor polypeptide family. Shown is (labeled ar2), (labeled tfec), (labeled crf28), (labeled

crf210), labeled crf29), (labeled il10r1), (labeled ar1n), (labeled il10r2), (labeled ar1c), (labeled gr2), (labeled hudirs1ec), (labeled gr1). Amino acids identical to the CRF2-12 polypeptide are boxed. By examining the regions of the boxed amino acids, the skilled artisan can readily identify conserved domains between the polypeptides. These conserved domains are preferred embodiments of the present invention.

FIG. 7 shows the nucleotide sequence (SEQ ID NO: 1) and the deduced amino acid sequence (SEQ ID NO: 2) of human CRF2-12.

FIG. 8 shows the nucleotide sequence (SEQ ID NO: 1) and the deduced amino acid sequence (SEQ ID NO: 2) of human CRF2-12 using an alternative, three-letter, notation sometimes used to describe amino acid sequences.

FIG. 9 represents the single letter nucleotide sequence (SEQ ID NO: 3) which fully describes and defines the codon usages possible for encoding the deduced amino acid sequence (SEQ ID NO: 2) of human CRF2-12.

FIG. 10 shows how the redundant code defines possible individual codons encoding the deduced amino acid sequence (SEQ ID NO: 2) of human CRF2-12.

FIG. 11 shows the 3'UTR portion of CRF2-12 (SEQ ID NO: 4).

FIG. 12 shows the nucleotide sequence (SEQ ID NO: 5) of mouse CRF2-12 cDNA, with capital letters representing a predicted coding sequence.

FIG. 13 shows a deduced amino acid sequence (SEQ ID NO: 6) for mouse CRF2-12.

FIG. 14A shows the deduced amino acid sequence (SEQ ID NO:2) of human CRF2-12, where the first boxed region, region a, constitutes the signal sequence, the first non-boxed region, region b, constitutes the predicted intracellular domain, the second boxed region, region c, constitutes the predicted transmembrane domain, and the second non-boxed region, region d, constitutes the predicted extracellular domain of human CRF2-12. Potential glycosylation sites are underlined as well as the C-terminal intracellular Tyr-based motif which is also homologous to those within the IFN- α R2c intracellular domain and is likely to participate in Stat activation.

FIG. 14B shows a phylogenetic tree derived from an alignment of the extracellular domains of the class II cytokine receptors, which assigns CRF2-12 to a subgroup with several other receptors including CRF2-8, CRF2-9, CRF2-10, IL-10R1 and IFN- γ R1. Alignment was generated by the program PILEUP of the Wisconsin Package Version 9.1, Genetics Computer Group (GCG), Madison, Wisconsin was used with the following parameters: the gap creation penalty 1, the gap extension penalty 1. The CLUSTAL X program was used to create the phylogenetic tree.

FIG. 14C shows the organization of the CRF2-12 gene. The gene is composed of 7 exons and its structure correlates well with the common conserved architecture of other genes encoding CRF members. The first exon contains the 5'-UTR and the signal peptide. Exons 2, 3, 4 and 5 and a part of exon 6 encode the extracellular domain. Exon 6 also encodes the transmembrane domain and the beginning of the intracellular domain. Exon 7 covers the rest of the intracellular domain and the 3'-UTR.

FIG. 14D a schematic diagram of human chromosome 1, shows that the CRF2-12 gene and the IL-22R1 gene are transcribed in the same direction toward the telomere and are positioned approximately 10 kb apart. Chromosomal localization of the CRF2-12 gene and its close neighbor, the IL-22R1 gene as well as the ideogram of human chromosome 1 was generated from the NCBI database. The genes are transcribed in the same direction as indicated by the arrow. Schematic exon/intron structure of the CRF2-12 gene is shown. Coding regions of exons are shaded and the segments corresponding to 5' and 3' untranslated regions are left open.

FIG. 15A and B, as shown in these figures, CRF2-12 appears to be constitutively expressed in a variety of cell lines and tissues. FIG 15A is a northern blot, and shows that CRF2-12 mRNA is expressed at variable levels in all the tumor cell lines examined, including hematopoietic (HL-60, K-562, MOLT-4 and Raji) and non-hematopoietic (HeLa, SW480, A549, and G-361) cell lines.

Northern blotting was performed on two blots containing mRNA isolated from: (A) human cancer cell lines (promyelocytic leukemia HL-60, epitheloid carcinoma HeLa S3, lymphoblastic leukemia MOLT-4, Burkitt's lymphoma Raji, colorectal adenocarcinoma, SW480, lung carcinoma A549 and melanoma G361) and (B) normal human fetal tissues (heart, kidney, skin, small intestine) and adult lung. Arrows pointing to the CRF2-12 and the b-actin transcripts. Equal RNA loading was assessed by evaluating the expression of the b-actin gene.

FIG. 15B is a northern blot, and shows that CRF2-12 was also expressed by various normal tissues, including heart, kidney, skin, small intestine, and lung. CRF2-12 mRNA was also present in skeletal muscle and liver.

FIG. 16 shows the nucleotide sequence (SEQ ID NO: 7) and the deduced amino acid sequence (SEQ ID NO: 8) of IFN- λ 1.

FIG. 17 shows the nucleotide sequence (SEQ ID NO: 9) and the deduced amino acid sequence (SEQ ID NO: 10) of IFN- λ 2.

FIG. 18 shows the nucleotide sequence (SEQ ID NO: 11) and the deduced amino acid sequence (SEQ ID NO: 12) of IFN- λ 3.

FIG. 19A shows a sequence alignment of polypeptides IFN- λ 1 (SEQ ID NO: 8), IFN- λ 2 (SEQ ID NO: 10) and IFN- λ 3 (SEQ ID NO: 12). Identical residues are boxed. The consensus sequence is shown on the bottom. Positions of corresponding introns are indicated by arrows. By examining the regions of the boxed amino acids, the skilled artisan can readily identify conserved domains between the polypeptides. These conserved domains are preferred embodiments of the present invention. Amino acid (a.a.) residues are numbered starting from the Met residue (signal peptide a.a. are included).

FIG. 19B shows a phylogenetic tree for IFN- λ proteins generated as described in the Figure 14 legend.

FIG. 19C is a schematic showing the gene structure and chromosomal localization for IFN- λ genes as well as the direction of transcription (arrows). Coding regions of exons are shaded and the segments corresponding to 5' and 3' untranslated regions are left open.

FIG. 20A, from left to right, is a diagrammatic illustration of transmembrane receptors encoded by cDNA expression vectors encoding i) intact CRF2-12 ii) a chimeric CRF2-12/ γ R1 receptor that has the CRF2-12 extracellular domain fused to the transmembrane and intracellular domains of the human IFN- γ R1 chain, iii) CRF2-4, the second chain of the human IL-10 and IL-22 receptor complexes and iv) a FLAG tagged chimeric IL-10R1/CRF2-12 receptor that has the IL-10R1 extracellular domain fused to the transmembrane and intracellular domains of CRF2-12.

FIG. 20B is a diagrammatic illustration of IFN- λ s and their derivatives. From top to bottom, i) represents IFN- λ s; ii) represents IFN- λ s tagged at the N-terminus with the FLAG epitope (FL-IFN- λ s); iii) represents IFN- λ s tagged at the C-terminus with the Arg-Arg-Ala-Ser-Val-Ala sequence that contains the consensus amino acid motif recognizable by the catalytic subunit of the cAMP-dependent protein kinase (FL-IFN- λ s-P).

FIG. 20C is a western blot showing the expression of FL-I IFN- λ s and FL-IFN- λ 1-P in COS cells. Western blotting analysis with anti-FLAG antibody of conditioned media from COS-1 cells transfected with plasmids pEF-SPFL (lane 1, mock), pEF-FL-IFN- λ 1 gene (lane 2, FL-IFN- λ 1 gene), pEF-FL-IFN- λ 2 gene (lane 3, FL-IFN- λ 2 gene),

pEF-FL-IFN- λ 3 gene (lane 4, FL-IFN- λ 3 gene), pEF-FL-IFN- λ 1 (lane 5, FL-IFN- λ 1), pEF-FL-IFN- λ 2 (lane 6, FL-IFN- λ 2), pEF-FL-IFN- λ 3 (lane 7, FL-IFN- λ 3). FL-IFN- λ 1-P was purified from conditioned media by affinity chromatography and evaluated by Western blotting with anti-FLAG antibody (lane 8). Lane 9 represents an autoradiograph of the SDS-PAGE gel containing radiolabeled FL-IFN- λ 1-P. The molecular weight markers are shown on the left.

FIG. 21 MHC class I antigen expression, ligand binding and EMSA performed on hamster cells.

A. Four cell lines used in these experiments are schematically shown (top panels): the parental Chinese hamster 16-9 cells and cells expressing the human CRF2-12/gR1 chimeric receptor or human IL-10R2 alone or both receptors together. The cells were left untreated (open areas, thick lines) or treated with conditioned media (100 ml) from COS cells transfected with plasmid pEF-FL-IFN- λ 1 (FL-IFN- λ 1; shaded areas, thin lines) or with E. coli produced IFN- λ 1 (IFN- λ 1E.coli, 100 ng; open areas, thin lines) and the ability of IFN- λ 1 to upregulate MHC class I antigen expression was demonstrated by flow cytometry (panels A, B, C and D). The cells were also tested for their ability to bind FL-IFN- λ s (panels E, F, G, H and I). The cells were incubated for three hours at 20° C with conditioned medium from COS-1 cells transfected with one of the following plasmids: the control vector pEF-SPFL (open areas, thick lines); the pEF-FL-IFN- λ 1 (FL-IFN- λ 1; open areas, thin lines; panels E, F, G and H) or the pEF-FL-IFN- λ 2 (FL-IFN- λ 2; shaded areas, thin lines; panel I). Specificity binding was demonstrated by ligand binding competition. Cells expressing both chains were co-

incubated with either FL-IFN- λ 1 or FL-IFN- λ 2 (100 ml of conditioned media from COS cells expressing FL-IFN- λ 1/2) and with E. coli produced IFN- λ 1 lacking the FLAG epitope (IFN- λ 1E.coli, 1 mg/ml) (open areas, thin lines; panels H and I). Ligand binding to the cell surface was determined by flow cytometry with anti-FLAG antibody (Sigma) as the primary antibody and fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Santa Cruz) as the secondary antibody. The ordinate represents relative cell numbers, and the abscissa relative fluorescence (logarithmic scale).

FIG. 21B. The hamster cells described in FIG. 21A row 1 were left untreated or treated with various stimuli: conditioned media (100ml) from COS cells transfected with plasmids pEF-FL-IFN- λ 1/2/3 gene (FL-IFN- λ sgene), pEF-FL-IFN- λ 1/2/3 (FL-IFN- λ s), PEF-IFN- λ 1 (IFN- λ 1COS). Numbers 1, 2 and 3 in treatment lanes reflect which IFN- λ protein (IFN- λ 1, IFN- λ 2 and IFN- λ 3, respectively) was used for the treatment. E. coli produced IFN- λ 1 was also evaluated (IFN- λ 1E.coli). Anti-CRF2-4 antibody (1mg) was added to the cells 30 minutes before IFN- λ treatment.

FIG. 22 shows the results of experiments wherein ligand was crosslinked to hamster cells transfected with receptor subunit combinations. Untreated ^{32} P-labeled FL-IFN- λ 1-P ([^{32} P]FL-IFN- λ 1-P) was loaded as a control (lane 1). ([^{32} P]FL-IFN- λ 1-P) was also incubated in DMEM medium with 10% FBS and crosslinking in solution was performed as described (32) (lane 2). The untransfected and transfected hamster cells described in Figure 22 row 1 were incubated with ^{32} P-labeled FL-IFN- λ 1-P with or without addition of a 100-fold excess of unlabeled IFN- λ 1 (competitor), washed,

harvested and crosslinked. The extracted crosslinked complexes were analyzed on 7.5% SDS-PAGE. The molecular weight markers are shown on the left.

FIG. 23A shows western blots, wherein the ability of chimeric FL-IL-10R1/CRF2-12 receptor to activate Stat in response to IL-10 and IFN- α in HT-29 cells was analyzed.

FIG. 23B shows an EMSA gel, wherein modified ISGF3 binding to ISRE in response to IL-10 and IFN- α in HT-29 cells expressing the chimeric FL-IL-10R1/CRF2-12 receptor was analyzed.

FIG 23C shows EMSA gels, wherein A549 cells response to IFN- λ 1 and IFN- α was measured by their ability to modify binding to GAS and ISRE DNA probes as indicated. Positions of Stat DNA-binding complexes in EMSAs are indicated by arrows.

FIG. 23D are flow cytometry graphs showing the level of MHC class I antigen expression in HeLa, HT29 and A549 in response to treatment with conditioned medium from COS cells transfected with plasmid pEF-FL-IFN- λ 1 (FL-IFN- λ 1; shaded areas, thin lines) or treatment with Hu-IFN-a (100 un/ml; open areas, thin lines) as compared to control cells left untreated (open areas, thick lines). The ordinate represents relative cell numbers, and the abscissa relative fluorescence (logarithmic scale).

FIG. 24A and B one of several biological activities of IFN- λ s. FIG. 24A shows cell survival and antiviral protection in response to IFN- λ 1. An equal amount of cells were plated in all wells and treated with two-fold serial dilutions of ligands, as indicated

(rows A and C). Twenty-four hours later the virus was added in all wells except for the first seven wells in row F. Row C shows that HT29 cells exhibit a dose dependent survival response to vesicular stomatitis virus (VSV) in response to administration of increasing IFN- λ 1 concentrations. Row A shows the dose dependent response to IFN- α , a positive control.

FIG. 24B shows the change in IFN- λ s mRNA expression in virus-infected cells. HeLa cells were left untreated (lane 3) or infected with either Dengue virus (DV, lanes 4-6) Sindbis virus (SV, lanes 7-9) or vesicular stomatitis virus (VSV, lanes 10-12). HT29 cells were left untreated (lane 13) or infected with VSV (lanes 14-16). HuH7 cells were left untreated (lane 17) or infected with DV (lanes 18-20) or SV (lanes 21-23). Cells were collected at post-infection times shown in the Figure, RNA was isolated and RT-PCR was performed as described in Materials and Methods. Water was used as a negative control for RT-PCR (lane 2). 1kb ladder was run in lanes 1 and 24.

FIG. 25 is a schematic model of IFN- λ s' interaction with the CRF2-12 receptor system. In one aspect of the invention IFN- λ s are monomers. In another embodiment of the present invention, IFN- λ s are multimers of and between the three members of the IFN- λ family. In another embodiment of the present invention a multimer may contain other members of the cytokine family, including the IFN and IL-10 families of cytokines. A functional IFN- λ receptor complex may include two receptor chains, for example, the unique CRF2-12 (IFN- λ R1) chain and the CRF2-4 (or IL-10R2) chain. The IL-10R2 chain is a shared common chain for at least three receptor complexes, the IL-10, IL-22 and IFN- λ receptor complexes. When expressed alone, neither chain of the

IFN- λ receptor complex is capable of IFN- λ binding. Expression of both chains is required for ligand binding and for assembling of the functional receptor complex. Ligand binding leads to the formation of the heterodimeric receptor complex and to the initiation of a signal transduction cascade. The CRF2-4 chain is associated with Tyk2 (Kotenko, S. V., Izotova, L. S., Mirochnitchenko, O. V., Esterova, E., Dickensheets, H., Donnelly, R. P. & Pestka, S. (2001) *J Biol Chem* 276, 2725-2732 and Levy, D. E. & Garcia-Sastre, A. (2001) *Cytokine Growth Factor Rev* 12, 143-156) and the IFN- λ R1 chain is likely to interact with Jak1. Upon the ligand-induced heterodimerization of IFN- λ receptor chains receptor-associated Jaks crossactivate each other, phosphorylate the IFN- λ R1 intracellular domain and, thus, initiate the cascade of signal transduction events. Stat1, Stat2, Stat3 and Stat5 are activated by IFN- λ leading to activation of biological activities, such as upregulation of MHC class I antigen expression and induction of antiviral protection.

FIG. 26. Antiviral response. (A) Antiviral protection in response to IFN- λ 1 was evaluated on either HT29, A549 or HaCaT cells infected with vesicular stomatitis virus (VSV) using cytopathic effect (CPE) reduction assay performed in 96-well microtiter plate. IFN- α was used as a positive control. Antiviral activity is shown as the amount of IFN- α or IFN- λ 1 for 50% protection of the cells from CPE. (B) Expression of ISRE-controlled genes encoding 2',5'-oligoadenylate synthetase (2',5'-OAS) and MxA protein in response to IFN- α and IFN- λ 1 was evaluated by RT-PCR. Cells were treated for 16 h with indicated ligands, RNA was isolated and subjected to RT-PCR. (C) The hamster cells expressing IFN- λ R1/ λ R1 + IL-10R2 were left untreated or treated

with various stimuli: conditioned media from COS cells containing FL- IFN- α ; IFN- λ 2 (1000 u/ml); and conditioned media from untreated HT29 cells (HT29) or EMCV-infected HT-29 or A549 cells (HT29+EMCV and A549+EMCV). Where indicated STAT1 Ab was added to shift the mobility of the STAT-DNA-binding complexes.

FIG. 27 shows further amino acid sequences for IFN- λ 1, IFN- λ 2 and IFN- λ 3.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides isolated nucleic acid molecules comprising a novel family of cytokines designated IFN- λ , their specific functional receptor complex, and characterization of their signaling and biological activity.

The present invention further provides two receptor proteins, CRF2-12 and IL-10R2 (CRF2-4), that comprise a functional receptor complex for these novel ligands.

The present invention further provides a novel IFN- α/β -independent ligand-receptor system which upon engagement leads to the establishment of an antiviral state.

All references cited herein are incorporated herein by reference in their entirety, including the figures and sequences.

Three closely positioned genes on human chromosome 19 encode distinct but highly homologous proteins which we designated IFN- λ 1, IFN- λ 2 and IFN- λ 3 based to their ability to induce antiviral protection. Expression of these proteins is induced upon viral infection. A receptor complex which is utilized by all three IFN- λ proteins for signaling is a further embodiment of the present invention. This complex is composed of two subunits, a novel receptor designated IFN- λ R1 or CRF2-12, and a second subunit, IL-10R2 or CRF2-4, which is also a shared receptor component for the IL-10 and IL-22 receptor complexes. Binding of IFN- λ s to the receptor complex induces signaling through the Jak-Stat signal transduction pathway, including activation of the ISGF3 transcription complex, the same complex that is activated by signaling through

the IFN- α/β receptor complex. Stat recruitment is largely mediated by the IFN- α 2c intracellular domain (Kotenko, S. V., Izotova, L. S., Mirochnitchenko, O. V., Lee, C. & Pestka, S. (1999) *Proc Natl Acad Sci U S A* 96, 5007-5012 and Prejean, C. & Colamonici, O. R. (2000) *Semin. Cancer Biol* 10, 83-92).

It is interesting to note that there is a Tyr-based motif (Tyr517MetAlaArgStop) at the C-terminus of the IFN- λ R1 intracellular domain which is also present at the end of the IFN- α 2c intracellular domain (Tyr512IleMetArgStop) which is competent in Stat activation during IFN- α signaling ((39); Y. Ge, S.V.K. and S. Pestka unpublished). This motif is also conserved in mouse IFN- λ R1 (TyrLeuValArgStop)³. Thus, the IFN- λ and IFN- α/β signaling pathways at least partially overlap. As a consequence, at least some of their biological activities are similar, including induction of antiviral protection and up-regulation of MHC class I antigen expression. According with their antiviral activity, the expression of IFN- λ s mRNAs is induced by viral infections in several cell lines and in dendritic cells in response to polyI:C treatment.

The signal transduction pathways and biological activities of IFN- λ s and IFN- α overlap. In another embodiment of the present invention, an IFN- λ . or a combination of IFN- λ s may be used as a substitute, or in combination with, IFN- α . In another aspect of the present invention, IFN- λ s can synergize with IFN- α in mediating resistance to viral infections (or plays an independent primary role in establishment of resistance to certain viruses), and other conditions for which IFN- α is effective.

In another embodiment of the present invention, an IFN- λ or a combination of IFN- λ s may be used to treat, ameliorate or prevent conditions for which IFN- α is not effective, for example, viral infections of the gastrointestinal tract.

A new receptor protein designated as "CRF2-12", one possible nucleotide sequence which can encode the complete CRF2-12 protein is Figure 1 and the complete polypeptide CRF2-12 has the amino acid sequence in Figure 2. Automated analysis of the protein sequence using the "PIX" package (<http://www.hgmp.mrc.ac.uk>) revealed the presence of the following features in the CRF2-12 polypeptide: region a) a leader sequence/signal peptide comprising residues 1-20 or thereabouts; region b) an extracellular region between residues 20-228 or thereabouts; region c) a transmembrane region between residues 228-244 or thereabouts; and region d) an intracellular region/cytoplasmic tail region between residues 244-520 or thereabouts. The CRF2-12 gene is expressed in spleen, placenta and other tissues and is located on human chromosome 1, between 20.2 million bases and 20.4 million bases, adjacent to and telomeric of the gene for IL22R1 receptor chain, in the cytogenic region 1p36.11-1p36.12, as shown in Figure 3. The present invention provides methods for detecting genetic abnormalities in patients, developing knock-out mice or transgenically altered mice, see Figures 12 and 13, treatment of a variety of disorders including but not limited to inflammatory disorders, malignant disorders and disorders associated with autoimmunity. In addition, the invention includes diagnostic kits comprising several antibodies such as ELISA kits, or 'in situ hybridization'. Finally, the invention includes

therapeutic agents that comprise complementary cDNA, RNA sequences or variants thereof.

The 208 amino acid polypeptide fragment of CRF2-12 region b shares significant identity and similarity with the corresponding extracellular portion of members of the class-II cytokine receptor family. As illustrated in Figure 4, marked similarity exists between the extracellular portion of CRF2-12 and that of IL22R1. The 208 amino polypeptide fragment of CRF2-12 region b therefore represents the extracellular domain of a new member of the class-II cytokine receptor family. The similarity between CRF2-12 and other members of the IL-10 receptor family is shown in Figure 5, while its broader relationship with other members of the class-II cytokine receptor family is shown in Figure 6.

As used herein, unless otherwise specified, the term cytokine includes proteins which are variously termed “interferons”, “interleukins”, “colony-stimulating factors”, “tumor necrosis factors” and so on. Indeed, these terms are frequently used interchangeably with the term cytokine. Cytokines are known to elicit biological activities via binding to specific membrane-bound receptors.

As used herein, unless otherwise specified, the term Extracellular Domains includes those of the receptors composed of one or more proteins, which have proteins outside the cell.

As used herein, unless otherwise specified, the term Transmembrane Domains includes those of the receptors composed of one or more proteins, which extend through the cell membrane.

As used herein, unless otherwise specified, the term Intracellular Domains includes those of the receptors composed of one or more proteins, which extend into the cytoplasm of the cell.

The present invention describes and defines isolated polypeptides of greater than or equal to 70% identity to the amino acid reference sequence revealed in Figure 2, covering both the whole polypeptide and specific regions "a", "b", "c" and "d" as defined below.

Region "a", to be residues 1-19, 1-20, 1-21 and/or 1-22, and may be termed herein the signal sequence.

Region "b" to be residues 19-226, 19-227, 19-228, 19-230, 20-226, 20-227, 20-228, 20-229, 20-230, 21-226, 21-227, 21-228, 21-229, 21-230, 22-226, 22-227, 22-228, 22-229, 22-230, and may be termed herein the extracellular domain.

Region "c" to be residues 226-242, 226-243, 226-244, 226-245, 226-246, 227-242, 227-243, 227-244, 227-245, 227-246, 228-242, 228-243, 228-244, 228-245, 228-246, 229-242, 229-243, 229-244, 229-245, 229-246, 230-242, 230-243, 230-244, 230-245, 230-246, and may be termed herein the transmembrane domain.

Region "d" to be residues 242-520, 243-520, 244-520, 245-520, 246-520, and may be termed herein the intracellular domain.

The present invention also describes and defines isolated polypeptides comprising at least 12 contiguous amino acids of the polypeptide sequence shown in Figure 2, selected from groups "a", "b", "c" and/or "d".

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

In the present invention, a "secreted" protein of the present invention refers to a protein capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as a proteins of the present invention released into the extracellular space without necessarily containing a signal sequence. If the secreted proteins of the present invention is released into the extracellular space, the secreted proteins of the present invention can undergo extracellular processing to

produce a "mature" proteins of the present invention. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

A "membrane" associated polypeptide of the present invention may be utilized as a polypeptide integrated in a lipid membrane, such as a membrane-bound polypeptide, an intracellular polypeptide expressed in the cell's secretory pathway, a polypeptide expressed in the plasma membrane at the cell surface or as a polypeptide integrated synthetically into membrane-like structures such as in liposomes or micelles.

The polynucleotides of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotides can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. The polynucleotides of the present invention may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

Polypeptides of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isoesters, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides of the present invention may be modified by either natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in the polypeptides of the present invention, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in the polypeptides of the present invention. Also, the polypeptides of the present invention may contain many types of modifications. Polypeptides of the present invention may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides of the present invention may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation,

prenylation, racemization, selenoylation, sulfation. transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS--STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

Polypeptides of the present invention "having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptides of the present invention (e.g., anti-viral activity, ability to bind an antibody which binds a polypeptide of the present invention, ability to bind to CRF2-12, or as described in the example section herein), including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptides of the present invention, but rather substantially similar to the dose-dependence in the activity as compared to the polypeptides of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptides of the present invention.)

"Regulatory regions" is intended to include promoters, enhancers and/or other expression control elements (e.g., polyadenylation signals), which may be in any effective relationship with the coding sequence. Such regulatory sequences are

described, for example, in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc.

IFN- λ s are expressed mainly in all cells, and particularly in dendritic cells natural cytokine producing cells. Expression of the proteins and polypeptides of the present invention may be regulated in any effective manner. For example, by double stranded RNA, anti-sense technology, as well as cytokines and effective small molecules.

By upregulating the level of CRF2-12 expression in virus-infected cells or in tumor cells we can force these cells undergo apoptosis or antiproliferative effect in response to IFN- λ s. Enhanced expression of CRF2-12 can be, perhaps, induced by some agents, or simply achieved by targeted delivery of CRF2-12 expression construct into virus- or other pathogen-infected cells, or malignant cells. CRF2-12 expression can be replaced by expression of a chimeric receptor, such as IL-10R1/CRF2-12 described above. The extracellular domain of such chimeric receptor can be substituted by the extracellular domain of various receptors. If such chimeric receptors are expressed in target cells (malignant cells or pathogen-infected cells), these cells can be killed by the treatment with the appropriate ligand which binds to the extracellular domain of the

chimeric receptor and induce signaling through the CRF2-12 intracellular domain. Some cancers express autocrine growth factors to maintain cancer growth, or express certain cytokines such as IL-10 to suppress the immune system. Thus, by using the extracellular domain of an appropriate receptor in combination with the intracellular domain of CRF2-12, cancer cells expressing such chimeric receptor can be killed by their own growth factors or cytokines. The extracellular domain of IL-10R1 seems to be most useful since many cancers, particularly advanced cancers, produce high level of IL-10.

Therapeutic Indications

The first embodiment is the use of IFN- λ s to induce antiviral protection to treat viral infections in both humans and other mammals. Such protection may be induced for the treatment of an ongoing infection or for prophylaxis of anticipated infections, including but not limited to common recurring infections such as influenza, and circumstances requiring emergency prophylaxis, such as a bioweapon attack.

Another embodiment is the use of IFN- λ s to induce apoptosis to treat hyperproliferative disorders in both humans and other mammals. And in addition, to withdraw interferon lambda in order to prevent apoptosis, for the treatment of hypoproliferative disorders in humans and other mammals. It is understood that the stated administration or withdrawal of IFN lambda also included other methods of manipulation of the IFN lambda/receptor signaling mechanism which have similar effects to direct ligand manipulation. Such means might include, but are not limited to antibody moieties or other binding structures which bind the receptor, mimicking or

blocking ligand effects and or interference with downstream signaling events resulting from receptor engagement. It will be realized that hypo and hyper proliferative disorders will include all human malignancies and other overgrowths such as benign tumors and cysts in addition to disease causing or promoting systems which include the over-growth or under-growth of one or more individual cell types. Such systems may include any and all disorders of the immune system, including autoimmune diseases, the digestive system, the haematopoietic system, the nervous and neuronal system, the cardiovascular system, the respiratory system, tooth eruption and other such bodily mechanisms, such as growth and development of fetuses and juveniles where the natural, regular and programmed selective growth and death of cells may become disrupted.

A further embodiment is the control of the glycosylation of IFN- λ 1 as a target for drug development to induce either an antiviral effect or induce cell death to treat either humans or other mammals. Other mechanisms for manipulating the affinity or selective action of interferon lambda, such as changing of one or more amino acids of the IFN-lambda peptide sequence, or insertion or removal of short peptide elements to/from the natural IFN-lambda sequence are also envisaged.

Another embodiment is the use of IFN- λ s to upregulate MHC class I antigen expression and, thus, stimulate immune response against viruses, other pathogens and tumors in both humans and other mammals.

A final embodiment is the use of novel sequences in gene therapy to treat either humans or other mammals, including but not limited to the substitution of interferon lambda sequences from other mammals to the human sequence in order to manipulate

the ligand affinity for its natural receptor, or to induce therapeutic signaling or blocking of an unrelated receptor. Furthermore, the construction of chimeric molecules, comprised of IFN lambda sequences and peptide sequenced from human or other mammalian cytokines, growth factors etc for the purpose of therapy, research and/or teaching is also envisaged.

The present invention also includes variations of CRF2-12. It will be recognized by someone trained in the art that mutations can exist in a peptide sequence, which do not affect the function of the protein. Often, these occur naturally but can be produced artificially by substituting one or more amino acid residues for the natural ones. Such so-called "conservative" substitutions include but are not limited to; the substitution of one acidic amino acid for another (e.g. Aspartic acid for glutamic acid), one non-polar amino acid for another (e.g. Glycine for alanine), one hydrophobic amino acid for another (e.g. Leucine for isoleucine), one basic amino acid for another (e.g. Lysine for arginine), and so on. Examples of amino acids in similar groups are widely known and accepted in the field. Such conservative changes in the peptide sequence therefore fall within the scope of this invention. It will be further recognized by someone trained in the art that mutations, resulting or created as above, can exist in a peptide sequence which alter the function of a protein and/or its ability to interact with other proteins. In particular, the mutation may alter the ability of the receptor to bind a natural ligand and/or alter the consequences of ligand binding by preventing the receptor from transmitting a signal or by enhancing the signal. Such non-conservative changes fall within the scope of this invention and are included in it.

The present invention also describes and defines isolated nucleic acid molecules, which encode the CRF2-12 polypeptides, its fragments and mutants. An illustrative nucleic acid sequence which can encode CRF2-12 is given in Figure 1. This nucleic acid sequence will encode all the CRF2-12 fragments and peptides described in regions "a", "b", "c" and "d". Translated products of nucleic acid sequences are usually quoted in a single-letter code, as shown in Figure 2, and Figure 7 illustrates the translation of the nucleic acid sequence revealed in Figure 1. An alternative, three-letter, notation is sometimes used to describe the translation of nucleic acid sequence and this is shown in Figure 8, for CRF2-12.

As is widely recognized in the art, there is redundancy in the translation of nucleic acid sequences. Often, several triplet codons may be translated to the identical amino acid. The present invention presupposes that natural variation at any of the triplet codons may result in a nucleic acid sequence which varies at one or more positions from the illustrative sequence revealed in Figure 1 will still result in an identical translation product to that revealed in Figure 2, indeed that such variations are not only possible but likely, especially between individuals from different ethnic backgrounds. Such redundancy will also apply to the natural or man-made conservative and/or non-conservative amino-acid changes referred to above. The IUPAC convention for writing nucleic acid sequences allows for the use of single letters which may define two or more nucleotides, in addition to the four unique nucleotides (A, C, G, T). These are widely known in the art. Figure 9, reveals the single-letter nucleic acid sequence, which fully describes and defines the possible extent of variation in a gene encoding CRF2-12,

while Figure 10 expands this to show how the redundant code defines possible individual codons.

As is widely recognized in the art, portions of genes which encode peptides (exons) are usually separated from one another by intervening, non-coding nucleic acid sequences (introns). Such introns are integral parts of the gene. The coding sequence for CRF2-12 revealed in Figure 1 is distributed within at least seven exons and six intervening introns. The present invention incorporates these exons and introns to form part of the gene describing CRF2-12. Also widely recognized is the fact that following the coding portion of a gene comes a non-coding portion, which is a part of the last exon, the so-called "3'UTR". The present invention describes and defines the 3'UTR for the CRF2-12 gene and this is revealed in Figure 11.

As is widely recognized in the art, multi-exon genes, such as the gene for CRF2-12, may exist as splice variants, where one or more coding exons are missing, resulting in a shorter translation product, or added, resulting in a translation product which can be either longer or shorter. The present invention provides for the naturally-occurring presence of such splice variants and incorporates them. In its primary form, the gene product CRF2-12 is expected to be found embedded within the membranes of cells. However, it will be recognized by those knowledgeable in the art that splice variants can occur which exclude the transmembrane and intracellular portions of the protein, resulting in a protein which is freed into the extracellular milieu. Such variants would result in a soluble CRF2-12 and so soluble CRF2-12 is included in the scope of this invention.

As is widely recognized in the art, individuals may vary in one or more nucleotide residues within a given gene, such as the CRF2-12 gene. Such natural variations are often referred to as “allelic variants.” These variants are useful for the prediction and monitoring of diseases, particularly diseases involving CRF2-12 or adjacent genes.

As is common practice in the art, cell-bound receptor structures similar in nature to CRF2-12, are often produced from an artificially constructed polynucleotide, such that the product of said nucleotide comprises the specific invention (in this case the extracellular portion of CRF2-12 or parts thereof) operationally and functionally joined to a protein or protein fragment with the property of improving the pharmacokinetics of the specific invention. A molecule frequently used in this way is the so-called Fc region of human immunoglobulin, resulting in a chimeric molecule (in this case CRF2-12 and Fc) encoded from a single polynucleotide. An example of this in clinical use today is the hybrid TNF(p75) receptor / Fc molecule. The present invention anticipates the use of the CRF2-12 gene for the construction of such chimeric polynucleotides, which are thereby incorporated in the scope of the invention.

As is common knowledge in the art, cell-surface receptors of the CRF family, such as the interleukin-10 receptor, the interleukin-20 receptor and the interleukin-22 receptor exist functionally as two or more individual polypeptide chains. Furthermore, it will be appreciated that synthetic chimeric polynucleotides can be produced which encode the extracellular portions of one or more of the known members of the CRF family (including, but not limited to: interleukin-10 receptor alpha, interleukin-10

receptor beta, interleukin-20 receptor alpha, interleukin-20 receptor beta, interleukin-22 receptor alpha, interferon gamma receptor components, interleukin alpha/beta receptor components) in association with CRF2-12. Such chimeric polypeptides might represent the natural specificity of CRF2-12 or a novel specificity which depend upon the presence of the CRF2-12 polypeptide. The present invention therefore incorporates such chimeric molecules and their resultant specificities where, in addition to CRF2-12 said chimeric polypeptide contains sequences from one or more other members of the CRF family and constitute novel, soluble receptor entities. It is anticipated that such novel soluble receptor entities would find use in the treatment of diseases described previously.

The present invention, CRF2-12 and its various embodiments, constitute a receptor for IFN- λ . The receptor nature of CRF2-12 will allow other ligands to be identified by processes commonly used in the art. For example, one aspect of the present invention includes a method of screening for CRF2-12 ligands which includes the steps of binding a polypeptide including CRF2-12 to a solid, inert matrix and passing over the resultant complexes a biological mixture which contains a potential CRF2-12 ligand, whereby the ligand is retained and then harvesting the ligand in a purified form using methods well-established in the art (such as ion-exchange) and identified. Since the identification of CRF2-12 ligand is wholly dependent upon the present invention, CRF2-12 ligands are therefore included in the scope of the present invention.

Polypeptides and Fragments

The invention further provides an isolated IFN- λ 1 polypeptide having the amino acid sequence encoded by pEF-FL-IFN- λ 1 gene, an isolated IFN- λ 1 polypeptide having the amino acid sequence encoded by pEF-FL-IFN- λ 1, an isolated IFN- λ 1 polypeptide having the amino acid sequence in SEQ ID NO: 8, or a peptide or polypeptide comprising a portion of the above polypeptides.

The invention further provides an isolated IFN- λ 2 polypeptide having the amino acid sequence encoded by pEF-FL-IFN- λ 2 gene, an isolated IFN- λ 2 polypeptide having the amino acid sequence encoded by pEF-FL-IFN- λ 2, an isolated IFN- λ 2 polypeptide having the amino acid sequence in SEQ ID NO: 10, or a peptide or polypeptide comprising a portion of the above polypeptides.

The invention further provides an isolated IFN- λ 3 polypeptide having the amino acid sequence encoded by pEF-FL-IFN- λ 3 gene, an isolated IFN- λ 3 polypeptide having the amino acid sequence encoded by pEF-FL-IFN- λ 3, an isolated IFN- λ 3 polypeptide having the amino acid sequence in SEQ ID NO: 12, or a peptide or polypeptide comprising a portion of the above polypeptides.

The invention further provides an isolated polypeptide comprising an amino acid sequence of the full-length IFN- λ 1 polypeptide having the complete amino acid sequence of SEQ ID NO: 8. The invention further provides an isolated polypeptide comprising the amino acid sequence of the full-length IFN- λ 1 polypeptide having the complete amino acid sequence of SEQ ID NO: 8 excepting the N-terminal methionine

(i.e., residues 2-200 of SEQ ID NO: 8). The invention further provides an isolated polypeptide comprising the amino acid sequence of the mature IFN- λ 1 polypeptide shown as residues 23-200 of SEQ ID NO: 8. The invention further provides an isolated polypeptide comprising an IFN- λ 1 polypeptide encoded by the genomic fragment encoding the IFN- λ 1 gene contained in plasmid pEF-FL-IFN- λ 1 gene. The invention further provides an isolated polypeptide comprising an IFN- λ 1 polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 1. The invention further provides an isolated polypeptide comprising a mature IFN- λ 1 polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 1. The polypeptides of the present invention also include polypeptides having an amino acid sequence at least 80% identical, more preferably at least 90% identical, and still more preferably 95%, 96%, 97%, 98% or 99% identical to those described above, as well as polypeptides having an amino acid sequence with at least 90% similarity, and more preferably at least 95% similarity, to those above.

The invention further provides an isolated polypeptide comprising the IFN- λ 2 polypeptide having the complete amino acid sequence of SEQ ID NO: 10. The invention further provides an isolated polypeptide comprising the IFN- λ 2 polypeptide having the complete amino acid sequence of SEQ ID NO: 10 excepting the N-terminal methionine (i.e., residues 2-196 of SEQ ID NO: 10). The invention further provides an isolated polypeptide comprising the mature IFN- λ 2 polypeptide shown as residues 23-196 of SEQ ID NO: 10. The invention further provides an isolated polypeptide comprising the IFN- λ 2 polypeptide shown as residues M12-196 of SEQ ID NO: 10.

The invention further provides an isolated polypeptide comprising the IFN- λ 2 polypeptide shown as residues A13-196 of SEQ ID NO: 10. The invention further provides an isolated polypeptide comprising the mature IFN- λ 2 polypeptide shown as residues P31-196 of SEQ ID NO: 10. The invention further provides an isolated polypeptide comprising the IFN- λ 2 polypeptide shown as residues M92-196 of SEQ ID NO: 10. The invention further provides an isolated polypeptide comprising the IFN- λ 2 polypeptide shown as residues A93-196 of SEQ ID NO: 10. The invention further provides an isolated polypeptide comprising the mature IFN- λ 2 polypeptide shown as residues P113-196 of SEQ ID NO: 10. The invention further provides an isolated polypeptide comprising a IFN- λ 2 polypeptide coded by plasmid pEF-FL-IFN- λ 2gene. The invention further provides an isolated polypeptide comprising a polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 2. The invention further provides an isolated polypeptide comprising a mature polypeptide encoded by the cDNA contained in clone pEF-FL-IFN- λ 2. The polypeptides of the present invention also include polypeptides having an amino acid sequence at least 80% identical, more preferably at least 90% identical, and still more preferably 95%, 96%, 97%, 98% or 99% identical to those described above, as well as polypeptides having an amino acid sequence with at least 90% similarity, and more preferably at least 95% similarity, to those above.

The invention further provides an isolated polypeptide comprising a IFN- λ 3.1 polypeptide having the complete amino acid sequence of SEQ ID NO: 12. The invention further provides an isolated polypeptide comprising a IFN- λ 3 polypeptide

having the complete amino acid sequence of SEQ ID NO: 12 excepting the N-terminal methionine (i.e., residues 2-196 of SEQ ID NO: 12). The invention further provides an isolated polypeptide comprising a mature IFN- λ 3 polypeptide shown as residues P23-196 of SEQ ID NO: 12. The invention further provides an isolated polypeptide comprising a IFN- λ 3 polypeptide shown as residues M6-196 of SEQ ID NO: 12. The invention further provides an isolated polypeptide comprising a IFN- λ 3 polypeptide shown as residues M12-196 of SEQ ID NO: 12. The invention further provides an isolated polypeptide comprising a IFN- λ 3 polypeptide shown as residues P7-196 of SEQ ID NO: 12. The invention further provides an isolated polypeptide comprising a IFN- λ 3 polypeptide shown as residues A13-196 of SEQ ID NO: 12. The invention further provides an isolated polypeptide comprising a mature IFN- λ 3 polypeptide shown as residues P31-196 of SEQ ID NO: 12. The invention further provides an isolated polypeptide comprising a IFN- λ 3 polypeptide coded by plasmid pEF-FL-IFN- λ 3 gene. The invention further provides an isolated polypeptide comprising a polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 3. The invention further provides an isolated polypeptide comprising a mature polypeptide encoded by the cDNA contained in clone pEF-FL-IFN- λ 3. The polypeptides of the present invention also include polypeptides having an amino acid sequence at least 80% identical, more preferably at least 90% identical, and still more preferably 95%, 96%, 97%, 98% or 99% identical to those described above, as well as polypeptides having an amino acid sequence with at least 90% similarity, and more preferably at least 95% similarity, to those above.

An additional embodiment of this aspect of the invention relates to a peptide or polypeptide which comprises the amino acid sequence of an epitope-bearing portion of a polypeptide of the present invention having an amino acid sequence described above. Peptides or polypeptides having the amino acid sequence of an epitope-bearing portion of a polypeptide of the invention include portions of such polypeptides with at least six or seven, preferably at least nine, and more preferably at least about 30 amino acids to about 50 amino acids, although epitope-bearing polypeptides of any length up to and including the entire amino acid sequence of a polypeptide of the invention described above also are included in the invention.

In another embodiment, the invention provides an isolated antibody that binds specifically to a polypeptide having an amino acid sequence described above. The invention further provides methods for isolating antibodies that bind specifically to a polypeptide having an amino acid sequence as described herein. Such antibodies are useful therapeutically as described below.

To improve or alter the characteristics of the polypeptides of the present invention, protein engineering may be employed. In addition, recombinant DNA technology known to those skilled in the art, including, for example, site directed mutagenesis, can be used to create novel mutant proteins or "muteins" including single or multiple amino acid substitutions, deletions, additions or fusion proteins.

Modified polypeptides of the present invention can show, e.g., enhanced or decreased (i.e., blocking) activity or increased stability. In addition, they may be purified in higher yields, show better solubility than the corresponding natural polypeptide, and be more

effective therapeutically, at least under certain purification, storage and administration conditions.

Unless otherwise specified, a "polypeptide fragment" refers to an amino acid having a sequence which is a portion of that contained in SEQ ID NO: 2, SEQ ID NO:8 , SEQ ID NO: 10 or SEQ ID NO: 12, or is encoded by the nucleic acid sequence in plasmids pEF-CRF2-12/ γ R1+CRF2-4, pEF-IL-10R1/IFN- λ R1, pEF-CRF2-12/ γ R1, pEF-FLCRF2-12, pEF-CRF2-12, pEF-CRF2-12/ γ R1, pEF-FL-IFN- λ 1, pEF-FL-IFN- λ 2, pEF-FL-IFN- λ 3, pEF-FL-IFN- λ 1gene, pEF-FL-IFN- λ 2gene or pEF-FL-IFN- λ 3gene.

The protein or polypeptide fragments of the present invention may be complete within itself, or it may be comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region.

Representative examples of polypeptide fragments of the receptor of the present invention include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 20-228, 228-244 or 244-520 or the end of the coding region of SEQ ID NO: 2.

In another aspect of the present invention, differences as well as similarities between sequences may comprise a preferred embodiment of a protein or polypeptide of the present invention. Conserved domains or sequences may indicate that the conserved domain or sequence is indispensable for function. Differences in the amino acid composition of proteins or polypeptides are also an important aspect of the present invention. For example, a difference in an amino acid at a single position may trigger an

unwanted immunological response against a protein or polypeptide. This may occur, for example, when the protein or polypeptide is being used therapeutically. In addition, an amino acid difference may affect the potency or the functionality of the proteins or polypeptides of the present invention.

Representative examples of polypeptide or polypeptide fragments of the IFN- λ s of the present invention include, for example, polypeptides comprising, or alternatively consisting of:

R38-L47 of SEQ ID NO: 8, wherein Q49 may be any polar or charged amino acid, for example K;

F39-L47 of SEQ ID NO: 8, wherein Q49 may be any polar or charged amino acid, for example K;

S49-E58 of SEQ ID NO: 8, wherein S49 may be any amino acid having a small side chain, i.e., G, A, S, C, or the like, preferably S or A and most preferably S, R54 may be any charged amino acid, i.e., D, E, K or R, preferably R or K, and most preferably R, L57 may be any hydrophobic amino acid, i.e., A, V, F, P, M, I or L, preferably L or I, most preferably L;

F50-E59 of SEQ ID NO: 8, wherein R54 may be any charged amino acid, i.e., D, E, K or R, preferably R or K, and most preferably R, L57 may be any hydrophobic amino acid, i.e., A, V, F, P, M, I or L, preferably L or I, most preferably L, E59 may be any charged amino acid, i.e., D, E, K or R, preferably E or K, most preferably E;

A90-K100 of SEQ ID NO: 8, wherein E92 may be any charged amino acid, i.e., D, E, K or R, preferably E or Q, and most preferably E, L95 may be any hydrophobic amino acid, i.e., A, V, F, P, M, I or L, preferably L or V, most preferably L;

W149-A156 of SEQ ID NO: 8;

G163-T170 of SEQ ID NO: 8;

N172-K182 of SEQ ID NO: 8;

An exact match for W77-L191 of SEQ ID NO: 8;

An exact match for W149-L191 of SEQ ID NO: 8;

An exact match for C34-T170 of SEQ ID NO: 8;

An exact match for C34-T178 of SEQ ID NO: 8;

An exact match for C34-T200 of SEQ ID NO: 8;

An exact match for W77-T170 of SEQ ID NO: 8;

An exact match for W77-T178 of SEQ ID NO: 8;

An exact match for W77-T200 of SEQ ID NO: 8;

An exact match for W149-T170 of SEQ ID NO: 8;

An exact match for W149-T178 of SEQ ID NO: 8;

An exact match for W149-T200 of SEQ ID NO: 8;

C37-E61 of SEQ ID NO: 10, wherein Q48 may be any polar or charged amino acid, for example D, E, K, R, S, T, Y, H, C, N, Q, W, preferably Q or K, most preferably, Q, R55 may be any charged amino acid, i.e., D, E, K or R, preferably R or K, most preferably R, L60 may be any hydrophobic amino acid, i.e., A, V, F, P, M, I or L, preferably L or I, most preferably L;

C37-E62 of SEQ ID NO: 10, wherein Q48 may be any polar or charged amino acid, for example D, E, K, R, S, T, Y, H, C, N, Q, W, preferably Q or K, most preferably, Q, R55 may be any charged amino acid, i.e., D, E, K or R, preferably R or K, most preferably R, L60 may be any hydrophobic amino acid, i.e., A, V, F, P, M, I or L, preferably L or I, most preferably L, E62 may be any charged amino acid, i.e., D, E, K or R, preferably E or K, most preferably E;

W80-P91 of SEQ ID NO: 10, wherein R83 may be any charged amino acid, i.e., D, E, K or R, preferably R or K, most preferably R, R88 may be any charged amino acid, i.e., D, E, K or R, preferably R or Q, most preferably R;

W154-A161 of SEQ ID NO: 10, wherein Y156 may be any polar amino acid, i.e., S, T, Y, H, C, N, Q, W, preferably Y or H, most preferably Y;

K164-T175 of SEQ ID NO: 10, wherein S166 may be any polar amino acid, i.e., S, T, Y, H, C, N, Q, W, preferably S or T, most preferably S;

N177-T183 of SEQ ID NO: 10;

An exact match for C37-T175 of SEQ ID NO: 10;

An exact match for C37-T183 of SEQ ID NO: 10;

An exact match for C37-V196 of SEQ ID NO: 10;

An exact match for W80-T175 of SEQ ID NO: 10;

An exact match for W80-T183 of SEQ ID NO: 10;

An exact match for W80-V196 of SEQ ID NO: 10;

An exact match for W154-T175 of SEQ ID NO: 10;

An exact match for W154-T183 of SEQ ID NO: 10;

An exact match for W154-V196 of SEQ ID NO: 10;

C37-E61 of SEQ ID NO: 12, wherein Q48 may be any polar or charged amino acid, for example D, E, K, R, S, T, Y, H, C, N, Q, W, preferably Q or K, most preferably, Q, R55 may be any charged amino acid, i.e., D, E, K or R, preferably R or K, most preferably R, L60 may be any hydrophobic amino acid, i.e., A, V, F, P, M, I or L, preferably L or I, most preferably L;

C37-E62 of SEQ ID NO: 12, wherein Q48 may be any polar or charged amino acid, for example D, E, K, R, S, T, Y, H, C, N, Q, W, preferably Q or K, most preferably, Q, R55 may be any charged amino acid, i.e., D, E, K or R, preferably R or K, most preferably R, L60 may be any hydrophobic amino acid, i.e., A, V, F, P, M, I or L, preferably L or I, most preferably L, E62 may be any charged amino acid, i.e., D, E, K or R, preferably E or K, most preferably E;

W80-P91 of SEQ ID NO: 12, wherein R83 may be any charged amino acid, i.e., D, E, K or R, preferably R or K, most preferably R, R88 may be any charged amino acid, i.e., D, E, K or R, preferably R or K, most preferably R;

W154-A161 of SEQ ID NO: 12, wherein Y156 may be any polar amino acid, i.e., S, T, Y, H, C, N, Q, W, preferably Y or H, most preferably Y;

K164-T175 of SEQ ID NO: 12, wherein S166 may be any polar amino acid, i.e., S, T, Y, H, C, N, Q, W, preferably S or T, most preferably S;

N177-T183 of SEQ ID NO: 12;

An exact match for C37-T175 of SEQ ID NO: 12;

An exact match for C37-T183 of SEQ ID NO: 12;

An exact match for C37-V196 of SEQ ID NO: 12;

An exact match for W80-T175 of SEQ ID NO: 12;

An exact match for W80-T183 of SEQ ID NO: 12;

An exact match for W80-V196 of SEQ ID NO: 12;

An exact match for W154-T175 of SEQ ID NO: 12;

An exact match for W154-T183 of SEQ ID NO: 12;

An exact match for W154-V196 of SEQ ID NO: 12.

In another embodiment the present invention, in any given polypeptide of the present invention, a specific amino acid is an exact match at a particular position. These amino acids distinguish between the forms of IFN- λ , and between allelic variations of a single form.

In another set of embodiments, the following positions, each independently or in combination, is preferred: position 2 of SEQ ID NO: 8 is not T, most preferably it is A, position 4 of SEQ ID NO: 8 is not D, most preferably it is A, position 5 of SEQ ID NO: 8 is not C, most preferably it is W, position 6 of SEQ ID NO: 8 is not M, most preferably it is T, position 7 of SEQ ID NO: 8 is not P, most preferably it is V, position 11 of SEQ ID NO: 8 is not L, most preferably it is T, position 13 of SEQ ID NO: 8 is not A, most preferably it is V, position 14 of SEQ ID NO: 8 is not A, most preferably it is L, position 15 of SEQ ID NO: 8 is not V, most preferably it is G, position 17 of SEQ ID NO: 8 is not T, most preferably it is A, position 19 of SEQ ID NO: 8 is not T, most preferably it is A, position 21 of SEQ ID NO: 8 is not A, most preferably it is P, position 24 of SEQ ID NO: 8 is not V, most preferably it is T, position 25 of SEQ ID NO: 8 is not A, most preferably it is S, position 26 of SEQ ID NO: 8 is not R, most preferably it is K, position 27 of SEQ ID NO: 8 is not A, most preferably it is P, position 28 of SEQ ID NO: 8 is not L, most preferably it is T, position 29 of SEQ ID NO: 8 is not T, most preferably it is P, position 30 of SEQ ID NO: 8 is not D, most preferably it is T, position 31 of SEQ ID NO: 8 is not A, most preferably it is G, position 32 of SEQ ID NO: 8 is not R, most preferably it is K, position of SEQ ID NO: 8 37 is not A, most preferably it is G, position 38 of SEQ ID NO: 8 is not Q, most preferably it is R, position 48 of SEQ

ID NO: 8 is not Q, most preferably it is A, position 52 of SEQ ID NO: 8 is not R, most preferably it is K, position 54 of SEQ ID NO: 8 is not K, most preferably it is R, position 62 of SEQ ID NO: 8 is not L, most preferably it is K, position 65 of SEQ ID NO: 8 is not D, most preferably it is N, position 66 of SEQ ID NO: 8 is not T or C, most preferably it is W, position 67 of SEQ ID NO: 8 is not R or K, most preferably it is S, position 69 of SEQ ID NO: 8 is not R or H, most preferably it is S, position 71 of SEQ ID NO: 8 is not R, most preferably it is P, position 72 of SEQ ID NO: 8 is not L, most preferably it is V, position 75 of SEQ ID NO: 8 is not R, most preferably it is G, position 76 of SEQ ID NO: 8 is not T, most preferably it is N, position 81 of SEQ ID NO: 8 is not Q, most preferably it is L, position 89 of SEQ ID NO: 8 is not M, most preferably it is V, position 105 of SEQ ID NO: 8 is not T or S, most preferably it is A, position 106 of SEQ ID NO: 8 most preferably is A, position 107 of SEQ ID NO: 8 most preferably is G, position 111 of SEQ ID NO: 8 is not V or G, most preferably it is E, position 128 of SEQ ID NO: 8 is not F, most preferably it is L, position 129 of SEQ ID NO: 8 is not R, most preferably it is Q, position 142 of SEQ ID NO: 8 is not A or T, most preferably it is P, position 151 of SEQ ID NO: 8 is not Y, most preferably it is H, position 162 of SEQ ID NO: 8 is not P, most preferably it is A, position 182 of SEQ ID NO: 8 is not N, most preferably it is K, position 183 of SEQ ID NO: 8 is not C, most preferably it is Y, position 186 of SEQ ID NO: 8 is not S, most preferably it is D, position 188 of SEQ ID NO: 8 is not D, most preferably it is N, position 191 of SEQ ID NO: 8 is not V, most preferably it is L, position 192 of SEQ ID NO: 8 most preferably is R, position 193 of SEQ ID NO: 8 most preferably is T, position 194 of SEQ ID NO: 8 most preferably is S, position 195 of SEQ ID NO: 8 most preferably is T, position 196

of SEQ ID NO: 8 most preferably is H, position 197 of SEQ ID NO: 8 most preferably is P, position 198 of SEQ ID NO: 8 most preferably is E, position 199 of SEQ ID NO: 8 most preferably is S, position 200 of SEQ ID NO: 8 most preferably is T.

In another set of embodiments, the following positions, each independently or in combination, is preferred: position 2 of SEQ ID NO: 10 is not A, most preferably it is T; position 3 of SEQ ID NO: 10 is not A, most preferably it is G; position 4 of SEQ ID NO: 10 is not A, most preferably it is D; position 5 of SEQ ID NO: 10 is not W, most preferably it is C; position 6 of SEQ ID NO: 10 is not M, most preferably it is T; position 7 of SEQ ID NO: 10 is not V, most preferably it is P; position 11 of SEQ ID NO: 10 is not T, most preferably it is L; position 12 of SEQ ID NO: 10 is not L, most preferably it is M; position 13 of SEQ ID NO: 10 is not V, most preferably it is A; position 14 of SEQ ID NO: 10 is not L, most preferably it is A; position 15 of SEQ ID NO: 10 is not G, most preferably it is V; position 17 of SEQ ID NO: 10 is not A, most preferably it is T; position 19 of SEQ ID NO: 10 is not A, most preferably it is T; position 21 of SEQ ID NO: 10 is not P, most preferably it is A; position 24 of SEQ ID NO: 10 is not T, most preferably it is V; position 25 of SEQ ID NO: 10 is not S, most preferably it is A; position 26 of SEQ ID NO: 10 is not K, most preferably it is R; position 27 of SEQ ID NO: 10 most preferably is L; position 28 of SEQ ID NO: 10 is not R, most preferably it is H; position 29 of SEQ ID NO: 10 most preferably is G; position 30 of SEQ ID NO: 10 is not P, most preferably it is A; position 31 of SEQ ID NO: 10 is not T, most preferably it is L; position 33 of SEQ ID NO: 10 is not T, most preferably it is D; position 34 of SEQ ID NO: 10 is not G, most preferably it is A;

position 35 of SEQ ID NO: 10 is not K, most preferably it is R; position 40 of SEQ ID NO: 10 is not G, most preferably it is A; position 41 of SEQ ID NO: 10 is not R, most preferably it is Q; position 51 of SEQ ID NO: 10 is not A, most preferably it is Q; position 52 of SEQ ID NO: 10 is not S, most preferably it is A; position 55 of SEQ ID NO: 10 is not K, most preferably it is R; position 57 of SEQ ID NO: 10 is not R, most preferably it is K; position 65 of SEQ ID NO: 10 is not K, most preferably it is L; position 68 of SEQ ID NO: 10 is not N, most preferably it is D; position 69 of SEQ ID NO: 10 is not W, most preferably it is C; position 70 of SEQ ID NO: 10 is not S or K, most preferably it is R; position 72 of SEQ ID NO: 10 is not R or S, most preferably it is H; position 74 of SEQ ID NO: 10 is not P, most preferably it is R; position 75 of SEQ ID NO: 10 is not V, most preferably it is L; position 78 of SEQ ID NO: 10 is not G, most preferably it is R; position 79 of SEQ ID NO: 10 is not N, most preferably it is T; position 84 of SEQ ID NO: 10 is not L, most preferably it is Q; position 92 of SEQ ID NO: 10 is not V, most preferably it is M; position 108 of SEQ ID NO: 10 is not S or A, most preferably it is T; position 110 of SEQ ID NO: 10 most preferably is D; position 111 of SEQ ID NO: 10 most preferably is T; position 112 of SEQ ID NO: 10 is not G, most preferably it is D; position 116 of SEQ ID NO: 10 is not G or E, most preferably it is V; position 133 of SEQ ID NO: 10 is not L, most preferably it is F; position 134 of SEQ ID NO: 10 is not Q, most preferably it is R; position 147 of SEQ ID NO: 10 is not T or P, most preferably it is A; position 156 of SEQ ID NO: 10 is not H, most preferably it is Y; position 167 of SEQ ID NO: 10 is not A, most preferably it is P; position 187 of SEQ ID NO: 10 is not K, most preferably it is N; position 188 of SEQ ID NO: 10 is not Y, most preferably it is C; position 191 of SEQ ID NO: 10 is not D, most preferably it is

S; position 193 of SEQ ID NO: 10 is not N, most preferably it is D; position 196 of SEQ ID NO: 10 is not L, most preferably it is V.

In another set of embodiments, the following positions, each independently or in combination, is preferred: position 2 of SEQ ID NO: 12 is not A, most preferably it is T; position 3 of SEQ ID NO: 12 is not A, most preferably it is G; position 4 of SEQ ID NO: 12 is not A, most preferably it is D; position 5 of SEQ ID NO: 12 is not W, most preferably it is C; position 6 of SEQ ID NO: 12 is not T, most preferably it is M; position 7 of SEQ ID NO: 12 is not V, most preferably it is P; position 11 of SEQ ID NO: 12 is not T, most preferably it is L; position 12 of SEQ ID NO: 12 is not L, most preferably it is M; position 13 of SEQ ID NO: 12 is not V, most preferably it is A; position 14 of SEQ ID NO: 12 is not L, most preferably it is A; position 15 of SEQ ID NO: 12 is not G, most preferably it is V; position 17 of SEQ ID NO: 12 is not A, most preferably it is T; position 19 of SEQ ID NO: 12 is not A, most preferably it is T; position 21 of SEQ ID NO: 12 is not P, most preferably it is A; position 24 of SEQ ID NO: 12 is not T, most preferably it is V; position 25 of SEQ ID NO: 12 is not S, most preferably it is A; position 26 of SEQ ID NO: 12 is not K, most preferably it is R; position 27 of SEQ ID NO: 12 most preferably is L; position 28 of SEQ ID NO: 12 is not H, most preferably is R; position 29 of SEQ ID NO: 12 most preferably is G; position 30 of SEQ ID NO: 12 is not P, most preferably it is A; position 31 of SEQ ID NO: 12 is not T, most preferably it is L; position 33 of SEQ ID NO: 12 is not T, most preferably it is D; position 34 of SEQ ID NO: 12 is not G, most preferably it is A; position 35 of SEQ ID NO: 12 is not K, most preferably it is R; position 40 of SEQ ID

NO: 12 is not G, most preferably it is A; position 41 of SEQ ID NO: 12 is not R, most preferably it is Q; position 51 of SEQ ID NO: 12 is not A, most preferably it is Q; position 52 of SEQ ID NO: 12 is not S, most preferably it is A; position 55 of SEQ ID NO: 12 is not K, most preferably it is R; position 57 of SEQ ID NO: 12 is not R, most preferably it is K; position 65 of SEQ ID NO: 12 is not K, most preferably it is L; position 68 of SEQ ID NO: 12 is not N, most preferably it is D; position 69 of SEQ ID NO: 12 is not W, most preferably it is C; position 70 of SEQ ID NO: 12 is not S or R, most preferably it is K; position 72 of SEQ ID NO: 12 is not H or S, most preferably it is R; position 74 of SEQ ID NO: 12 is not P, most preferably it is R; position 75 of SEQ ID NO: 12 is not V, most preferably it is L; position 78 of SEQ ID NO: 12 is not G, most preferably it is R; position 79 of SEQ ID NO: 12 is not N, most preferably it is T; position 84 of SEQ ID NO: 12 is not L, most preferably it is Q; position 92 of SEQ ID NO: 12 is not M, most preferably it is V; position 108 of SEQ ID NO: 12 is not T or A, most preferably it is S; position 110 of SEQ ID NO: 12 most preferably is D; position 111 of SEQ ID NO: 12 most preferably is T; position 112 of SEQ ID NO: 12 is not G, most preferably it is D; position 116 of SEQ ID NO: 12 is not V or E, most preferably it is G; position 133 of SEQ ID NO: 12 is not F, most preferably it is L; position 134 of SEQ ID NO: 12 is not Q, most preferably it is R; position 147 of SEQ ID NO: 12 is not A or P, most preferably it is T; position 156 of SEQ ID NO: 12 is not H, most preferably it is Y; position 167 of SEQ ID NO: 12 is not A, most preferably it is P; position 187 of SEQ ID NO: 12 is not K, most preferably it is N; position 188 of SEQ ID NO: 12 is not Y, most preferably it is C; position 191 of SEQ ID NO: 12 is not D, most preferably it is

S; position 193 of SEQ ID NO: 10 is not N, most preferably it is D; position 196 of SEQ ID NO: 12 is not L, most preferably it is V.

In another set of embodiments, the positions described supra, may be reversly altered such that one IFN- λ type may more closely resemble another type, or alternatively, an allele.

Polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention.

For many proteins, including the extracellular domain of membrane associated protein or the mature form(s) of a secreted protein, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. However, even if deletion of one or more amino acids from the N-terminus or C-terminus of a protein results in modification of loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete protein generally will be retained when less than the majority of the residues of the complete protein are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Interferon gamma has been reported to show up to ten times higher activities by deleting 8-10 amino acid residues from the carboxy terminus of the protein (Dobeli et al., J. Biotechnology 7:199-216 (1988)). In the present case deletions of C-terminal amino acids of the IFN- λ polypeptides of the present invention may retain some biological activity, such as antiviral activity, modulation of MHC, apoptosis eliciting activity expression or binding to the receptor complex comprising IFN- λ R1.

However, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete protein generally will be retained when less than the majority of the residues of the complete protein are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

The present application is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence encoding the polypeptide described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence.

The present application is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence encoding the IFN- λ

polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence.

Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates an IFN- λ functional activity. By a polypeptide demonstrating an IFN- λ "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) an IFN- λ protein. Such functional activities include, but are not limited to, biological activity (e.g., anti-viral activity, modulation of MHC expression, immunomodulatory activity, regulator of tumor cell growth, ability to induce apoptosis), antigenicity (ability to bind, or compete with an IFN- λ polypeptide for binding, to an anti-IFN- λ antibody), immunogenicity (ability to generate antibody which binds to an IFN- λ polypeptide), ability to bind to and/or activate the receptor complex comprising IFN- λ R1, and the like.

The functional activity of the polypeptides of the present invention, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with a full-length IFN- λ polypeptide for binding to anti-IFN- λ antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or

radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, physiological correlates of IFN- λ binding to its substrates (signal transduction) can be assayed. In addition, assays described herein and otherwise known in the art may routinely be applied to measure the ability of IFN- λ polypeptides and fragments, variants derivatives and analogs thereof to elicit IFN- λ related biological activity (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope of the invention.

It also will be recognized by one of ordinary skill in the art that some amino acid sequences of the IFN- λ polypeptides can be varied without significant effect on the structure or function of the protein.

Thus, the invention further includes variations of the IFN- λ polypeptide which show substantial IFN- λ polypeptide activity or which include regions of IFN- λ protein such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, splice variants and type substitutions selected according to general

rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," Science 247:1306-1310 (1990), wherein the authors indicate that there are two main approaches for studying the tolerance of an amino acid sequence to change. The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection. The second approach uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene and selections or screens to identify sequences that maintain functionality.

Thus, the fragment, derivative or analog of the polypeptides of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12 may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol, or albumin), or (iv) one in which the additional amino acids are fused to the above form of the polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the above form of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Thus, the polypeptides of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation. As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein. Additional variant polypeptides of the present invention include expression variants that enhance secretion or increase the biological activity of the polypeptide of the present invention.

Amino acids in the polypeptides of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244:1081-1085 (1989).)

The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding, in vitro proliferative activity or interferon receptor activation.

Of special interest are substitutions of charged amino acids with other charged or neutral amino acids which may produce proteins with highly desirable improved characteristics, such as less aggregation. Aggregation may not only reduce activity but also be problematic when preparing pharmaceutical formulations, because aggregates can be immunogenic (Pinckard et al., *Clin. Exp. Immunol.* 2:331-340 (1967); Robbins et al., *Diabetes* 36: 838-845 (1987); Cleland et al., *Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377 (1993).)

Replacement of amino acids can also change the selectivity of the binding of a ligand to cell surface receptors. For example, Ostade et al., *Nature* 361:266-268 (1993) describes

certain mutations resulting in selective binding of TNF- α to only one of the two known types of TNF receptors. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith et al., J. Mol. Biol. 224:899-904 (1992) and de Vos et al. Science 255:306-312 (1992)).

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. The polypeptides may be produced, isolated and purified by any effective manner known in the art.

For example, recombinantly produced versions of IFN- λ s and soluble CRF2-12 polypeptides can be substantially purified by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural or recombinant sources using specific antibodies of the invention, described infra, in methods which are well known in the art of protein purification. By "% similarity" for two polypeptides is intended, for example, a similarity score produced by comparing the amino acid sequences of the two polypeptides. This may be done, for example, using the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711) and the default settings for determining similarity. Bestfit uses the local homology algorithm of Smith and Waterman (Advances in Applied Mathematics 2:482-489, 1981) to find the best segment of similarity between two sequences.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of a polypeptide of the invention it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence of the present invention. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the query sequence may be inserted into the subject sequence. These alterations of the subject sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As described in detail below, the polypeptides of the present invention can also be used to raise polyclonal and monoclonal antibodies, which are useful in assays for detecting IFN- λ protein expression and CRF2-12 protein expression, as described below, or as agonists and antagonists capable of enhancing or inhibiting IFN- λ protein function and CRF2-12 receptor or receptor complex function. Further, such polypeptides can be used in the yeast two-hybrid system to "capture" IFN- λ binding proteins, and proteins that bind to the intracellular, extracellular or the transmembrane domain of the CRF2-12 receptor or the receptor complex. Captured proteins are candidate agonists and

antagonists according to the present invention. The yeast two hybrid system is described in Fields and Song, Nature 340:245-246 (1989).

Among the especially preferred polypeptide fragments of the invention are fragments characterized by structural or functional attributes of the proteins and polypeptides of the present invention. Such fragments include amino acid residues that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, surface forming regions, and high antigenic index regions (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program).

Other preferred regions include: Garnier-Robson predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman predicted alpha-regions, beta-regions, and turn-regions; Kyte-Doolittle predicted hydrophilic regions; Eisenberg alpha and beta amphipathic regions; Emini surface-forming regions; Karplus-Schulz predicted flexible regions; and Jameson-Wolf high antigenic index regions, as predicted using the default parameters of these computer programs. Polynucleotides encoding these polypeptides are also encompassed by the invention.

It has been reported that Interferon alpha possesses a wide variety of antiviral, anti-proliferative and immunomodulative biological activities. These multiple activities of interferon are thought to be mediated through interaction with specific cell-surface receptors. The interferon receptor generally is believed to consists of more than one

individual polypeptide component and different parts of the interferon molecule are thought to contribute to certain interferon activities via interaction with distinct chains of the interferon receptor complex. Wang et al., J. Immunol 152:705-715; Uze et al. J. Mol. Biol 243:245-257 (1994). The structure-functional organization of the type 1 interferon molecule, the interferon receptor complex, and the role of distinct receptor chains in signal transduction has been analyzed. Danilkovitch et al. Hybridoma 16:69-75 (1997); Pontzer et al. J. Interferon Res 14: 133-141 (1994); Danilkovich et al., Immunology Letters 31:15-20 (1991). Polypeptide fragments of the present invention, therefore, may be used to mediate antiviral, antiproliferative, apoptotic and immunomodulative biological activities.

Polypeptide fragments from the C-terminus of Interferon-alpha2 exhibit antiproliferative activity on normal human peripheral blood lymphocytes. Epitopes involving amino acids 124-144 of the Interferon-alpha2 molecule are thought to be responsible for receptor binding and for the manifestation of interferon antiproliferative properties.

Danilkovich et al. Immunology Letters, supra. Polypeptide fragments from the carboxy-terminal region of Interferon-tau are thought to be involved in the antiviral activity by a mechanism and specificity shared by alpha Interferons. Pontzer et al. Proc. Natl. Acad. Sci. USA 87:5945-5949 (1990). Accordingly, polypeptide fragments of the present invention may be used as physiological regulators of tumor cell growth, anti-proliferative activity, anti-viral activity apoptotic activity and immunomodulatory activity.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID

NOS: 1-12 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention.

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12.

The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention, polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower stringency hybridization conditions defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human, mouse, rabbit or rat. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein.

Immunospecific binding excludes non-specific binding but does not necessarily exclude cross-reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means.

(See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Pat. No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 11, least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes.

Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et

al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to any effective method, including in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., supra; Wilson et al., supra, and Bittle et al., J. Gen. Virol., 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide. Anti-peptide antibody titer may also be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid.

Peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier-coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant

known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by any effective method. For example, by selection of anti-peptide antibodies by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

The polypeptides of the present invention (e.g., those comprising an immunogenic or antigenic epitope) can be fused to heterologous polypeptide sequences. For example, the polypeptides of the present invention (including fragments or variants thereof) may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. By way of another non-limiting example, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused with albumin (including but not limited to recombinant human serum albumin or fragments or variants thereof (see, e.g., U.S. Pat. No. 5,876,969, issued Mar. 2, 1999, EP Patent 0 413 622, and U.S. Pat. No. 5,766,883, issued Jun. 16, 1998, herein incorporated by reference in their entirety)).

Such fusion proteins as those described above may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988).

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Pat. Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, et al., *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo and Blasco, *Biotechniques* 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety).

Recombinant and Synthetic Production of Polypeptides of the Invention

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of polypeptides or fragments of polypeptides of the present invention by recombinant and synthetic techniques. The vector may be any effective vector. These include, for example, a phage, plasmid, viral or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides of the present invention, or encoding the polypeptides or polypeptied fragments of the present invention may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter. Any effective promoter may be used. These include the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs preferably also contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation in an effective position with respect to the DNA insert of the invention. The coding portion of the transcripts expressed by the constructs will preferably include a

translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Any effective marker may be used. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178)); insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, COS, 293 and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc., supra; pBS vectors, Phagescript vectors, pBluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815

(all available from Invitrogen, Caribad, Calif.). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by any effective means, including naked nucleic acid, use of a gene gun, calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that polypeptides or polypeptide fragments of the present invention may be expressed by a host cell lacking a recombinant vector.

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to stabilize and purify proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various

portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al., *J. Molecular Recognition* 8:52-58 (1995) and K. Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995).

The polypeptide and polypeptide fragments of the present invention may be recovered and purified from recombinant cell cultures in any effective manner. For example, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. For example, see Lin et al., "Purification of Recombinant Human Interferon Beta expressed in *E. coli*" *Methods in Enzymology* 119: 183-192 (1986), which is hereby incorporated by reference in its entirety. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Further methods that may be used for production and isolation of the polypeptides of the present invention are disclosed in U.S. Patent No. 6,433,145.

Polypeptides of the present invention include: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material, and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous

polynucleotides of the present invention. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences of the present invention via homologous recombination, resulting in the formation of a new transcription unit (see, e.g., U.S. Pat. No. 5,641,670, issued Jun. 24, 1997; U.S. Pat. No. 5,733,761, issued Mar. 31, 1998; International Publication No. WO 96/29411, published Sep. 26, 1996; International Publication No. WO 94/12650, published Aug. 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijistra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using any effective technique (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., NY., and Hunkapiller et al., Nature, 310:105-111 (1984)). For example, a polypeptide or a fragment of a polypeptide of the present invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, alpha-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, alpha-Abu, alpha-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine,

phenylglycine, cyclohexylalanine, alpha-alanine, fluoro-amino acids, designer amino acids such as alpha-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

Non-naturally occurring variants may be produced using art-known mutagenesis techniques, which include, but are not limited to oligonucleotide mediated mutagenesis, alanine scanning, PCR mutagenesis, site directed mutagenesis (see, e.g., Carter et al., Nucl. Acids Res. 13:4331 (1986); and Zoller et al., Nucl. Acids Res. 10:6487 (1982)), cassette mutagenesis (see, e.g., Wells et al., Gene 34:315 (1985)), restriction selection mutagenesis (see, e.g., Wells et al., Philos. Trans. R. Soc. London SerA 317:415 (1986)).

The invention encompasses polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄ ; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid

backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Pat. No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For

example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

As noted above, the polyethylene glycol may have a branched structure.

Branched polyethylene glycols are described, for example, in U.S. Pat. No. 5,643,575; Morpurgo et al., *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev et al., *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti et al., *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group

may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation

which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado et al., Crit. Rev. Thera. Drug Carrier Sys. 9:249-304 (1992); Francis et al., Intern. J. of Hematol. 68:1-18 (1998); U.S. Pat. No. 4,002,531; U.S. Pat. No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride (CISO.sub.2 CH.sub.2 CF.sub.3). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Pat. No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting

polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

The number of polyethylene glycol moieties attached to each protein of the invention (i.e., the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado et al., *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

The polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional

embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides having an identical sequence.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (i.e., polypeptides having different sequences). In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Any effective multimer may be formed. For example, in one embodiment of the present invention, IFN- λ s form multimers of and between two of the three members, including, for example, IFN- λ 1/IFN- λ 2, IFN- λ 1/IFN- λ 3 and IFN- λ 2/IFN- λ 3, or multimers with all three members of the IFN- λ family. In another embodiment of the present invention an effective multimer contains other members of the cytokine family, including the IFN and IL-10 families of cytokines.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as,

for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence. In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein of the present invention. In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Pat. No. 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in an -Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequences from another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the

invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

In another example, proteins of the invention are associated by interactions between FLAG polypeptide sequence contained in fusion proteins of the invention containing FLAG polypeptide sequence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in FLAG fusion proteins of the invention and anti-FLAG antibody.

Further methods that may be used for production multimers of the present invention are disclosed in U.S. Patent No. 6,433,145, and are hereby incorporated by reference in their entirety.

Antibodies

The polypeptides of the invention also relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment and/or an epitope of a polypeptide of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding).

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 molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In a specific embodiment, the immunoglobulin molecules of the invention are IgG1. In another specific embodiment, the immunoglobulin molecules of the invention are IgG4.

Antibodies of the invention include, but are 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.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and

fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Pat. Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention that they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues. Preferred epitopes of the invention include those that include a sequence homology between IFN- λ forms and alleles. Also preferred are epitopes that include sequence differences between IFN- λ forms and alleles. These can be ascertained by those of ordinary skill in the art, and are further described in the polypeptide section of the present specification.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a

specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein).

Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 5×10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein, or the ability of the antibody to block the biological activity of the CRF2-12 receptor or receptor complex, the ability to block binding of the polypeptides of the present invention to the CRF2-12 receptor or receptor complex, or the ability of the antibody to block signal transduction from the receptor or receptor complex. The antibodies may be directed either towards the receptor or the IFN- λ polypeptides of the present invention. In preferred embodiments, the antibody competitively inhibits binding to the epitope by

at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies that disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies that do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra, or in the EXAMPLES section infra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as

antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies that activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Pat. No. 5,811,097; Deng et al., *Blood* 92(6):1981-1988 (1998); Chen et al., *Cancer Res.* 58(16):3668-3678 (1998); Harrop et al., *J. Immunol.* 161(4):1786-1794 (1998); Zhu et al., *Cancer Res.* 58(15):3209-3214 (1998); Yoon et al., *J. Immunol.* 160(7):3170-3179 (1998); Prat et al., *J. Cell. Sci.* 111(Pt2):237-247 (1998); Pitard et al., *J. Immunol. Methods* 205(2):177-190 (1997); Liautard et al., *Cytokine* 9(4):233-241 (1997); Carlson et al., *J. Biol. Chem.* 272(17):11295-11301 (1997); Taryman et al., *Neuron* 14(4):755-762 (1995); Muller et al., *Structure* 6(9):1153-1167 (1998); Bartunek et al., *Cytokine* 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al.,

Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO. 92/08495; WO 91/14438; WO 89/12624; U.S. Pat. No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal

antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples. In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention. Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')₂ fragments of the

invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles that carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* 182:41-50 (1995); Ames et al., *J. Immunol. Methods* 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.* 24:952-958 (1994); Persic et al., *Gene* 187 9-18 (1997); Burton et al., *Advances in Immunology* 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409;

5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are

known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Pat. Nos 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species that bind the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al. U.S. Pat. No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; International Publication No. W091/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the

art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice

rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Pat. Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, Calif.) and Genpharm (San Jose, Calif.) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *Bio/technology* 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444; (1989) and Nissinoff, *J. Immunol.* 147(8):2429-2438 (1991)). For

example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

Polynucleotides Encoding Antibodies

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques* 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*. John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from

a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778; Bird, Science 242:423-42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra et al., Science 242:1038-1041 (1988)).

Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4.degree. C., adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4.degree. C., washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a

particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%-20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ^{32}P or ^{125}I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating a surface, for example the well of a 96 well microtiter plate, with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., ^3H or ^{125}I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the

antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., ³H or ¹²⁵I) in the presence of increasing amounts of an unlabeled second antibody.

Diagnosis and Imaging

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical

symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{125}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{112}In), and technetium (^{99}Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled

molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S. W. Burchiel et al. "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S. W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Pat. No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patent using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Kits

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified

antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another embodiment, a kit comprises a set of antibodies that can specifically bind, and thus distinguish between, the IFN- λ 1, IFN- λ 2 and/or the IFN- λ 3 polypeptides of the present invention. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with each antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptides of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another embodiment, a kit comprises a set of antibodies that can specifically bind, and thus distinguish between, alleles of IFN- λ 1, IFN- λ 2 and/or the IFN- λ 3 polypeptides of the present invention. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with each antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptides of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit

may include a recombinantly produced or chemically synthesized polypeptide antigen.

The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound

labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, Mo.).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

Fusion Proteins

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptides of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptides of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on

trafficking signals, the polypeptides can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to the polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptides of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

The polypeptides of the present invention may be fused with heterologous polypeptide sequences. For example, the polypeptides of the present invention may be fused parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), or albumin (including but not limited to recombinant albumin), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the

constant regions of the heavy or light chains of mammalian immunoglobulins (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric polypeptide or polypeptide fragment of the present invention alone (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). Polynucleotides comprising or alternatively consisting of nucleic acids which encode these fusion proteins are also encompassed by the invention

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a

pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, Calif., 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Immunophenotyping

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Pat. No. 5,985,660; and Morrison et al., Cell, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the

screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art.

Therapeutic Uses

The present invention is further directed to antibody-based therapies which involve administering an effective amount of antibodies of the present invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases,

disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., KDI, IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of

disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.

Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO

86/05807; PCT Publication WO 89/01036; and U.S. Pat. No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant

cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic*

Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG

initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., *Methods in Enzymol.* 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, W138, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of

replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgprt- or aprt-cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 (Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev,

Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215); and hygromycin, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical

selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids

of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Pat. No. 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entirety.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Pat. Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT

publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337-11341(1992) (said references incorporated by reference in their entireties).

As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of the polypeptides of the present invention may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to the polypeptides of the present invention may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988)). The polypeptides of the present invention fused or conjugated to an antibody having disulfide-linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug

discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, Calif., 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the FLAG tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and

nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Pat. No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{111}In or ^{99}Tc .

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi . A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not

limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- α , TNF- β , lymphotoxin alpha, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi et al., Int. Immunol., 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"),

granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-5.6 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies 84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Pat. No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Nucleic Acid Molecules

By "nucleotide sequence" of a nucleic acid molecule or polynucleotide is intended, for a DNA molecule or polynucleotide, a sequence of deoxyribonucleotides, and for an RNA molecule or polynucleotide, the corresponding sequence of ribonucleotides (A, G, C and U), where each thymidine deoxyribonucleotide (T) in the specified deoxyribonucleotide sequence is replaced by the ribonucleotide uridine (U).

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells, purified (partially or substantially) DNA molecules in solution and synthetic polynucleotides. Isolated RNA molecules include in vivo or in vitro RNA transcripts of the DNA molecules of the present invention. However, a nucleic acid contained in a clone that is a member of a library (e.g., a genomic or cDNA library) that has not been isolated from other members of the library (e.g., in the form of a homogenous solution containing the clone and other members of the library) or a chromosome isolated or removed from a cell or cell lysate (e.g., a "chromosome spread", as in a karyotype), is not "isolated" for the purposes of

this invention. As discussed further herein, isolated nucleic acid molecules according to the present invention may be produced naturally, recombinantly or synthetically.

The stringency of the hybridization and wash depend primarily on the salt concentration and temperature of the solutions. In general, to maximize the rate of annealing of the probe with its target, the hybridization is usually carried out at salt and temperature conditions that are 20 - 25°C below the calculated T_m of the of the hybrid. Wash conditions should be as stringent as possible for the degree of identity of the probe for the target. In general, wash conditions are selected to be approximately 12 - 20°C below the T_m of the hybrid. In regards to the nucleic acids of the current invention, a moderate stringency hybridization is defined as hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 µg/ml denatured salmon sperm DNA at 42°C, and wash in 2X SSC and 0.5% SDS at 55°C for 15 minutes. A high stringency hybridization is defined as hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 µg/ml denatured salmon sperm DNA at 42°C, and wash in 1X SSC and 0.5% SDS at 6-5°C for 15 minutes. Very high stringency hybridization is defined as hybridization in 6X SSC, SX Denhardt's solution, 0.5% SDS and 100 µg/ml denatured salmon sperm DNA at 42°C, and wash in 0.1 X SSC and 0.5% SDS at 65°C for 15 minutes.

As one of ordinary skill would appreciate, due to the possibilities of sequencing errors discussed above, the actual complete polypeptides of the present invention may be somewhat longer or shorter. More generally, the actual open reading frame may be anywhere in the range of ± 20 amino acids, more likely in the range of ± 10 amino acids,

of that predicted from the methionine codon at the N-terminus shown in (SEQ ID NOS: 1, 3, 5, 7, 9, 11).

The present invention also relates to the genes of the present invention corresponding to SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and/or 12. The genes of the present invention can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the genes of the present invention from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and/or 12, using information from the sequences disclosed herein. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue. See also, for example, U.S. Patent Application Pub. No. 2002 039763, which is incorporated herein by reference in its entirety, including the sequences.

Unless otherwise specified, the term expression vector generally means any effective nucleic acid having a promoter operably linked to the nucleic acid molecule to be expressed and which in its part may be operably linked to an effective transcription

terminator, preferably such that the expression vector effectively expresses the nucleic acid molecule to be expressed given suitable conditions.

The nucleic acid compositions of the present invention can be prepared in any suitable manner.

The polypeptides encoded by the nucleic acid compositions of the present invention may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein. It is often advantageous to include a nucleic acid encoding an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, FLAG sequences, or an additional sequence for stability during recombinant production.

Nucleic acids encoding polypeptides of the present invention, particularly IFN- λ s, soluble receptor polypeptides and antibodies of the present invention, are preferably provided in an isolated form, and preferably are substantially purified.

The present invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a chimeric receptor comprising the extracellular domain of CRF2-12 and the intracellular domain of another membrane bound receptor. The present invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a chimeric receptor comprising the extracellular domain of CRF2-12 and the intracellular domain of another membrane bound tyrosine kinase receptor. The present invention also provides an isolated nucleic acid molecule

comprising a nucleotide sequence encoding a chimeric receptor comprising the extracellular domain of CRF2-12 and the intracellular domain of a cytokine receptor. The present invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a chimeric receptor comprising the extracellular domain of CRF2-12 and the intracellular domain of an IFN R1 type receptor. The present invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a chimeric receptor comprising the extracellular domain of CRF2-12 and the intracellular domain of IFN γ R1. The present invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the chimeric protein in plasmid pEF-CRF2-12/ γ R1. The present invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence complementary to any of these nucleotide sequences.

The present invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a chimeric receptor comprising the intracellular domain of CRF2-12 and the extracellular domain of another membrane bound receptor. The present invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a chimeric receptor comprising the intracellular domain of CRF2-12 and the extracellular domain of another membrane bound tyrosine kinase receptor. The present invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a chimeric receptor comprising the intracellular domain of CRF2-12 and the extracellular domain of a cytokine receptor; a nucleotide sequence encoding a chimeric receptor comprising the intracellular domain

of CRF2-12 and the extracellular domain of an IFN R1 type receptor. The present invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a chimeric receptor comprising the intracellular domain of CRF2-12 and the extracellular domain of IL-10R1. The present invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the chimeric protein in plasmid pEF-IL-10R1/IFN- λ R1. The present invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence complementary to any of these nucleotide sequences.

The present invention also provides an isolated nucleic acid molecule comprising a tandem vector having a nucleotide sequence encoding two receptors, wherein the first receptor is an R1 type receptor and the second receptor is an R2 type receptor, and wherein the expression of each receptor is controlled by separate promoters and polyadenylation signals. The present invention also provides an isolated nucleic acid molecule comprising a tandem vector having a nucleotide sequence encoding two receptors, wherein the first receptor comprises the extracellular domain of CRF2-12 and the second receptor is an R2 type receptor, and wherein the expression of each receptor is controlled by separate promoters and polyadenylation signals. The present invention also provides an isolated nucleic acid molecule comprising a tandem vector having a nucleotide sequence encoding two receptors, wherein the first receptor comprises the intracellular domain of CRF2-12 and the second receptor is an R2 type receptor, and wherein the expression of each receptor is controlled by separate promoters and polyadenylation signal. The present invention also provides an isolated nucleic acid

molecule comprising a tandem vector having a nucleotide sequence encoding two receptors, wherein the first receptor comprises CRF2-12 and the second receptor is an R2 type receptor, and wherein the expression of each receptor is controlled by separate promoters and polyadenylation signals. The present invention also provides an isolated nucleic acid molecule comprising any of the above tandem vectors, wherein the R2 type receptor comprises CRF2-4. The present invention also provides an isolated nucleic acid molecule comprising the tandem vector pEF-CRF2-12/ γ R1+CRF2-4. The present invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence complementary to any of these nucleotide sequences.

The present invention also provides isolated nucleic acid molecules comprising a polynucleotide encoding at least a portion of one of the IFN- λ polypeptides having the complete amino acid sequence shown of SEQ ID NOS: 8, 10 and 12.

The nucleotide sequence determined by sequencing the IFN- λ 1 clone, which is shown in FIG. 16 (SEQ ID NO: 7), contains an open reading frame encoding a full length polypeptide of 200 amino acid residues, including an initiation codon encoding an N-terminal methionine.

The nucleotide sequence determined by sequencing the IFN- λ 2 clone, which is shown in FIG. 17 (SEQ ID NO: 9), contains an open reading frame encoding a full length polypeptide of 196 amino acid residues, including an initiation codon encoding an N-terminal methionine.

The nucleotide sequence determined by sequencing the IFN- λ 3 clone, which is shown in FIG. 18 (SEQ ID NO: 11), contains an open reading frame encoding a full length polypeptide of 196 amino acid residues, including an initiation codon encoding an N-terminal methionine.

The present invention also provides isolated nucleic acid molecules encoding the complete amino acid sequence excepting the N-terminal methionine shown in SEQ ID NOS: 8, 10 and 12, which molecules also can encode additional amino acids fused to the N-terminus of the amino acid sequences of IFN- λ 1, IFN- λ 2 and/or IFN- λ 3.

Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the IFN- λ 1 polypeptide having the complete amino acid sequence of SEQ ID NO: 8. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the IFN- λ 1 polypeptide having the complete amino acid sequence of SEQ ID NO: 8 excepting the N-terminal methionine (i.e., residues 2-200 of SEQ ID NO: 8). Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the mature IFN- λ 1 polypeptide shown as residues 23-200 of SEQ ID NO: 8. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence of the genomic fragment encoding the complete IFN- λ 1 gene contained in plasmid pEF-FL-IFN- λ 1 gene. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the complete polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 1; a nucleotide sequence encoding the mature polypeptide encoded by the human cDNA contained in

plasmid pEF-FL-IFN- λ 1. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence complementary to any of these nucleotide sequences.

Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the IFN- λ 2 polypeptide having the complete amino acid sequence of SEQ ID NO: 10. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the IFN- λ 2 polypeptide having the complete amino acid sequence of SEQ ID NO: 10 excepting the N-terminal methionine (i.e., residues 2-196 of SEQ ID NO: 10). Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the mature IFN- λ 2 polypeptide shown as residues 23-196 of SEQ ID NO: 10. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding IFN- λ 2 polypeptide (residues M12-196 of SEQ ID NO: 10). Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding IFN- λ 2 polypeptide residues A13-196 of SEQ ID NO: 10. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the mature IFN- λ 2 polypeptide shown as residues P31-196 of SEQ ID NO: 10. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding IFN- λ 2 polypeptide (residues M92-196 of SEQ ID NO: 10). Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding IFN- λ 2 polypeptide residues A93-196 of SEQ ID NO: 10. Another aspect of the

invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the mature IFN- λ 2 polypeptide shown as residues P113-196 of SEQ ID NO: 10. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence of the genomic fragment encoding the complete IFN- λ 2 gene contained in plasmid pEF-FL-IFN- λ 2 gene. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 2. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the mature polypeptide encoded by the cDNA contained in clone pEF-FL-IFN- λ 2. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence complementary to any of these nucleotide sequences.

Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the IFN- λ 3 polypeptide having the complete amino acid sequence of SEQ ID NO: 12. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the IFN- λ 3 polypeptide having the complete amino acid sequence of SEQ ID NO: 12 excepting the N-terminal methionine (i.e., residues 2-196 of SEQ ID NO: 12). Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the mature IFN- λ 3 polypeptide shown as residues P23-196 of SEQ ID NO: 12. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding IFN- λ 3 polypeptide (residues M6-196 of SEQ ID NO:

12). Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding IFN- λ 3 polypeptide (residues M12-196 of SEQ ID NO: 12). Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding IFN- λ 3 polypeptide residues P7-196 of SEQ ID NO: 12. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding IFN- λ 3 polypeptide residues A13-196 of SEQ ID NO: 12. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the mature IFN- λ 3 polypeptide shown as residues P31-196 of SEQ ID NO: 12. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence of the genomic fragment encoding the complete IFN- λ 3 gene contained in plasmid pEF-FL-IFN- λ 3 gene. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 3. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a mature polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 3. Another aspect of the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence complementary to any of these nucleotide sequences.

The invention also provides isolated nucleic acid molecules comprising a polynucleotide encoding at least a portion of one of the IFN- λ polypeptides described in the "Polypeptides" section of the present disclosure.

Further embodiments of the invention include isolated nucleic acid molecules that comprise a polynucleotide having a nucleotide sequence at least 80%, 85%, or 90% identical, more preferably at least 91%, 92%, 93%, and 94% and most preferably at least 95%, 96%, 97%, 98% or to any of the nucleotide sequences, above, or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide of the present invention. This polynucleotide of the present invention, which hybridizes under stringent conditions does not hybridize to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues. An additional nucleic acid embodiment of the invention relates to an isolated nucleic acid molecule comprising a polynucleotide that encodes the amino acid sequence of an epitope-bearing portion of a polypeptide having an amino acid sequence described above.

A further aspect of the invention is a DNA sequence that represents the complete upstream and downstream regulatory regions of the genes of the present invention. DNA constructs containing the regulatory region are also provided. Further, host cells comprising such constructs, which cells are in vitro or in vivo, are also encompassed by the present invention.

The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of polypeptides or peptides of the present invention by recombinant techniques.

Leader and Mature Sequences

The amino acid sequence of the complete proteins of the present invention includes a leader sequence and a mature protein, as shown in FIGS. 14A and 19A. Accordingly, the present invention provides nucleic acid molecules encoding a mature form of the proteins of the present invention having the polypeptide sequence of SEQ ID NOS:2, 6, 8, 10 and/or 12. Polynucleotides encoding the mature forms are also encompassed by the invention.

According to the current understanding in the art, once export of a growing protein chain across the rough endoplasmic reticulum has been initiated, proteins secreted by mammalian cells have the signal or secretory leader sequence cleaved from the complete polypeptide to produce a secreted "mature" form of the protein. In some instances, proteins having a signal or leader sequence may be retained intracellularly or at the cell surface. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequences encoding mature polypeptides of the present invention having the amino acid sequence encoded by the human cDNA in clones pEF-FL-IFN- λ 1, pEF-FL-IFN- λ 2 and/or pEF-FL-IFN- λ 3 and genomic fragments encoded by pEF-FL-IFN- λ 1 gene, pEF-FL-IFN- λ 2 gene and/or pEF-FL-IFN- λ 3 gene. By the "mature polypeptides" of the present invention having the amino acid sequence encoded by the

human cDNA in clones pEF-FL-IFN- λ 1, pEF-FL-IFN- λ 2 and/or pEF-FL-IFN- λ 3 and genomic fragments encoded by pEF-FL-IFN- λ 1 gene, pEF-FL-IFN- λ 2 gene and/or pEF-FL-IFN- λ 3 gene is meant the mature form(s) of the proteins of the present invention produced by expression in a mammalian cell (e.g., COS cells, as described below) from the open reading frame encoded by the human DNA sequence of the clone contained in the vector or a portion of the DNA sequence of the clone contained in the vector fused to a heterologous signal sequence.

Methods for predicting whether a protein has a secretory leader as well as the cleavage point for that leader sequence are available. For instance, the method of McGeoch (Virus Res. 3:271-286 (1985)) uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje (Nucleic Acids Res. 14:4683-4690 (1986)) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2 where +1 indicates the amino terminus of the mature protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80% (von Heinje, supra). However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, or in the form of DNA. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the genes of the present invention of interest in the genome).

In addition, isolated nucleic acid molecules of the invention include DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encodes a polypeptides of the present invention of the present invention. Of course, the genetic code and species-specific codon preferences are well known in the art. Thus, it would be routine for one skilled in the art to generate the degenerate variants described above, for instance, to optimize codon expression for a particular host (e.g., change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*, yeast, bacillus, plants, or any other effective host).

Treatment

The IFN- λ compositions of the present invention have been shown to activate the CRF2-12 receptor and receptor complex of the present invention, activating many of the same signal-transduction pathway elements as treatment with IFN-alpha. However the CRF2-12 receptor and receptor complex of the present invention is not responsive to IFN-alpha or beta. Accordingly, IFN- λ compositions of the present invention should have a clinical advantage to treatment with other interferons. One of these advantages derives from the widespread presence of receptors responsive to IFN- λ s, particularly since there is an area of non-overlap between the IFN-alpha/beta receptor complex

distribution and the IFN- λ receptor complex distribution. Accordingly, the compositions of the present invention may encounter greater clinically efficacy for certain therapies, known in the art, for which this family of cytokines has been found to be useful. Some of these uses are listed in U.S. Patent No. 6,472,512, which is incorporated herein by reference in its entirety.

Thus, the invention provides a method of treatment of an individual in need of an increased level of interferon activity comprising administering to such an individual a pharmaceutical composition of the present invention in an amount effective to increase the interferon activity level in such an individual.

Compositions of the invention may also be useful in treating diseases, disorders, and/or conditions of the immune system. Therefore, it will be appreciated that conditions caused by a decrease in the standard or normal level of interferon activity in an individual, particularly disorders of the immune system, can be treated by administration of polynucleotides, polypeptides, or agonists or antagonists of the compositions of the present invention. Thus, the invention also provides a method of treatment of an individual in need of an increased level of interferon activity comprising administering to such an individual a pharmaceutical composition comprising a therapeutic amount of polynucleotides, polypeptides, or agonists or antagonists of compositions of the present invention, effective to increase the interferon activity level in such an individual.

Particularly preferred is treatment of individuals that, for any reason, but preferably one unrelated to the effectiveness of IFN- λ , are unresponsive to IFN-alpha, IFN-beta, IFN-omega, KDI, and other cytokines or interferons.

Particularly preferred are conditions that may be treated, prevented, ameliorated or diagnosed through the biological effects of a composition of the present invention. These include, for example, conditions responsive to modulation of the JAK-Stat pathway; conditions responsive to modulation of the ISFG-3 complex; and other such activities modulated by receptors responsive to polypeptides and fragments of the present invention, preferably CRF2-12 and the CRF2-12 receptor complex.

In another embodiment, the allelic type of an individual may be determined for a gene or combination of genes of the present invention. The allelic type may be used in the treatment, prevention, amelioration or diagnosis of disorders related to the compositions of the present invention. For example, it may be determined that an individual has an allelic type that is non-functional. In such case, administration of a functional composition in accordance with the present invention would be effective in the treatment of the diagnosed condition. In another embodiment, the allelic type is determined prior to administration of a composition of the present invention, and the allelic type administered is matched to the allelic type of the individual to which the composition is to be administered. In such manner, immune reactions may be avoided against the composition, or the incidence of such occurrences decreased.

The allelic type of an individual may be determined in any effective manner. For example, the following techniques may be useful in such determination: immunotyping

of material from the individual, measurement of biological effects of material obtained from the individual, PCR of genomic or other effective nucleic acid from the individual, RT-PCR of mRNA or other effective nucleic acid from the individual (e.g., from a biopsy, or the like), RFLP, repeat polymorphism linkage (ca repeats, or the like), sequencing, hybridization, or any other effective technique.

Compositions of the present invention may be used clinically for any anti-viral therapy for which they are effective. For example, in the treatment of AIDS, viral hepatitis including chronic hepatitis B, hepatitis C, papilloma viruses (e.g., condyloma acuminatum, laryngeal papillomatosis), viral encephalitis, and in the prophylaxis of rhinitis and respiratory infections.

In a specific embodiment, compositions of the present invention are useful in the treatment, prevention, detection and/or diagnosis of an unregulated growth, e.g., a cancer, some examples would include hairy cell leukemia, bladder carcinoma, cervical carcinoma, acute myeloid leukemia, osteosarcoma, basal cell carcinoma, glioma, renal cell carcinoma, multiple myeloma, melanoma, and Hodgkin's disease.

Compositions of the present invention are believed to be capable of stimulating natural killer cell activity. Accordingly, they may be used to treat any infection for which they are effective. Among the conditions that may find useful treatment by the present invention are *Cryptosporidium parvum* infection and multidrug-resistant pulmonary tuberculosis.

Compositions of the present invention are also believed to be useful as immunotherapeutic agents. Depending on whether an agonist or antagonist is used, the effect the compositions of the present invention may find use as immunosuppressive or immunoenhancing agents.

Because of their immunomodulatory function, compositions of the present invention may find use as protective agents when administered prior to chemotherapy. They may also find use in treatment of hyperproliferation of lymphocytes, myeloid progenitors and bone marrow stem cells, as occurs, for example, in chronic myelogenous leukemia.

The compositions of the present invention may also find use in the prevention of graft vs. host rejection, or to curtail the progression of autoimmune diseases, such as arthritis, multiple sclerosis, systemic lupus or diabetes. The polynucleotides, polypeptides, or agonists or antagonists of the present invention are also useful in the treatment of allergies in mammals, e.g., by inhibiting the humoral response.

Compositions of the present invention may be used as an adjuvant or coadjuvant to enhance or stimulate the immune response in cases of prophylactic or therapeutic vaccination.

Further, there is provided a method of treating infection in a patient comprising administering an effective amount of a composition of the present invention to a patient in need of anti-infective therapy. In a preferred embodiment the infection is of viral,

bacterial, or parasitic etiology. In a particularly preferred embodiment, the infection is a viral infection.

Further, there is provided a method of treating cancer in a patient comprising administering an effective amount of a composition of the present invention to a patient in need of anti-cancer therapy.

Further, there is provided a method of immunotherapy in a patient comprising administering an effective amount of a composition of the present invention to a patient in need of immunotherapy.

Compositions of the present invention, may be useful in treating diseases, disorders, and/or conditions of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer or some autoimmune diseases, disorders, and/or conditions, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, compositions of the present invention, can be used as a marker or detector of a particular immune system disease or disorder.

Compositions of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of hematopoietic cells. Compositions of the present invention could be used to increase differentiation and

proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein diseases, disorders, and/or conditions (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, compositions of the present invention can also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, Compositions of the present invention could be used to treat or prevent blood coagulation diseases, disorders, and/or conditions (e.g., afibrinogenemia, factor deficiencies), blood platelet diseases, disorders, and/or conditions (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, Compositions of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment or prevention of heart attacks (infarction), strokes, or scarring.

Compositions of the present invention may also be useful in treating, preventing, and/or diagnosing autoimmune diseases, disorders, and/or conditions. Many autoimmune diseases, disorders, and/or conditions result from inappropriate recognition

of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of compositions of the present invention that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune diseases, disorders, and/or conditions.

Examples of autoimmune diseases, disorders, and/or conditions that can be treated, prevented, and/or diagnosed or detected by the compositions of the present invention include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, and/or diagnosed by compositions of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

Compositions of the present invention may also be used to treat, prevent, and/or diagnose organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs

by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of compositions of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, compositions of the present invention may also be used to modulate inflammation. For example, compositions of the present invention may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat, prevent, and/or diagnose inflammatory conditions, both chronic and acute conditions, including chronic prostatitis, granulomatous prostatitis and malacoplakia, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

Hyperproliferative Disorders

Compositions of the present invention can be used to treat, prevent, and/or diagnose hyperproliferative diseases, disorders, and/or conditions, including neoplasms. Compositions of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, compositions of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative diseases, disorders, and/or conditions can be treated, prevented, and/or diagnosed. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating, preventing, and/or diagnosing hyperproliferative diseases, disorders, and/or conditions, such as a chemotherapeutic agent.

Examples of hyperproliferative diseases, disorders, and/or conditions that can be treated, prevented, and/or diagnosed by using compositions of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative diseases, disorders, and/or conditions can also be treated, prevented, and/or diagnosed by compositions of the present invention. Examples of such hyperproliferative diseases, disorders, and/or conditions include, but are not limited to: hypergammaglobulinemia, lymphoproliferative diseases, disorders, and/or conditions, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenström's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

One preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

Thus, the present invention provides a method for treating cell proliferative diseases, disorders, and/or conditions by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating cell-proliferative diseases, disorders, and/or conditions in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding a polynucleotide of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts

upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes" is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is

preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention concurrently to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating

cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

As used herein, the term "concurrently" has the same meaning as its definition of U.S. Patent No. 6,248,725, which is incorporated herein by reference in its entirety.

The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating one or more of the described diseases, disorders, and/or conditions. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail herein. Those of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

In particular, the antibodies, fragments and derivatives of the present invention are useful for treating a subject having or developing cell proliferative and/or differentiation diseases, disorders, and/or conditions as described herein. Such treatment

comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors, for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against a composition of the present invention, fragments or regions thereof, for both immunoassays and therapy of diseases, disorders, and/or conditions related to compositions of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.

Moreover, polypeptides of the present invention may find one inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph I B, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed

to compositions of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al., Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

Compositions of the present invention may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said compositions may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues. Moreover, in another preferred embodiment of the present invention, said compositions, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, (See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998) Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by reference).

Compositions of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Such therapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

Compositions of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in

activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

Cardiovascular Disorders

Nucleic acids of the present invention may be used to treat, prevent, and/or diagnose cardiovascular diseases, disorders, and/or conditions, including peripheral artery disease, such as limb ischemia. Other disorders listed in US Patent No. 6472,512.

The present invention provides for treatment of diseases, disorders, and/or conditions associated with neovascularization by administration of a composition of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman et al., *Medicine*, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of treating an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a composition of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists may be utilized in a variety of additional methods in order to therapeutically treat or prevent a cancer or tumor. Cancers which may be treated or diagnosed with compositions of the present invention are listed in U.S. Patent No. 6,472,512, which is incorporated herein by reference in its entirety.

Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily.

The compositions of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP-PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-

17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff. J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaininolmidazole; and metalloproteinase inhibitors such as BB94.

Diseases at the Cellular Level

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated, prevented, and/or diagnosed by compositions of the present invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as papilloma

viruses, hepatitis virus, herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, polynucleotides, polypeptides, agonists and/or antagonists of the present invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated, prevented, and/or diagnosed by a composition of the present invention include, but are not limited to, progression, and/or metastases of malignancies and related diseases, disorders, and/or conditions such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., fungoides mycosis, Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary

carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated, prevented, and/or diagnosed by a composition of the present invention include AIDS; neurodegenerative diseases, disorders, and/or conditions (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Wound Healing and Epithelial Cell Proliferation

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing a composition of the present invention, for therapeutic purposes,

for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Compositions of the present invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Compositions of the present invention, could be used to promote dermal reestablishment subsequent to dermal loss

Compositions of the present invention could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. Other uses of the compositions of the present invention as they relate to wound healing and epithelial cell proliferation are listed in U.S. Patent No. 6,472,512, which is incorporated herein by reference in its entirety.

Neurological Diseases

Nervous system diseases, disorders, and/or conditions, which can be treated with compositions of the present invention, include, but are not limited to, nervous system injuries, and diseases, disorders, and/or conditions which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian

patients) Other uses of the compositions of the present invention as they relate to treatment of nervous system related conditions are listed in U.S. Patent No. 6,472,512, which is incorporated herein by reference in its entirety.

In a specific embodiment, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention of the invention are used to treat, prevent, detect, and/or diagnose multiple sclerosis.

The compositions of the invention which are useful for treating, preventing, and/or diagnosing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, compositions of the invention which elicit any of the following effects may be useful according to the invention: (1) increased survival time of neurons in culture; (2) increased sprouting of neurons in culture or in vivo; (3) increased production of a neuron-associated molecule in culture or in vivo, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron dysfunction in vivo. Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, the method set forth in Arakawa et al. (J. Neurosci. 10:3507-3515 (1990)); increased sprouting of neurons may be detected by methods known in the art, such as, for example, the methods set forth in Pestronk et al. (Exp. Neurol. 70:65-82 (1980)) or Brown et al. (Ann. Rev. Neurosci. 4:17-42 (1981)); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody

binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

Infectious Disease

Compositions of the present invention can be used to treat, prevent, and/or diagnose infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated, prevented, and/or diagnosed. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, compositions of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picomaviridae, Poxviridae (such as Smallpox or Vaccinia),

Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, condyloma acuminatum, laryngeal papillomatosis, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts, fungoides mycosis), and viremia.

polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, compositions of the invention are used to treat: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment compositions of the invention are used to treat, prevent, and/or diagnose AIDS.

In a specific embodiment, compositions of the present invention can be used to treat, detect, prevent and/or diagnose diseases associated with Hepatitis virus (e.g., chronic hepatitis C, chronic hepatitis B, chronic active hepatitis, and/or hepatitis D);

diseases associated with Papilloma virus (e.g., condyloma acuminatum, warts, and/or laryngeal papillomatosis)

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but are not limited to, the following Gram-Negative and Gram-positive bacteria, bacterial families and fungi: Actinomyces (e.g., Norcardia), Acinetobacter, Cryptococcus neoformans, Aspergillus, Bacillaceae (e.g., Bacillus anthraxis), Bacteroides (e.g., Bacteroides fragilis), Blastomycosis, Bordetella, Borrelia (e.g., Borrelia burgdorferi), Brucella, Candidia, Campylobacter, Chlamydia, Clostridium (e.g., Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium tetani), Coccidioides, Corynebacterium (e.g., Corynebacterium diphtheriae), Cryptococcus, Dermatocycoses, E. coli (e.g., Enterotoxigenic E. coli and Enterohemorrhagic E. coli), Enterobacter (e.g. Enterobacter aerogenes), Enterobacteriaceae (Klebsiella, Salmonella (e.g., Salmonella typhi, Salmonella enteritidis, Salmonella paratyphi), Serratia, Yersinia, Shigella), Erysipelothrix, Haemophilus (e.g., Haemophilus influenza type B), Helicobacter, Legionella (e.g., Legionella pneumophila), Leptospira, Listeria (e.g., Listeria monocytogenes), Mycoplasma, Mycobacterium (e.g., Mycobacterium leprae and Mycobacterium tuberculosis), Vibrio (e.g., Vibrio cholerae), Neisseriaceae (e.g., Neisseria gonorrhea, Neisseria meningitidis), Pasteurellaceae, Proteus, Pseudomonas (e.g., Psuedomonas aeruginosa), Rickettsiaceae, Spirochetes (e.g., Treponema spp., Leptospira spp., Borrelia spp.) Shigella spp., Staphylococcus (e.g., Staphylococcus

aureus), Meningioccus, Pneumococcus and Streptococcus (e.g., Streptococcus pneumoniae and Groups A,B, and C Streptococci), and Ureaplasmas. These bacterial, parasitic, and fungal families can cause diseases or symptoms, including, but not limited to: antibiotic-resistant infections, bacteremia, endocarditis, septicemia, eye infections (conjunctivitis) tuberculosis, uveitis, gingivitis, bacterial diarrhea, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, dental caries, Reiter's Disease, respiratory tract infections (e.g., Whooping Cough or Empyema), sepsis, Lyme Disease, Cat-Scratch Disease, dysentery, paratyphoid fever, food poisoning, Legionella disease, chronic and acute inflammation, erythema, yeast infections, typhoid, pneumonia, gonorrhea, meningitis (e.g., meningitis types A and B), chlamydia, syphilis, diphtheria, leprosy, burcellosis, peptic ulcers, anthrax, spontaneous abortion, birth defects, lung infections, ear infections, deafness, blindness, lethargy, malaise, vomiting, chronic diarrhea, Crohn's disease, colitis, vaginosis, sterility, pelvic inflammatory disease, candidiasis, paratuberculosis, tuberculosis, lupus, botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections or noscomial infections. Compositions of the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments,

Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by a composition of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis,

Leishmaniasis, theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis.

Preferably, treatment or prevention using a composition of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a composition of the present invention, and returning the cells to the patient (ex vivo therapy).

Regeneration

Compositions of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) the regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and

skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, Compositions of the present invention may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. Compositions of the present invention of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated, prevented, and/or diagnosed include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using Compositions of the present invention to proliferate and differentiate nerve cells. Diseases that could be treated, prevented, and/or diagnosed using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic diseases, disorders, and/or conditions (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated, prevented, and/or diagnosed using Compositions of the present invention.

Chemotaxis

Compositions of the present invention may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

Compositions of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat, prevent, and/or diagnose inflammation, infection, hyperproliferative diseases, disorders, and/or conditions, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat, prevent, and/or diagnose wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat, prevent, and/or diagnose wounds.

It is also contemplated that Compositions of the present invention may inhibit chemotactic activity. These molecules could also be used to treat, prevent, and/or diagnose diseases, disorders, and/or conditions. Thus, Compositions of the present invention could be used as an inhibitor of chemotaxis.

Formulations

The compositions of the present invention may be formulated and dosed in any effective manner consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the polypeptide alone), the site of delivery of the polypeptide composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" of polypeptide to be administered may be determined by such considerations.

One embodiment of the present invention provides for pharmaceutical compositions comprising a polypeptide of the present invention. The invention also provides for pharmaceutical compositions comprising combinations of polypeptides of the present invention. In one embodiment, the invention provides for a pharmaceutical compositions comprising a combination of polypeptides of the present invention, wherein the ratio of IFN- λ 1:IFN- λ 2:IFN- λ 3 is described by the formula m:n:o, wherein m may be any number between 0 and 1000, n may be any number between 0 and 1000 and o may be any number between 0 and 1000, and wherein at least one of m, n or o is different from 0. The invention also provides for pharmaceutical compositions comprising a combination of polypeptides of the present invention, wherein the ratio of IFN- λ 1:IFN- λ 2:IFN- λ 3 is described by the formula m:n:o, wherein m may be any number between 0 and 1000, n may be any number between 0 and 1000 and o may be any number between 0 and 1000, and wherein at least two of m, n or o are different from 0. The invention further provides for pharmaceutical compositions comprising a

combination of polypeptides of the present invention, wherein the ratio of IFN- λ 1:IFN- λ 2:IFN- λ 3 is described by the formula m:n:o, wherein m may be any number between 0 and 1000, n may be any number between 0 and 1000 and o may be any number between 0 and 1000, and wherein all three of m, n and o are different from 0. The combination may further comprise antibodies of the present invention. The combination may also further comprise an agonist and/or antagonist, or a combination of agonists and/or antagonists of the CRF2-12 receptor or the CRF2-12 receptor complex. In a preferred embodiment, the combination further comprises an antibody to the extracellular domain of CRF2-12 that acts as an agonist of the CRF2-12 receptor or the CRF2-12 receptor complex. The combination may further comprise other cytokines, interleukins and/or other pharmaceuticals, including anti-inflammatory agents.

The invention also provides for pharmaceutical compositions comprising an antibody of the present invention. The invention also provides for pharmaceutical compositions comprising combinations of antibodies of the present invention. In one embodiment, the invention provides a composition comprising a combination of anti-IFN- λ 1 specific antibody, anti-IFN- λ 2 specific antibody and anti-IFN- λ 3 specific antibody, wherein the ratio of anti-IFN- λ 1 specific antibody: anti-IFN- λ 2 specific antibody: anti-IFN- λ 3 specific antibody is described by the formula x:y:z, wherein x may be any number between 0 and 1000, y may be any number between 0 and 1000 and z may be any number between 0 and 1000, and wherein at least one of x, y or z is different from 0. The invention also provides for pharmaceutical compositions comprising a combination of anti-IFN- λ 1 specific antibody, anti-IFN- λ 2 specific

antibody and anti-IFN- λ 3 specific antibody, wherein the ratio of anti-IFN- λ 1 specific antibody: anti-IFN- λ 2 specific antibody: anti-IFN- λ 3 specific antibody is described by the formula $x:y:z$, wherein x may be any number between 0 and 1000, y may be any number between 0 and 1000 and z may be any number between 0 and 1000, and wherein at least two of x , y or z are different from 0. The invention also provides for pharmaceutical composition comprising a combination of anti-IFN- λ 1 specific antibody, anti-IFN- λ 2 specific antibody and anti-IFN- λ 3 specific antibody, wherein the ratio of anti-IFN- λ 1 specific antibody: anti-IFN- λ 2 specific antibody: anti-IFN- λ 3 specific antibody is described by the formula $x:y:z$, wherein x may be any number between 0 and 1000, y may be any number between 0 and 1000 and z may be any number between 0 and 1000, and wherein all three of x , y or z are different from 0. The combination may further comprise an IFN- λ or a combination of IFN- λ s of the present invention. The combination may also further comprise an agonist and/or antagonist, or a combination of agonists and/or antagonists of the CRF2-12 receptor or the CRF2-12 receptor complex. In a preferred embodiment, the combination further comprises an antibody to the extracellular domain of CRF2-12 that acts as an agonist of the CRF2-12 receptor or the CRF2-12 receptor complex. The combination may further comprise other cytokines, interleukins and/or other pharmaceuticals, including anti-inflammatory agents.

Any effective amount of the polypeptides or polypeptide fragments of the present invention may be administered to an individual in need thereof. As a general proposition, the total pharmaceutically effective amount administered parenterally per dose will be in the range of about 1 μ g/kg/day to 10 mg/kg/day of patient body weight,

although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day. If given continuously, the composition is typically administered at a dose rate of about 1 µg/kg/hour to about 50 µg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing the polypeptides or polypeptide fragments of the invention may be administered by any effective route, including, for example, orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray.

By "pharmaceutically acceptable carrier" is meant any effective pharmaceutically acceptable carrier, preferably a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

The term "parenteral" as used herein refers to any effective parenteral mode of administration, including modes of administration such as intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The compositions may also suitably be administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable

polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers* 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and R. Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., *Id.*) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

In a preferred embodiment, compositions of the invention are formulated in a biodegradable, polymeric drug delivery system, for example as described in U.S. Pat. Nos. 4,938,763; 5,278,201; 5,278,202; 5,324,519; 5,340,849; and 5,487,897 and in International Publication Numbers WO01/35929, WO00/24374, and WO00/06117 which are hereby incorporated by reference in their entirety. In specific preferred embodiments the compositions of the invention are formulated using the ATRIGEL Biodegradable System of Atrix Laboratories, Inc. (Fort Collins, Colo.).

Examples of biodegradable polymers which can be used in the formulation of compositions of the present invention, include but are not limited to, polylactides, polyglycolides, polycaprolactones, polyanhydrides, polyamides, polyurethanes, polyesteramides, polyorthoesters, polydioxanones, polyacetals, polyketals, polycarbonates, polyorthocarbonates, polyphosphazenes, polyhydroxybutyrates, polyhydroxyvalerates, polyalkylene oxalates, polyalkylene succinates, poly(malic acid), poly(amino acids), poly(methyl vinyl ether), poly(maleic anhydride), polyvinylpyrrolidone, polyethylene glycol, polyhydroxycellulose, chitin, chitosan, and

copolymers, terpolymers, or combinations or mixtures of the above materials. The preferred polymers are those that have a lower degree of crystallization and are more hydrophobic. These polymers and copolymers are more soluble in the biocompatible solvents than the highly crystalline polymers such as polyglycolide and chitin which also have a high degree of hydrogen-bonding. Preferred materials with the desired solubility parameters are the polylactides, polycaprolactones, and copolymers of these with glycolide in which there are more amorphous regions to enhance solubility. In specific preferred embodiments, the biodegradable polymers which can be used in the formulation of compositions are poly(lactide-co-glycolides).

Polymer properties such as molecular weight, hydrophobicity, and lactide/glycolide ratio may be modified to obtain the desired drug release profile (See, e.g., Ravivarapu et al., *Journal of Pharmaceutical Sciences* 89:732-741 (2000), which is hereby incorporated by reference in its entirety).

It is also preferred that the solvent for the biodegradable polymer be non-toxic, water miscible, and otherwise biocompatible. Examples of such solvents include, but are not limited to, N-methyl-2-pyrrolidone, 2-pyrrolidone, C2 to C6 alkanols, C1 to C15 alcohols, diols, triols, and tetraols such as ethanol, glycerine propylene glycol, butanol; C3 to C15 alkyl ketones such as acetone, diethyl ketone and methyl ethyl ketone; C3 to C15 esters such as methyl acetate, ethyl acetate, ethyl lactate; alkyl ketones such as methyl ethyl ketone, C1 to C15 amides such as dimethylformamide, dimethylacetamide and caprolactam; C3 to C20 ethers such as tetrahydrofuran, or solketal; tweens, triacetin, propylene carbonate, decylmethylsulfoxide, dimethyl sulfoxide, oleic acid, 1-

dodecylazacycloheptan-2-one, Other preferred solvents are benzyl alcohol, benzyl benzoate, dipropylene glycol, tributyrin, ethyl oleate, glycerin, glycofural, isopropyl myristate, isopropyl palmitate, oleic acid, polyethylene glycol, propylene carbonate, and triethyl citrate. The most preferred solvents are N-methyl-2-pyrrolidone, 2-pyrrolidone, dimethyl sulfoxide, triacetin, and propylene carbonate because of the solvating ability and their compatibility.

Additionally, formulations comprising compositions of the present invention and a biodegradable polymer may also include release-rate modification agents and/or pore-forming agents. Examples of release-rate modification agents include, but are not limited to, fatty acids, triglycerides, other like hydrophobic compounds, organic solvents, plasticizing compounds and hydrophilic compounds. Suitable release rate modification agents include, for example, esters of mono-, di-, and tricarboxylic acids, such as 2-ethoxyethyl acetate, methyl acetate, ethyl acetate, diethyl phthalate, dimethyl phthalate, dibutyl phthalate, dimethyl adipate, dimethyl succinate, dimethyl oxalate, dimethyl citrate, triethyl citrate, acetyl tributyl citrate, acetyl triethyl citrate, glycerol triacetate, di(n-butyl) sebacate, and the like; polyhydroxy alcohols, such as propylene glycol, polyethylene glycol, glycerin, sorbitol, and the like; fatty acids; triesters of glycerol, such as triglycerides, epoxidized soybean oil, and other epoxidized vegetable oils; sterols, such as cholesterol; alcohols, such as C6-C12 alkanols, 2-ethoxyethanol. The release rate modification agent may be used singly or in combination with other such agents. Suitable combinations of release rate modification agents include, but are not limited to, glycerin/propylene glycol, sorbitol/glycerine, ethylene oxide/propylene

oxide, butylene glycol/adipic acid, and the like. Preferred release rate modification agents include, but are not limited to, dimethyl citrate, triethyl citrate, ethyl heptanoate, glycerin, and hexanediol. Suitable pore-forming agents that may be used in the polymer composition include, but are not limited to, sugars such as sucrose and dextrose, salts such as sodium chloride and sodium carbonate, polymers such as hydroxylpropylcellulose, carboxymethylcellulose, polyethylene glycol, and polyvinylpyrrolidone. Solid crystals that will provide a defined pore size, such as salt or sugar, are preferred.

In specific preferred embodiments the compositions of the invention are formulated using the BEMA BioErodible Mucoadhesive System, MCA MucoCutaneous Absorption System, SMP Solvent MicroParticle System, or BCP BioCompatible Polymer System of Atrix Laboratories, Inc. (Fort Collins, Colo.).

Sustained-release compositions also include liposomally entrapped polypeptides. Liposomes containing a polypeptide of the present invention are prepared by methods known, for example: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for effective polypeptide therapy.

A liposomally entrapped polypeptide of the present invention can be produced for clinically effective formulations. Modified interferons, such as PEG-Intron (Schering-Plough INTRON) are clinically effective and can be used in therapies with other agents, such as Robavirin.

The polypeptide of the present invention may be administered in combination with other known anti-viral, immunomodulatory and anti-proliferative therapies, such as IL-2, alpha interferon, KDI, Ribavirin and temozolomide.

For parenteral administration, in one embodiment, the polypeptide of the invention is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting the polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The polypeptide is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers may result in the formation of polypeptide salts.

Polypeptide to be used for therapeutic administration must be sterile. Sterility may be achieved in any effective manner. For example, by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Compositions of the present invention ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized polypeptide using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

EXAMPLES

EXAMPLE 1

Cloning of the CRF2-12 cDNA, expression pattern and protein characterization.

A search of the GenBank database was performed with the TBLASTN program using as a query a consensus amino acid motif shared by extracellular domains of class II cytokine receptor family members. A genomic fragment from human chromosome 1 encoding a novel amino acid (a.a.) sequence with homology to the consensus motif was identified. This genomic fragment mapped in the vicinity of a known member of the family, namely, the IL-22R1 (CRF2-9) gene (1p36+11 chromosomal region). According to the present invention this fragment represented an exon of a novel receptor from this family of receptors.

The existence of a gene (Hs1_4548_30_10_1) in this region was predicted by GenomeScan (NCBI web site)(5). However, according to the present invention the last four exons were predicted incorrectly by the program, and the last exon encoding intracellular domain of the receptor of the present invention is different and distinct from that provided by GenomeScan.

Several ESTs were positioned in this genomic region representing the 3' untranslated region (3'-UTR) of a novel gene (the UniGene designation Hs.105866), which we predicted to be contingent with the Hs1_4548_30_10_1 gene.

In accordance with the present invention, several sets of primers were designed and used for RT-PCR with several human cDNA libraries as a template to obtain the receptor of the present invention. The primers and a human placental cDNA library (Clontech, catalog # HL4025AH) were used to obtain the genomic organization and exon-intron junctions of the CRF2-12 gene.

Primers

5'-CCAGAACTTCAGCGTGACCTGAC-3' (R12-10 forward primer, R12-10F),

5'-CCAGGATGTGACCTATTTGTGGC-3' (R12-11F),

5'-CCAGCAAGAAGCAGGTGGGCTTAG-3' (R12-13 reverse primer, R12-13R),

5'-TTCGGGACACTGAACGTGTAGATG-3' (R12-14R).

Libraries containing cDNA isolated from HeLa cells (Clontech, catalog # HL4048AH) or from various human tissues, including placenta, fetal brain and liver, EBV-transformed lymphocytes, and leukocytes (Clontech, catalog # HL4025AH, HL4028AH, HL4029AH, HL4006AE and HL4050AH) were used for nested PCR to assess the presence of CRF2-12 cDNA in these libraries.

A first round was performed with R12-10F and R12-13R primers followed by the second round with R12-11F and R12-14R primers and the PCR product of the first round as a template.

The placental cDNA library was selected and further used for amplification of CRF2-12 cDNA by nested PCR. First round was performed with primers:

5'-GCCGCGGCAGGAAGGCCATGGCG-3' (R12-1F) and

5'-CATCCTCCTCCTCTTCGTCCTCTG-3' (R12-16R).

Then the PCR product of the first round was used as a template for the second round of PCR amplification with either primers.

5'-GCCGGTACCATGGCGGGGCCCCGAGCGCTGG-3' (R12-2F) and

5'-AGGGCTAGCCAGTTGGCTTCTGGGACCTCC-3' (R12-9R)

or

5'-GCCGGATCCCCGTCTGGCCCCTCCCCAGAA-3' (R12-3F) and

5'-AGGGCTAGCCAGTTGGCTTCTGGGACCTCC-3' (R12-9R)

to clone the extracellular domain of the CRF2-12 protein into either plasmid pEF3-IL-10R1/ γ R1 (Kotenko, et al., (1997) EMBO J 16, 5894-5903) with the use of KpnI and NheI restriction endonucleases, resulting in plasmid pEF-CRF2-12/ γ R1, or plasmid pEF3-FL γ R2/ α R2c (Kotenko, et al., (1999) Proc Natl Acad Sci U S A 96, 5007-5012) with the use of BamHI and NheI restriction endonucleases, resulting in plasmid pEF-FL-CRF2-12/ α R2c.

The intracellular domain of IFN- α 2c was replaced by the intracellular domain of IFN- γ R1 by cloning of the fragment containing the intracellular domain of IFN- γ R1 from plasmid pEF-CRF2-12/ γ R1 into plasmid pEF-FL-CRF2-12/ α 2c with the use of NheI and BssHII restriction endonucleases, resulting in plasmid pEF-FL-CRF2-12/ γ R1.

Cloning of the CRF2-12 intracellular domain.

Primers

5'-TCTAAGCCCACCTGCTTCTTGCTG-3' (R12-12F),

5'-CTGGCTAGCCCTGGTGCTGCCATCGC-3' (R12-19F),

5'-AGGCAGCAGCAGCATCAGATTCGG-3' (R12-8R) and

5'-GGGTCTAGATCACCTGGCCATGTAATGCCCCA-3' (R12-7R).

Nested PCR of the same placental cDNA library with the above primers (R12-12F and R12-8R primers for the first round and R12-19F and R12-7R primers for the second round) to clone the CRF2-12 intracellular domain into the pCR2.1 vector (Invitrogen).

The CRF2-12 intracellular domain was then cloned into plasmids pEF-CRF2-12/ γ R1 and pEF-FL-CRF2-12/ γ R1 with the use of NheI and NotI restriction endonucleases, resulting in plasmids pEF-CRF2-12 and pEF-FLCRF2-12, respectively.

Plasmid pEF-CRF (pEF-CRF2-4 or pEF-IL-10R2) was previously described (Kotenko, et al., (1997) EMBO J 16, 5894-5903). Plasmid pEF-CRF2-11 (or pEF2-IL-

20R2) was obtained by cloning PCR-derived CRF2-11 cDNA fragment into the pcDEF3 vector (Goldman 1996) with the use of KpnI and EcoRI restriction endonucleases.

Accordingly, a human cDNA of the receptor of the present invention was obtained having SEQ. ID. NO. 1 and encoding a receptor having an amino acid sequence in SEQ. ID.NO. 2. The novel receptor of the present invention was designated CRF2-12 (12th member of the class II cytokine receptor family; Figure 14A).

EXAMPLE 2

CRF2-12 characterization.

In accordance with the present invention, CRF2-12 was found to possess a characteristic signal peptide of about 18 a.a. and a 23 a.a. transmembrane domain (227 to 249 a.a.), which divides the receptor into a 208 a.a. extracellular domain and a relatively long 271 a.a. intracellular domain (Figure 14A).

Alignment of the extracellular domains of the class II cytokine receptors assigned CRF2-12 in a subgroup with several other receptors, CRF2-8, CRF2-9, CRF2-10, IL-10R1 and IFN- γ R1 (Figure 14B).

In accordance with the present invention, the entire CRF2-12 gene of the invention is composed of 7 exons (Figure 14C). The structure of the gene of the present invention was found to correlate with the common conserved architecture of other genes encoding CRF members (Kotenko 2002).

The first exon of the CRF2-12 gene of the present invention contains the 5'-UTR and the signal peptide. Exons 2, 3, 4 and 5 and a part of exon 6 of the CRF2-12 gene of the present invention encode the extracellular domain. Exon 6 of the CRF2-12 gene of the present invention also encodes the transmembrane domain and the beginning of the intracellular domain. Exon 7 of the CRF2-12 gene of the present invention encodes the rest of the intracellular domain and the 3'-UTR.

Both genes, the CRF2-12 gene and the IL-22R1 gene, are transcribed in the same direction toward the telomere and positioned approximately 10 kb apart. All exon/intron and intron/exon splice sites within of the CRF2-12 gene of the present invention conform well to the consensus motifs (exon/GT-intron-AG/exon).

EXAMPLE 3

CRF2-12 expression.

To determine what cell types express CRF2-12, a panel of RNA derived from various tumor cell lines was evaluated.

Expression of CRF2-12 mRNA.

Northern blotting was performed as described (Gallagher, et al., (2000) *Genes Immun* 1, 442-450 and Kotenko, et al., (2001) *J Biol Chem* 276, 2725-2732) with the use of a CRF2-12 probe corresponding to the coding region of the CRF2-12 cDNA.

Northern blotting was performed on two blots containing mRNA isolated from: human cancer cell lines (promyelocytic leukemia HL-60, epitheloid carcinoma HeLa S3,

lymphoblastic leukemia MOLT-4, Burkitt's lymphoma Raji, colorectal adenocarcinoma, SW480, lung carcinoma A549 and melanoma G361) and normal human fetal tissues (heart, kidney, skin, small intestine) and adult lung (Figure 15A and 15B). Arrows point to the CRF2-12 and the b-actin transcripts. Equal RNA loading was assessed by evaluating the expression of the b-actin gene.

CRF2-12 mRNA was expressed at variable levels in all of the cell lines examined (Figure 15A). These included both hematopoietic (HL-60, K-562, MOLT-4 and Raji) and non-hematopoietic (HeLa, SW480, A549, and G-361) cell lines. Of these cell lines, Raji cells displayed the highest levels of CRF2-12 gene expression. CRF2-12 was also expressed by various normal tissues, including heart, kidney, skin, small intestine, and lung (Figure 15B). CRF2-12 mRNA was also present in skeletal muscle and liver. Therefore, CRF2-12 appears to be constitutively expressed in a variety of cell lines and tissues.

EXAMPLE 4

Construction of tandem vectors encoding two receptors and construction of a chimeric CRF2-12/IFN- γ R1 receptor.

To assay for the CRF2-12 receptor ligands, a chimeric CRF2-12/IFN- γ R1 (CRF2-12/ γ R1) receptor (Figure 20A) was created. The chimeric CRF2-12/IFN- γ R1 receptor was expressed with different intact R2 chains in various combinations (CRF2-4 and CRF2-11) in 16-9 hamster cells.

To ensure that both receptors were expressed in a single transfected cell, tandem vectors, in which expression of CRF2-12/ γ R1 and either CRF2-4 or CRF2-11 is controlled by separate promoters and polyadenylation signal, were constructed. Tandem vectors encoding two receptors, CRF2-12/ γ R1 and either CRF2-4 or CRF2-11, in which the expression of each receptor is controlled by separate promoters and polyadenylation signals were created as follows. The fragment containing the EF-1 α promoter, the CRF2-12 coding sequence, and the bovine growth hormone (BGH) polyadenylation signal was released from the pEF-CRF2-12/ γ R1 vector by digestion with BsaI and BssHII restriction endonucleases and ligated into the BsaI and MluI sites of either the pEF-CRF2-4 or pEF-CRF2-11 plasmid. The resulting plasmids were designated pEF-CRF2-12/ γ R1+CRF2-4 and pEF-CRF2-12/ γ R1+CRF2-11, respectively.

EXAMPLE 6

Testing known cytokines for CRF2-12 specificity.

Plasmids pEF-CRF2-12/ γ R1+CRF2-4 and pEF-CRF2-12/ γ R1+CRF2-11 were transfected into hamster cells and cellular pools of G418-resistant clones were collected. The ability of different cytokines to activate the chimeric receptor and the ability of resultant receptor complexes to transduce IFN- γ -like signaling in the transfected hamster cells was tested. In particular, the chimeric receptor was tested for responsiveness to different ligands utilizing EMSA to detect Stat1 activation and FACS to evaluate MHC class I antigen expression, the activities characteristic for IFN- γ signaling. Signaling by

IL-19, IL-20, IL-22 and IL-24 in cells expressing CRF2-12/ γ R1 and either CRF2-4 or CRF2-11 chains was not observed.

EXAMPLE 7

CRF2-12 ligand identification and cloning.

In accordance with the present invention, a group of highly conserved between themselves proteins named IFN- λ 1, IFN- λ 2 and IFN- λ 3 which also demonstrated very limited primary homology to members of both type I IFN and IL-10 families, were identified. (Figs. 16, 17, 18 and 19, SEQ. ID NO. 7, SEQ. ID NO. 8, SEQ. ID NO. 9, SEQ. ID NO. 10, SEQ. ID NO. 11, SEQ. ID NO. 12).

Genes for these three novel cytokines were cloned as follows.

Human genomic DNA and the following primers were used to amplify IFN- λ genes:

5'-GCGGTACCATGGCTGCAGCTTGGACCGTGG-3' (ifnl-1F),

5'-GCGGTACCATGACTGGGGACTGCACGCCAGTG-3' (ifnl-2F),

5'- GCGGATCCTGTCCCCACTTCCAAGCCCACC-3' (ifnl-5F),

5'- GCGGATCCTGTGCGCCAGGCTCCGCGGGGCT-3' (ifnl-6F),

5'-CGGAATTCAGGTGGACTCAGGGTGGGTTGA-3' (ifnl-3R) and

5'-CGGAATTCAGACACACAGGTCCCCACTGGCAACACA-3' (ifnl-4R).

First round was performed with either ifnl-1F and ifnl-3R primers or ifnl-2F and ifnl-4R primers followed by the second round with either ifnl-5F and ifnl-3R primers or ifnl-6F and ifnl-4R primers and corresponding PCR products of the first round as a template. Resulting PCR products were cloned into the pEF-SPFL vector with the use of BamHI and EcoRI restriction endonucleases, generating plasmid pEF-FL-IFN- λ 1gene, pEF-FL-IFN- λ 2gene and pEF-FL-IFN- λ 3gene.

The PCR-derived genomic fragments which encode mature IFN- λ 1, IFN- λ 2, IFN- λ 3 proteins (Pro23 (Figure 19A) was predicted to be the first a.a. of the mature proteins) were cloned into plasmid pEF-SPFL, resulting in plasmids pEF-FL-IFN- λ 1gene, pEF-FL-IFN- λ 2gene and pEF-FL-IFN- λ 3gene.

Plasmid pEF-SPFL encodes the signal peptide derived from the human IFN- γ R2 chain followed by the FLAG epitope. The pEF-FL-IFN- λ 1gene, pEF-FL-IFN- λ 2gene and pEF-FL-IFN- λ 3gene constructs abutted each of IFN- λ s reading frames to the frame of the FLAG epitope (FL-IFN- λ s).

IFN- λ s cDNAs were subsequently cloned by RT-PCR using as a template mRNAs from COS-1 cells independently transfected with plasmids pEF-FL-IFN- λ 1gene, pEF-FL-IFN- λ 2gene and pEF-FL-IFN- λ 3gene. cDNA fragments encoding mature IFN- λ s proteins were cloned into the same plasmid pEF-SPFL resulting in plasmids pEF-FL-IFN- λ 1, pEF-FL-IFN- λ 2 and pEF-FL-IFN- λ 3.

The same primers as for cloning genomic fragments and total RNA isolated from COS-1 cells transfected with plasmids pEF-FL-IFN- λ 1gene, pEF-FL-IFN- λ 2gene and

pEF-FL-IFN- λ 3 gene were used for RT-PCR to obtain IFN- λ 1, IFN- λ 2 and IFN- λ 3 cDNA fragments, which were subsequently cloned into plasmid pEF-SPFL with the use of BamHI and EcoRI restriction endonucleases, resulting in plasmids pEF-FL-IFN- λ 1, pEF-FL-IFN- λ 2 and pEF-FL-IFN- λ 3. These plasmids encode IFN- λ molecules tagged at their N-terminus with the FLAG epitope (FL-IFN- λ).

The PCR product obtained with primers:

ifnl-5F and

5'-

GCGAATTCATGCGACGGATGCCCCGCCGAATTGAGGTGGACTCAGGGTGGG-3'

(ifnl-7R).

and plasmid pEF-FL-IFN- λ 1 as a template was digested with BamHI and EcoRI restriction endonucleases and cloned into corresponding sites of the pEF-SPFL vector, resulting in plasmid pEF-FL-IFN- λ 1-P. This plasmid encodes FL-IFN- λ tagged at its C-terminus with the Arg-Arg-Ala-Ser-Val-Ala sequence (FL-IFN- λ -P), that contains the consensus amino acid sequence recognizable by the catalytic subunit of the cAMP-dependent protein kinase (Li, et al., (1989) Proc Natl Acad Sci U S A 86, 558-562).

The PCR product obtained with primers 5'-

GATAGTACTTCCAAGCCCACCACA-3' (ifnl-5F) and ifnl-3R and plasmid pEF-FL-

IFN- λ 1 as a template was digested with ScaI and EcoRI restriction endonucleases and cloned into XmnI and EcoRI sites of the pMAL-p2x vector (New England Biolabs),

resulting in plasmid pMAL-IFN- λ 1. This plasmid encodes MBP (maltose-binding protein)-IFN- λ fusion protein which is secreted to the periplasm of *E. coli* cells. The fusion protein was purified from the periplasm by the cold osmotic shock method according to the manufacturer's protocol.

In accordance with the present invention, IFN- λ 1, IFN- λ 2, IFN- λ 3 were found to be encoded by genes including five exons, which resemble the structural organization of genes encoding IL-10-related cytokines.

Genes encoding three members of this family, designated IFN- λ 1, IFN- λ 2 and IFN- λ 3, (Figs. 16, 17, 18 and 19, SEQ. ID NO. 7, SEQ. ID NO. 8, SEQ. ID NO. 9, SEQ. ID NO. 10, SEQ. ID NO. 11, SEQ. ID NO. 12) were found to be clustered on human chromosome 19 (19q13+13 region).

EXAMPLE 8

Expression of IFN- λ s from pEF-FL-IFN- λ 1 gene, pEF-FL-IFN- λ 2 gene and pEF-FL-IFN- λ 3 gene.

COS-1 cells were transiently transfected with pEF-FL-IFN- λ 1 gene, pEF-FL-IFN- λ 2 gene and pEF-FL-IFN- λ 3 gene plasmids and three days after transfection conditioned media was collected and subjected to Western blotting and biological assays (Figures 20, 21, 22, 23 and 24). Western blotting revealed that FL-IFN- λ s were secreted from COS cells and migrated on the SDS-PAGE gel as several bands in the region of about 35 kDa for IFN- λ 1 and as a single band of about 25 kDa for IFN- λ 2 and IFN- λ 3

proteins (Figure 20C). In accordance with the present invention, this observation suggests glycosylation of IFN- λ 1. Further in accordance with the present invention, a potential site for N-linked glycosylation (Asn-X-Thr/Ser) is present in IFN- λ 1 (Figure 19A).

COS cells conditioned media containing IFN- λ s were used to treat hamster cells expressing CRF2-12/ γ R1 and either CRF2-4 or CRF2-11 chains. All three proteins were able to induce Stat activation and up-regulate MHC class I antigen expression, but only in cells expressing both CRF2-12/ γ R1 and CRF2-4 chains (Figure 21A, 23). Expression of neither CRF2-12/ γ R1 nor CRF2-4 alone rendered cells responsive to IFN- λ treatment.

Three days after transfection, conditioned media from COS-1 cells transiently transfected with expression plasmids were collected. FLAG-tagged proteins in the conditioned media were identified by Western blotting with anti-FLAG epitope specific M2 monoclonal antibody (Sigma) as described (Kotenko, et al., (2000) Proc Natl Acad Sci U S A 97, 1695-1700).

Conditioned media from COS-1 cells independently transiently transfected with plasmids pEF-FL-IFN- λ 1, pEF-FL-IFN- λ 2 and pEF-FL-IFN- λ 3 demonstrated indistinguishable biological activities and Western blotting pattern as conditioned media from COS-1 cells independently transfected with plasmids pEF-FL-IFN- λ 1gene, pEF-FL-IFN- λ 2gene and pEF-FL-IFN- λ 3gene (Figure 20C, 21A, 21B and 23).

In accordance with another embodiment of the present invention, the use of flow cytometry was used to detect binding of FLAG epitope-tagged IFN- λ s to the cells expressing both CRF2-12/ γ R1 and CRF2-4 chains (Figure 21A).

In accordance with another embodiment of the present invention, FL-IFN- λ s were found to compete for receptor binding (Figure 22). Cells expressing either chain alone did not bind FL-IFN- λ s as measured by FACS.

In yet another embodiment of the present invention, the entire IFN- λ 1 cDNA was cloned into the pcDEF3 vector Goldman, et al., (1996) Biotechniques 21, 1013-1015, resulting in the plasmid pEF-IFN- λ 1. Conditioned medium of COS-1 cells transiently transfected with this expression vector competed for binding with FL-IFN- λ s and was positive in MHC class I induction experiments and in the EMSA experiments (Figure 21A), demonstrating that IFN- λ s are secreted with their own signal peptides.

In another embodiment of the present invention, recombinant IFN- λ 1 was produced in *E. coli*. The recombinant IFN- λ 1 produced in *E. coli* was found to be active in all the experiments described above (Figure 21).

EXAMPLE 9

In another embodiment of the present invention, the interaction between IFN- λ and its receptor was characterized by crosslinking (Figure 22). Crosslinking experiments were performed with radiolabeled IFN- λ (Figure 20B and C) and the IFN- λ receptor chains expressed in hamster cells (Figure 22).

COS cell-expressed FL-IFN- λ 1-P was purified to homogeneity and radiolabeled (Figure 20B and C). The FL-IFN- λ 1-P protein was transiently expressed in COS cells and purified from conditioned media by immunoaffinity chromatography with the anti-FLAG M1 gel (Sigma) according to the manufacturer's suggested protocols. FL-IFN- λ 1-P was labeled with [^{32} P]ATP and used for crosslinking to cells as described (Li, et al., (1989) Proc Natl Acad Sci U S A 86, 558-562; Kotenko, et al., (1995) J Biol Chem 270, 20915-20921; Pestka, et al., (1999) Protein Expr Purif 17, 203-214; Kotenko, et al., (2001) J Immunol 166, 7096-7103).

Parental hamster cells, cells expressing either the chimeric human CRF2-12/ γ R1 chain, the intact human CRF2-4 chain, or both, were incubated with radiolabeled FL-IFN- λ 1-P. The cells were washed to remove unbound ligand, and bound ligand was crosslinked to the cells. After crosslinking, the cells were lysed and crosslinked complexes were resolved on 7.5% SDS-PAGE (Figure 22).

The appearance of several labeled crosslinked complexes was observed only in cells expressing both IFN- λ 1 receptor chains. Neither parental cells nor cells expressing either receptor chain alone were able to bind the ligand. (Figure 22, lanes 3, 4 and 5.)

Specificity of binding was shown by a competition assay with an excess of unlabeled IFN- λ 1 (Figure 22, lane 7). The major radiolabeled band in the region of 35 kDa corresponds to free FL-IFN- λ 1-P, and results from ligand bound to the cells but not crosslinked to receptors. A distinct crosslinked complex migrating in the region of 200 kDa which is not present in the ligand crosslinked to itself in solution, likely represents

oligomers of IFN- λ 1 and both receptor chains, formed as a result of the association of the IFN- λ 1 receptor chains induced by ligand binding.

FL-IFN- λ 1-P was also crosslinked to itself in solution to determine whether it can oligomerize. Patterns of radiolabeled bands for crosslinked or untreated ligand are identical (Figure 22, lanes 1 and 2), strongly suggesting that IFN- λ 1 is a monomer in solution. The crosslinking experiments demonstrated that IFN- λ 1 requires the presence of the both IFN- λ receptor chains on the cell surface and is unable to interact with either chain expressed alone.

Thus, we identified a specific novel ligand-receptor complex, making a first step in assigning functions for these novel cytokines. The CRF2-12 protein can also be designated IFN- λ R1 based on its function as the R1 type subunit in the IFN- λ receptor complex.

EXAMPLE 10

Signal transduction and biological activity.

Cytokines which utilize class II cytokine receptors for signaling primarily activate the Jak-Stat signal transduction pathway (Kotenko, S. V. (2002) Cytokine Growth Factor Rev. 13, 223-240 and Kotenko, S. V. & Pestka, S. (2000) Oncogene 19, 2557-2565). Activation of this pathway is generally considered essential for induction of cytokine-responsive genes.

To define which Stats are activated by the IFN- λ s, we examined the activation of several Stats in wild-type HT-29 cells and an HT-29 clone, 1084, that overexpresses a chimeric receptor IL-10R1/IFN- λ R1 (10R1/ λ R1) composed of the extracellular domain of the IL-10R1 chain linked to the intracellular domain of IFN- λ R1 (CRF2-12). This chimeric receptor is able to bind IL-10 and dimerize with CRF2-4 (IL-10R2), a common second chain for the IL-10, IL-22 and IFN- λ receptor complexes, to transduce a signal. The Stat proteins that are activated by signaling through this chimeric receptor upon IL-10 treatment represent those that are activated by signaling through the intact IFN- λ receptor complex. Stat activation was measured by immunoprecipitating specific Stat proteins from whole cell lysates, and then western blotting with phosphotyrosine-specific antibodies.

HT-29 cells do not normally express IL-10R1, therefore treatment with IL-10 did not induce activation of any Stats in these cells (Figure 23A and B). However, these cells do express IL-22R1 (CRF2-9), and treatment with IL-22 resulted in the activation of Stat1, Stat2 (weakly), Stat3 and Stat5. The parental HT-29 cell line was also fully responsive to IFN- α because IFN- α treatment activated Stat1, Stat2 and Stat3 in these cells.

In 1084 cells, treatment with IL-10 induced activation (tyrosine phosphorylation) of Stat1, Stat2, Stat3 and Stat5.

Accordingly, since the chimeric receptor binds IL-10 and transduce an IFN- λ signal, in another embodiment of the present invention, modulation of the activity of the

intracellular domain of CRF2-12, be it in a chimeric receptor by binding of the ligand specific for the extracellular domain, or in a CRF2-12 receptor by binding by an IFN- λ , or another agonist or antagonist of the receptor, results in modulation of Stat1, Stat2, Stat3 and Stat5.

Activation of Stat2 is a unique feature of type I IFNs signaling. Thus, in another embodiment of the present invention, the pattern of Stat activation through the 10R1/ λ R1 chimeric receptor suggested that signaling through the IFN- λ receptor complex activates IFN- α -like responses.

EXAMPLE 11

Type-I IFNs are unique insofar as they induce the activation of ISGF3. This transcription factor complex is composed of three subunits: Stat1, Stat2, and ISGF3 γ (p48 or IRF-9, interferon regulatory factor 9). Activation of ISGF3 by type I IFNs induces expression of a distinct subset of IFN-responsive genes (Darnell, et al., (1994) Science 264, 1415-1421). To determine if IFN- λ activates ISGF3, we treated wild-type HT-29 and A549 cells with IFN- λ and then measured ISGF3 activity using an interferon-stimulated response element (ISRE) present in the proximal promoter region of the ISG-15 gene. Treatment with IFN- λ s, like treatment with IFN- α , induces ISGF3 activity in HT-29 and A549 cells (Figure 23B and C). Accordingly, in accordance with another embodiment of the present invention, treatment with IFN- λ s induces ISGF3 activity.

EXAMPLE 12

IFN- λ s also induce tyrosine phosphorylation of Stat1 and Stat2 in HT-29 cells as measured by western blotting with anti-pY-Stat antibodies. Accordingly, in yet another embodiment of the present invention, IFN- λ s induce tyrosine phosphorylation of Stat1 and Stat2.

To determine whether IFN- λ and IFN- α possess overlapping biological activities, HT29 colorectal adenocarcinoma, HeLa cervix adenocarcinoma, A549 lung carcinoma, and HaCaT keratinocyte cells were treated with both ligands and MHC class I antigen expression was evaluated. Both IFN- λ and IFN- α were able to up-regulate expression of MHC class I antigens to comparable levels in all tested cell lines (Figure 21A). Accordingly, in another embodiment of the present invention, IFN- λ , agonist and antagonist thereof modulate expression of MHC class I antigens.

EXAMPLE 13

IFN- λ 1 induces cellular antiviral protection.

Antiviral protection in response to IFN- λ 1 was evaluated on HT29 cells infected with vesicular stomatitis virus (Figure 24A, row C). IFN- α was used as a positive control (Figure 24A, row A). Equal amount of cells were plated in all wells and treated with two-fold serial dilutions of indicated ligands (Figure 24A, rows A and C). Twenty-four hours later the virus was added in all wells except for the first seven wells in row F. As can be seen from Figure 24A, IFN- λ 1 was more effective than IFN- α in protecting cells from viral infection.

Accordingly, in another embodiment of the present invention, IFN- λ s, alone or in combination, other agonists of CRF2-12, and modulators of the intracellular domain of CRF2-12, are effective in providing protection from viral infection. In another embodiment of the present invention, IFN- λ 1, IFN- λ 2 or IFN- λ 3, or effective combinations thereof, induces overlapping signaling and biological activities with those of IFN- α in intact cells expressing an effective receptor.

EXAMPLE 14

Expression of IFN- λ s mRNA was evaluated by RT-PCR in virus-infected cells.

HeLa cells were left untreated (lane 3) or infected with either Dengue virus (DV, lanes 4-6) Sindbis virus (SV, lanes 7-9) or vesicular stomatitis virus (VSV, lanes 10-12). HT29 cells were left untreated (lane 13) or infected with VSV (lanes 14-16). HuH7 cells were left untreated (lane 17) or infected with DV (lanes 18-20) or SV (lanes 21-23). Cells were collected at post-infection times shown in the Figure, RNA was isolated and RT-PCR was performed as described in Materials and Methods. Water was used as a negative control for RT-PCR (lane 2). 1kb ladder was run in lanes 1 and 24.

The expression of IFN- λ 1, IFN- λ 2 and IFN- λ 3 mRNAs was detected in a number of cell lines infected with various viruses (Figure 24B).

It was also found that dendritic cells, which are important producers of IFN- α (Siegal, et al., (1999) Science 284, 1835-1837) expressed IFN- λ 1 mRNA in response to treatment with polyI:C.

Accordingly, in another embodiment of the present invention, the presence and/or quantity of IFN- λ 1, IFN- λ 2 and/or IFN- λ 3 present, or mRNA expressed, is an indicator of infection, in particular viral infection. In another aspect of the present invention, IFN- λ expression is modulated in response to viral infections. In another embodiment of the present invention, the cytokines designated IFN- λ s play a functional IFN- α independent role in an antiviral cellular defense.

EXAMPLE 15

Signaling through the CRF2-12 intracellular domain induces apoptosis.

We also evaluated whether biological activities induced by IFN- λ s through the intact IFN- λ receptor complex composed of CRF2-12 and CRF2-4 chains, can be reproduced in cells expressing the FL-IL-10R1/CRF2-12 chimeric receptor upon IL-10 treatment. HT-29 cells expressing FL-IL-10R1/CRF2-12 were treated with various amounts of IL-10. Three days later the cells were collected to measure the level of MHC class I antigen expression. Surprisingly, cells treated with IL-10 at concentration of about 10ng/ml and higher, appeared round-shaped, not attached to the plastic, and apparently did not proliferate, suggesting that the cells underwent apoptosis in response to IL-10. None of these changes were observed in untreated cells, in cells treated with IL-22 or IFN- γ , or in parental HT-29 cells treated with IL-10.

The induction of apoptosis in HT-29 cells expressing FL-IL-10R1/CRF2-12 chain by IL-10 was subsequent confirmed: i) by staining apoptotic cells with propidium iodide, and ii) by demonstrating phosphatidylserine externalization in apoptotic cells

with the use of Annexin V-FITC and flow cytometry. The induction of apoptosis in these cells by IL-10 was dependent on the concentration of IL-10 and on the level of the FL-IL-10R1/CRF2-12 chain expression. Apoptotic effects were stronger at higher concentrations of IL-10 and in cell clones with higher level of FL-IL-10R1/CRF2-12 expression.

At lower concentration of IL-10 (about few ng/ml) cells demonstrated antiproliferative response and at concentration of IL-10 about 1 ng/ml demonstrated activities characteristic for IFN- λ (MHC class I antigen induction and antiviral activity).

Thus, with declining concentration of IL-10, various activities can be induced in cells starting from apoptosis, followed by antiproliferative effect and the induction of antiviral state. Accordingly, another embodiment of the present invention results that either apoptosis, antiproliferative effect or antiviral protection may be obtained by effectively modulating the activity of the intracellular domain of CRF2-12.

In all cells tested so far, the level of CRF2-12 expression appeared to be low. Thus, the fact that in all tested cell lines IFN- λ appeared to induce can be explained by the low level of CRF2-12 expression. Accordingly, in another embodiment of the present invention, when viruses or other natural or pharmaceutical agents modulate the level of CRF2-12 expression, IFN- λ , or other compositions that modulate effectively the activity of the intracellular domain of CRF2-12, may be used to induce antiviral protection, apoptosis or to have an antiproliferative effect.

EXAMPLE 16

Cloning of CRF2-11 cDNA

CRF2-11 cDNA was obtained by PCR as follows.

First round primers:

5'-TTTGGAAGAAACAATGTTCTAGGTC-3' (R11-2F) and

5'-CTTCACCTGGGCCCTTCCGC-3' (R11-7R)

were used to amplify a libraries containing cDNA isolated from human leukocytes (Clontech, catalog # HL4050AH)

Second round primers:

5'-CTGAGGGTACCAAATGCAGACTTTCACAATG-3' (R11-3F) and

5'-GGGAATTCATGAGATCCAGGCCCTGAGGAGTTC-3' (R11-6R)

and the PCR product of the first round as a template.

EXAMPLE 17

In another embodiment of the present invention IFN- λ 1 induces cellular antiviral protection. As shown in **Fig 26A**, IFN- λ 1 induced antiviral protection in HT29, A549 and HaCaT cells infected with vesicular stomatitis virus (VSV, an RNA rhabdovirus) and in HT29 cells infected with encephalomyocarditis virus (EMCV, an RNA picornavirus). In antiviral assays IFN- λ 1 demonstrated antiviral potency comparable with that of IFN- α , on the order of 10^7 - 10^8 IFN- α -relevant units per mg. Moreover,

when HT29 cells were pretreated with 500 pg/ml of IFN- λ 1 prior to VSV infection, the titer of VSV in the conditioned media of infected cells (48 h after infection) dropped more than 3 orders of magnitude in comparison to the VSV titer produced by control untreated cells. In cells pretreated with 1250 pg/ml the viral titer dropped more than 6 orders of magnitude as determined by viral killing curve (data not shown). Accordingly, in another aspect of the present invention IFN- λ s are used to induce antiviral protection.

In accordance with another embodiment of the present invention, IFN- λ 1 is capable of inducing transcription of type I IFN-responsive ISRE-controlled genes encoding 2',5'-OAS and MxA protein (**Fig. 26B**). Thus, in intact cells, IFN- λ induces overlapping signaling and biological activities with those of IFN- α . These signaling and biological activities, include, for example, upregulation of MHC class I antigen expression, induction of antiviral protection, and induction of IFN-regulated genes. Accordingly, in another aspect of the present invention IFN- λ s are used to induce expression of a protein that mediates antiviral protection. These proteins may include, for example, 2',5'-oligoadenylate synthetase (2',5'-OAS) and Mx proteins.

In another embodiment of the present invention HT29 cells or A549 cells infected with EMCV secrete IFN- λ specific activity capable of stimulating STAT1 activation through the CRF2-12- λ R1 and CRF2-4 heterodimer (**Fig. 26C**). Uninfected cells, in accordance with the present invention, do not produce IFN- λ activity. Accordingly, in another aspect of the present invention IFN- λ s are used to detect the presence of infected cells. In yet another embodiment of the present invention, antagonists are used to modulate inflammation.

Other materials and methods

See, for example, Kotenko, et al., *Nature Immunology* 4:69-77 (2003), which is incorporated by reference herein in its entirety.

Cells, transfection and cytofluorographic analysis.

The 16-9 hamster x human somatic cell hybrid line is the Chinese hamster ovary cell (CHO-K1) hybrid containing a translocation of the long arm of human Chromosome 6 encoding the human IFNGR1 (Hu-IFN- γ R1) gene and a transfected human HLA-B7 gene (Soh, J., Donnelly, R. J., Mariano, T. M., Cook, J. R., Schwartz, B. & Pestka, S. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 8737-8741). The cells were maintained in F12 (Ham) medium (Sigma) containing 10% heat-inactivated fetal bovine serum (Sigma). COS-1 cells, an SV40 transformed fibroblast-like simian CV-1 cell line, were maintained in DMEM medium (GibcoBRL) with 10% heat-inactivated fetal bovine serum.

Colorectal adenocarcinoma HT29 cells were maintained in DMEM medium (GibcoBRL) with 10% heat-inactivated fetal bovine serum.

Cells were transfected as previously described (Kotenko, et al., (1997) *EMBO J* 16, 5894-5903 and Kotenko, et al., (2000) *Proc Natl Acad Sci U S A* 97, 1695-1700). COS cell supernatants were collected at 72 hrs as a source of the expressed proteins.

To detect cytokine-induced MHC class I antigen (HLA-B7) expression, cells were treated with COS cell supernatants or purified recombinant proteins as indicated in the text for 72 hours and analyzed by flow cytometry.

Cell surface expression of the HLA-B7 antigen was detected by treatment with mouse anti-HLA (W6/32) (Barnstable, et al., (1978) Cell 14, 9-20) monoclonal antibody followed by fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Santa Cruz Biotechnology Inc.). The cells then were analyzed by cytofluorography as previously described (Kotenko, et al., (1999) Proc Natl Acad Sci U S A 96, 5007-5012).

Unless otherwise specified, whether a result is statistically significant, means that when appropriately measured in any effective manner, there is a probability of less than 50% that the result occurred by mere chance. Other preferred confidence levels include 70%, 75%, 80%, 85%, 90%, 95% and 99%.

Electrophoretic mobility shift assays (EMSAs) and Western and Northern blotting.

Cells were starved overnight in serum-free media and then treated with IL-10 or IL-22 as indicated in the text for 15 minutes at 37°C and used for EMSA experiments to detect activation of Stat1, Stat3 and Stat5 as described (Kotenko, S. V., Krause, C. D., Izotova, L. S., Pollack, B. P., Wu, W. & Pestka, S. (1997) EMBO J 16, 5894-5903). EMSAs were performed with a 22-base pair sequence containing a Stat1 α binding site corresponding to the GAS element in the promoter region of the human IRF-1 gene (5'-

GATCGATTTCCTCGAAATCATG-3') as described (Kotenko, et al. (1995) J Biol Chem 270, 20915-20921 and Kotenko, et al., (1996) J Biol Chem 271, 17174-17182).

Claims

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the CRF2-12 polypeptide having the complete amino acid sequence in SEQ ID NO: 2;

(b) a nucleotide sequence encoding residues 2-520 of SEQ ID NO: 2;

(c) a nucleotide sequence encoding residues 19-520 in SEQ ID NO: 2;

(d) a nucleotide sequence encoding the CRF2-12 polypeptide extracellular domain;

(e) a nucleotide sequence encoding the extracellular domain of the CRF2-12 protein in plasmid pEF-CRF2-12/ γ R1;

(f) a nucleotide sequence encoding the extracellular domain of the CRF2-12 protein in plasmid pEF3-FL γ R2/ α R2c;

(g) a nucleotide sequence encoding the CRF2-12 polypeptide intracellular domain;

(h) a nucleotide sequence encoding the intracellular domain of the CRF2-12 protein in plasmid pEF-CRF2-12;

(i) a nucleotide sequence encoding the intracellular domain of the CRF2-12 protein in plasmid pEF-FLCRF2-12; and

(j) a nucleotide sequence complementary to any of nucleotide sequences (a)-(i).

2. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a chimeric receptor comprising the extracellular domain of CRF2-12 and the intracellular domain of another membrane bound receptor;

(b) a nucleotide sequence encoding a chimeric receptor comprising the extracellular domain of CRF2-12 and the intracellular domain of another membrane bound tyrosine kinase receptor;

(c) a nucleotide sequence encoding a chimeric receptor comprising the extracellular domain of CRF2-12 and the intracellular domain of a cytokine receptor;

(d) a nucleotide sequence encoding a chimeric receptor comprising the extracellular domain of CRF2-12 and the intracellular domain of an IFN R1 type receptor;

(e) a nucleotide sequence encoding a chimeric receptor comprising the extracellular domain of CRF2-12 and the intracellular domain of IFN γ R1;

(f) a nucleotide sequence encoding the chimeric protein in plasmid pEF-CRF2-12/ γ R1; and

(g) a nucleotide sequence complementary to any of nucleotide sequences (a)-(f).

3. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a chimeric receptor comprising the intracellular domain of CRF2-12 and the extracellular domain of another membrane bound receptor;

(b) a nucleotide sequence encoding a chimeric receptor comprising the intracellular domain of CRF2-12 and the extracellular domain of another membrane bound tyrosine kinase receptor;

(c) a nucleotide sequence encoding a chimeric receptor comprising the intracellular domain of CRF2-12 and the extracellular domain of a cytokine receptor;

(d) a nucleotide sequence encoding a chimeric receptor comprising the intracellular domain of CRF2-12 and the extracellular domain of an IFN R1 type receptor;

(e) a nucleotide sequence encoding a chimeric receptor comprising the intracellular domain of CRF2-12 and the extracellular domain of IL-10R1;

(f) a nucleotide sequence encoding the chimeric protein in plasmid pEF-IL-10R1/IFN- λ R1; and

(g) a nucleotide sequence complementary to any of nucleotide sequences (a)-(f).

4. An isolated nucleic acid molecule comprising a tandem vector comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding two receptors, wherein the first receptor is an R1 type receptor and the second receptor is an R2 type receptor, and wherein the expression of each receptor is controlled by separate promoters and polyadenylation signals;

(b) a nucleotide sequence encoding two receptors, wherein the first receptor comprises the extracellular domain of CRF2-12 and the second receptor is an R2 type receptor, and wherein the expression of each receptor is controlled by separate promoters and polyadenylation signals;

(c) a nucleotide sequence encoding two receptors, wherein the first receptor comprises the intracellular domain of CRF2-12 and the second receptor is an R2 type receptor, and wherein the expression of each receptor is controlled by separate promoters and polyadenylation signals;

(d) a nucleotide sequence encoding two receptors, wherein the first receptor comprises CRF2-12 and the second receptor is an R2 type receptor, and wherein the expression of each receptor is controlled by separate promoters and polyadenylation signals;

(e) any of tandem vectors (a) – (d), wherein the R2 type receptor comprises CRF2-4;

(f) the tandem vector pEF-CRF2-12/ γ R1+CRF2-4; and

(g) a nucleotide sequence complementary to any of nucleotide sequences (a) –

(f).

5. An isolated nucleic acid molecule comprising a polynucleotide encoding at least a portion of an IFN- λ 1 polypeptide having the complete amino acid sequence shown in SEQ ID NO 8.

6. An isolated nucleic acid molecule comprising a polynucleotide encoding at least a portion of an IFN- λ 2 polypeptide having the complete amino acid sequence shown in SEQ ID NO 10.

7. An isolated nucleic acid molecule comprising a polynucleotide encoding at least a portion of an IFN- λ 2 polypeptide having the complete amino acid sequence shown in SEQ ID NO 12.

8. An isolated nucleic acid molecule comprising a polynucleotide comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the IFN- λ 1 polypeptide having the complete amino acid sequence in SEQ ID NO: 8;

(b) a nucleotide sequence encoding residues 2-200 of SEQ ID NO: 8

(c) a nucleotide sequence encoding residues 23-200 of SEQ ID NO: 8;

(d) a nucleotide sequence of the genomic fragment encoding the complete IFN- λ 1 gene contained in plasmid pEF-FL-IFN- λ 1 gene;

(e) a nucleotide sequence encoding the complete polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 1;

(f) a nucleotide sequence encoding the mature polypeptide encoded by the human cDNA contained in plasmid pEF-FL-IFN- λ 1; and

(g) a nucleotide sequence complementary to any of nucleotide sequences (a)-(f).

9. An isolated nucleic acid molecule comprising a polynucleotide comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the IFN- λ 2 polypeptide having the complete amino acid sequence in SEQ ID NO: 10;

(b) a nucleotide sequence encoding residues 2-196 of SEQ ID NO: 10;

(c) a nucleotide sequence encoding residues 23-196 of SEQ ID NO: 10;

(d) a nucleotide sequence encoding residues 12-196 of SEQ ID NO: 10;

(e) a nucleotide sequence encoding residues 13-196 of SEQ ID NO: 10;

(f) a nucleotide sequence encoding residues 31-196 of SEQ ID NO: 10;

(g) a nucleotide sequence encoding residues 92-196 of SEQ ID NO: 10;

(h) a nucleotide sequence encoding residues 93-196 of SEQ ID NO: 10;

- (i) a nucleotide sequence encoding residues 113-196 of SEQ ID NO: 10;
- (j) the nucleotide sequence of the genomic fragment encoding the complete IFN- λ 2 gene contained in plasmid pEF-FL-IFN- λ 2gene;
- (k) a nucleotide sequence encoding a polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 2;
- (l) a nucleotide sequence encoding the mature polypeptide encoded by the cDNA contained in clone pEF-FL-IFN- λ 2; and
- (m) a nucleotide sequence complementary to any of nucleotide sequences (a)-(l).

10. An isolated nucleic acid molecule comprising a polynucleotide comprising a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence encoding the IFN- λ 3 polypeptide having the complete amino acid sequence in SEQ ID NO: 12;
- (b) a nucleotide sequence encoding residues 2-196 of SEQ ID NO: 12;
- (c) a nucleotide sequence encoding residues 23-196 of SEQ ID NO: 12;
- (d) a nucleotide sequence encoding residues 6-196 of SEQ ID NO: 12;
- (e) a nucleotide sequence encoding residues 12-196 of SEQ ID NO: 12;
- (f) a nucleotide sequence encoding residues 7-196 of SEQ ID NO: 12;
- (g) a nucleotide sequence encoding residues 13-196 of SEQ ID NO: 12;

(h) a nucleotide sequence encoding residues 31-196 of SEQ ID NO: 12;

(i) the nucleotide sequence of the genomic fragment encoding the complete IFN- λ 3 gene contained in plasmid pEF-FL-IFN- λ 3 gene;

(j) a nucleotide sequence encoding a polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 3;

(k) a nucleotide sequence encoding a mature polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 3; and

(l) a nucleotide sequence complementary to any of nucleotide sequences (a)-(k).

11. A host cell containing a recombinant vector comprising a nucleic acid having a sequence selected from the sequences in claims 1-10.

12. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

the amino acid sequence of the full-length IFN- λ 1 polypeptide having the complete amino acid sequence of SEQ ID NO: 8;

amino acids 2-200 of SEQ ID NO: 8;

amino acids 23-200 of SEQ ID NO: 8;

an IFN- λ 1 polypeptide encoded by the genomic fragment encoding the IFN- λ 1 gene contained in plasmid pEF-FL-IFN- λ 1 gene;

an IFN- λ 1 polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 1; and

a mature IFN- λ 1 polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 1.

13. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

the complete amino acid sequence of SEQ ID NO: 10;

amino acids 2-196 of SEQ ID NO: 10;

amino acids 23-196 of SEQ ID NO: 10;

amino acids 12-196 of SEQ ID NO: 10;

amino acids 13-196 of SEQ ID NO: 10;

amino acids 31-196 of SEQ ID NO: 10;

amino acids 92-196 of SEQ ID NO: 10;

amino acids 93-196 of SEQ ID NO: 10;

amino acids 113-196 of SEQ ID NO: 10;

a IFN- λ 2 polypeptide encoded by plasmid pEF-FL-IFN- λ 2gene;

a polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 2; and

a mature polypeptide encoded by the cDNA contained in clone pEF-FL-IFN- λ 2.

14. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

a polypeptide having the complete amino acid sequence of SEQ ID NO: 12;

amino acids 2-196 of SEQ ID NO: 12;

amino acids 23-196 of SEQ ID NO: 12;

amino acids 6-196 of SEQ ID NO: 12;

amino acids 12-196 of SEQ ID NO: 12;

amino acids 7-196 of SEQ ID NO: 12;

amino acids 13-196 of SEQ ID NO: 12;

amino acids 31-196 of SEQ ID NO: 12;

an IFN- λ 3 polypeptide encoded by plasmid pEF-FL-IFN- λ 3gene;

a polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 3; and

a mature polypeptide encoded by the cDNA contained in clone pEF-FL-IFN- λ 3.

15. An isolated antibody that binds specifically to an epitope comprising a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 8, SEQ ID NO: 10 and SEQ ID NO: 12.

16. A composition comprising a polypeptide selected from the group consisting of IFN- λ 1, IFN- λ 2 and IFN- λ 3.

17. A composition comprising a combination of IFN- λ 1, IFN- λ 2 and IFN- λ 3 polypeptides, wherein the ratio of IFN- λ 1:IFN- λ 2:IFN- λ 3 is described by the formula m:n:o, wherein m may be any number between 0 and 1000, n may be any number between 0 and 1000 and o may be any number between 0 and 1000, and wherein at least one of m, n or o is different from 0.

18. A composition comprising an antibody selected from the group consisting of an anti-IFN- λ 1 antibody, an anti-IFN- λ 2 antibody and an anti-IFN- λ 3 antibody.

19. A composition comprising a combination of anti-IFN- λ 1 specific antibody, anti-IFN- λ 2 specific antibody and anti-IFN- λ 3 specific antibody, wherein the ratio of anti-IFN- λ 1 specific antibody: anti-IFN- λ 2 specific antibody: anti-IFN- λ 3 specific antibody is described by the formula x:y:z, wherein x may be any number between 0 and 1000, y may be any number between 0 and 1000 and z may be any number between 0 and 1000, and wherein at least one of x, y or z is different from 0.

20. A composition comprising an antibody specific for the extracellular domain of IFN- λ R1.

21. A method for identifying compounds capable of enhancing or inhibiting a biological activity of IFN- λ 1 comprising

- i) contacting a receptor which is activated by IFN- λ 1 with the candidate compound in the presence and in the absence of IFN- λ 1;
- ii) assaying for receptor activity in the presence of the candidate compound and IFN- λ 1;
- iii) assaying for receptor activity in the presence of the candidate compound and the absence of IFN- λ 1; and
- iv) comparing the activity to a standard level of activity, the standard being assayed when contact is made between the receptor and IFN- λ 1 in the absence of the candidate compound.

22. A method for identifying compounds capable of enhancing or inhibiting a biological activity of IFN- λ 2 comprising

- i) contacting a receptor which is activated by IFN- λ 2 with the candidate compound in the presence and in the absence of IFN- λ 2;
- ii) assaying for receptor activity in the presence of the candidate compound and IFN- λ 2;
- iii) assaying for receptor activity in the presence of the candidate compound and the absence of IFN- λ 2; and

iv) comparing the activity to a standard level of activity, the standard being assayed when contact is made between the receptor and IFN- λ 2 in the absence of the candidate compound.

23. A method for identifying compounds capable of enhancing or inhibiting a biological activity of IFN- λ 3 comprising

i) contacting a receptor which is activated by IFN- λ 3 with the candidate compound in the presence and in the absence of IFN- λ 3;

ii) assaying for receptor activity in the presence of the candidate compound and IFN- λ 3;

iii) assaying for receptor activity in the presence of the candidate compound and the absence of IFN- λ 3; and

iv) comparing the activity to a standard level of activity, the standard being assayed when contact is made between the receptor and IFN- λ 3 in the absence of the candidate compound.

24. A method for identifying compounds that block, or are agonists or antagonists of CRF2-12 comprising

i) contacting a receptor comprising CRF2-12 with a candidate compound in the presence and in the absence of an IFN- λ composition;

ii) assaying for receptor activity in the presence of the candidate compound and the IFN- λ composition;

iii) assaying for receptor activity in the presence of the candidate compound and the absence of the IFN- λ composition; and

iv) comparing the activity to a standard level of activity, the standard being assayed when contact is made between the receptor and the IFN- λ composition in the absence of the candidate compound.

25. The method of claim 23 wherein the IFN- λ composition is the composition of claim 16.

26. A diagnostic method useful during diagnosis of a disorder comprising

i) assaying the expression level of an mRNA or polypeptide selected from IFN- λ 1, IFN- λ 2, IFN- λ 3 and IFN- λ R1 in cells or body fluid of an individual; and

ii) comparing the expression level with a standard expression level,

whereby an increase or decrease in the assayed expression level compared to the standard expression level is indicative of a disorder.

27. A method for treating an individual in need of an increased level of interferon activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of a composition of claims 16, 17 or 18.

28. A method for treating an individual in need of a decreased level of interferon activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of a composition of claim 17.

29. A method of treating an individual in need of an increased level of activation of a receptor complex comprising CRF2-12, wherein the method comprises administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the receptor complex comprising CRF2-12.

30. A method of treating an individual in need of a decreased level of activation of a receptor complex comprising CRF2-12, wherein the method comprises administering to such an individual a composition comprising a therapeutically effective amount of an antagonist of the receptor complex comprising CRF2-12.

31. A method for testing a candidate composition as an antagonist or agonist of the IFN- λ R1 receptor complex, wherein the method comprises:

i) assaying for receptor activity in the presence of a negative control composition;

ii) assaying for receptor activity in the presence of a positive control composition;

iii) assaying for receptor activity in the presence of the candidate composition;

iv) assaying for receptor activity in the presence of the candidate composition and the positive control composition;

wherein the resulting activity from ii) is statistically greater than the resulting activity from i);

wherein the candidate composition is considered an agonist if the resulting activity from iii) is statistically greater than the resulting activity from i);

wherein the candidate composition is considered an antagonist if the resulting activity from iv) is statistically smaller than the resulting activity from ii);

wherein the candidate composition is considered neither an agonist nor an antagonist if the resulting activity from iv) is not statistically different from the resulting activity from ii) and the resulting activity from iii) is not statistically different from the resulting activity from i).

32. The method of claim 31 wherein the positive control composition comprises a composition selected from the group consisting of IFN- λ 1, IFN- λ 2, IFN- λ 3 and the composition of claim 17.

33. The method of claim 32 wherein the IFN- λ 1 composition is selected from the group consisting of:

the amino acid sequence of the full-length IFN- λ 1 polypeptide having the complete amino acid sequence of SEQ ID NO: 8;

amino acids 2-200 of SEQ ID NO: 8;

amino acids 23-200 of SEQ ID NO: 8;

an IFN- λ 1 polypeptide encoded by the genomic fragment encoding the IFN- λ 1 gene contained in plasmid pEF-FL-IFN- λ 1 gene;

an IFN- λ 1 polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 1; and

a mature IFN- λ 1 polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 1.

34. The method of claim 32 wherein the IFN- λ 2 composition is selected from the group consisting of:

the complete amino acid sequence of SEQ ID NO: 10;

amino acids 2-196 of SEQ ID NO: 10;

amino acids 23-196 of SEQ ID NO: 10;

amino acids 12-196 of SEQ ID NO: 10;

amino acids 13-196 of SEQ ID NO: 10;

amino acids 31-196 of SEQ ID NO: 10;

amino acids 92-196 of SEQ ID NO: 10;

amino acids 93-196 of SEQ ID NO: 10;

amino acids 113-196 of SEQ ID NO: 10;

a IFN- λ 2 polypeptide encoded by plasmid pEF-FL-IFN- λ 2gene;

a polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 2; and

a mature polypeptide encoded by the cDNA contained in clone pEF-FL-IFN- λ 2.

35. The method of claim 32 wherein the IFN- λ 3 composition is selected from the group consisting of:

a polypeptide having the complete amino acid sequence of SEQ ID NO: 12;

amino acids 2-196 of SEQ ID NO: 12;

amino acids 23-196 of SEQ ID NO: 12;

amino acids 6-196 of SEQ ID NO: 12;

amino acids 12-196 of SEQ ID NO: 12;

amino acids 7-196 of SEQ ID NO: 12;

amino acids 13-196 of SEQ ID NO: 12;

amino acids 31-196 of SEQ ID NO: 12;

an IFN- λ 3 polypeptide encoded by plasmid pEF-FL-IFN- λ 3gene;

a polypeptide encoded by the cDNA contained in plasmid pEF-FL-IFN- λ 3; and

a mature polypeptide encoded by the cDNA contained in clone pEF-FL-IFN- λ 3.

Fig.1

```

1   gccATGGCGG GGGCCGAGCG CTGGGGCCCC CTGCTCCTGT GCCTGCTGCA
51  GGCCGCTCCA GGGAGGCCCC GTCTGGCCCC TCCCCAGAAT GTGACGCTGC
101 TCTCCCAGAA CTTCAGCGTG TACCTGACAT GGCTCCCAGG GCTTGGCAAC
151 CCCCAGGATG TGACCTATTT TGTGGCCTAT CAGAGCTCTC CCACCCGTAG
201 ACGGTGGCGC GAAGTGGAAG AGTGTGCGGG AACCAAGGAG CTGCTATGTT
251 CTATGATGTG CCTGAAGAAA CAGGACCTGT ACAACAAGTT CAAGGGACGC
301 GTGCGGACGG TTTCTCCAG CTCCAAGTCC CCCTGGGTGG AGTCCGAATA
351 CCTGGATTAC CTTTTTGAAG TGGAGCCGGC CCCACCTGTC CTGGTGCTCA
401 CCCAGACGGA GGAGATCCTG AGTGCCAATG CCACGTACCA GCTGCCCCCC
451 TGCATGCCCC CACTGGATCT GAAGTATGAG GTGGCATTCT GGAAGGAGGG
501 GGCCGGAAAC AAGACCCTAT TTCCAGTCAC TCCCCATGGC CAGCCAGTCC
551 AGATCACTCT CCAGCCAGCT GCCAGCGAAC ACCACTGCCT CAGTGCCAGA
601 ACCATCTACA CGTTCAGTGT CCCGAAATAC AGCAAGTTCT CTAAGCCCAC
651 CTGCTTCTTG CTGGAGGTCC CAGAAGCCAA CTGGGCTTTC CTGGTGCTGC
701 CATCGCTTCT GATACTGCTG TTAGTAATTG CCGCAGGGGG TGTGATCTGG
751 AAGACCCTCA TGGGGAACCC CTGGTTTCAG CGGGCAAAGA TGCCACGGGC
801 CCTGGACTTT TCTGGACACA CACACCCTGT GGCAACCTTT CAGCCCAGCA
851 GACCAGAGTC CGTGAATGAC TTGTTCTCT GTCCCCAAAA GGAAGTACC
901 AGAGGGGTCA GGCCGACGCC TCGAGTCAGG GCCCCAGCCA CCCAACAGAC
951 AAGATGGAAG AAGGACCTTG CAGAGGACGA AGAGGAGGAG GATGAGGAGG
1001 ACACAGAAGA TGGCGTCAGC TTCCAGCCCT ACATTGAACC ACCTTCTTTC
1051 CTGGGGCAAG AGCACCAGGC TCCAGGGCAC TCGGAGGCTG GTGGGGTGGA
1101 CTCAGGGAGG CCCAGGGCTC CTCTGGTCCC AAGCGAAGGC TCCTCTGCTT
1151 GGGATTCTTC AGACAGAAGC TGGGCCAGCA CTGTGGACTC CTCCTGGGAC
1201 AGGGCTGGGT CCTCTGGCTA TTTGGCTGAG AAGGGGCCAG GCCAAGGGCC
1251 GGGTGGGGAT GGGCACCAAG AA1CTCTCCC ACCACCTGAA TTCTCCAAGG

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1451 AAGAGGAGGC GAGGGAATCA GAAATTGAGG ACAGCGATGC GGGCAGCTGG
1501 GGGGCTGAGA GCACCCAGAG GACCGAGGAC AGGGGCCCGA CATTGGGGCA
1551 TTACATGGCC AGGTGAgctg tcccccgaca tcccaccgaa tctgatgctg
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1651 cgtacctccc ctgggcggcg gaggggcatt gcacttcgg gaagtccacc
1701 tagcggctgt ttgcctgtcg ggctgagcaa caagatgccc ctccctcctg
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3701 gctatcatca gcatcatctt agacttgtat gaaataacca ctccgtttct
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4451 atatttaaac gatatcctac tgcttggttg tattacccta aactgtttaa
4501 ataaagagct ctatttttaa agaaaaagg tacaattga

Fig. 2

1 MAGPERWGPL LLCLLQAAPG RRLAPPQNV TLLSQNFSVY LTWLPGLGNP
51 QDVTYFVAYQ SSPTRRRWRE VEECAGTKEL LCSMMCLKKQ DLYNKFKGRV
101 RTVSPSSKSP WVESEYLDYL FEVEPAPPVL VLTQTEEILS ANATYQLPPC
151 MPPLDLKYEV AFWKEGAGNK TLFVTPHGQ PVQITLQPAA SEHHCLSART
201 IYTFVFKYS KFSKPTCFLI EVPEANWAFI VLPSLLILLI VIAAGGVIWK
251 TLMGNPWFQR AKMPRALDFS GHHPVATFQ PSRPESVNDL FLCPOKELTR
301 GVRPTPRVRA PATQQTRWKK DLAEDDEEED EEDTEDGVSF QPYIEPPSFL
351 QGEHQAPGHS EAGGVDSGRP RAPLVPSEGS SAWDSSDRSW ASTVDSSWDR
401 AGSSGYLAEK GPGQGGGDG HQESLPPPEF SKDSGFLEEL PEDNLSSWAT
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501 AESTQRTEDR GRTLGHYMAR

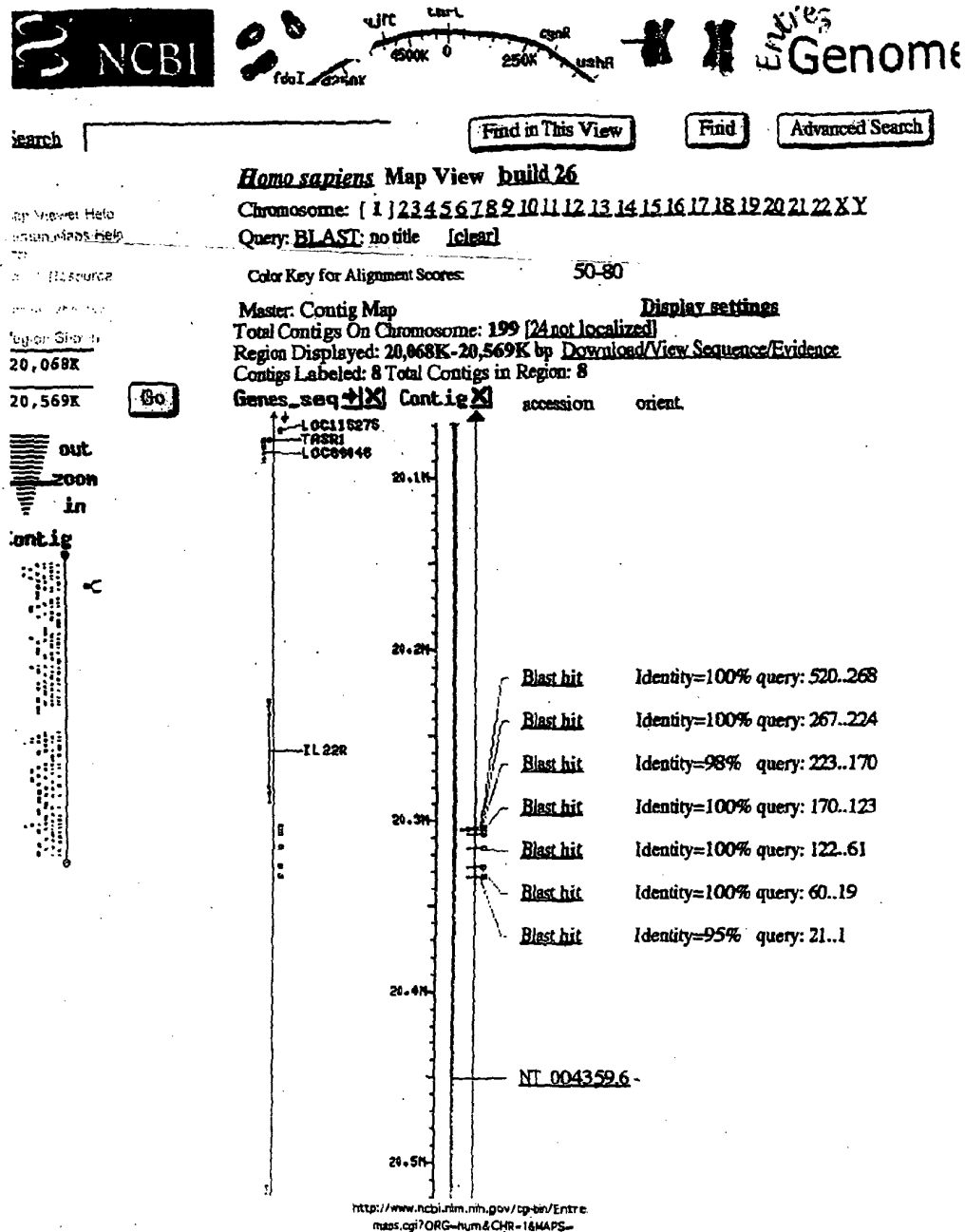


FIG. 3



Blast - Sequences results

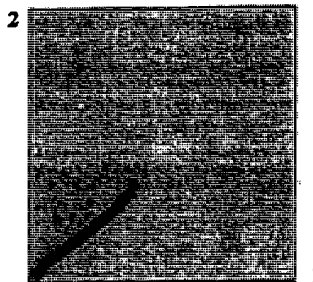
BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.1.2 [Oct-19-2000]

Matrix: BLOSUM62 gap open: 11 gap extension: 1
 x_dropoff: 50 expect: 10.0 wordsize: 3 Filter ☐ Align

-FIG. 4

Sequence 1 lcIHs1_4516_26_7_6 Length 423 (1..423)

Sequence 2 gi 7657691 Length 553 (1..553)



NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database

Score = 55.4 bits (131), Expect = 2e-06
 Identities = 54/190 (28%), Positives = 75/190 (39%), Gaps = 28/190 (14%)

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Query:      9  PLLLCLOAAGRP-----RLAPPQNVILLSONFSVILTWLPGLG-NPQDVITYFVAYQS 61
                L P N+T LS N  L W P G  VTY VY
Sbjct:     16  PLLLLLLAAPWGRAVPCVSGGLPKPANITFLSINMKNVLQWTPPEGLOGVKVITYTVOYFI 75
Tissue factor 33  *****

Query:     62  SPTRRRWREVEECAGTKELLCMMCLKKODLYNKFAGRVRTVSPSSKSPWVESEYLDYLF 121
                +++W  EC  C +  + D +++ +V+ + + S W ES
Sbjct:     76  YG-QKKWLNKSECRNINRTYCDLSA-ETSDYEHQYAKVKAIWGTKCKWRESGRFPYFPL 133
Tissue factor 76  ** *****

Query:     122  EVEPAPPVLVLTQTREILSANAT-----YQLPPCMPPL--DLKYEVAE----- 162
                E + PP + LT E+ +S  T          LP M + +LKY V+
Sbjct:     134  ETQIGPPEVALTTDEKSISVVLTAPEKWKRNPEFLVSMQOIYSNLKYNVSVLMTKSNRT 193
Tissue factor 134 *****

Query:     163  WKEGAGNKTLL 172
                W +  N TL
Sbjct:     194  WSQCVTNHTLL 203
Tissue factor 194 *****

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<http://www.ncbi.nlm.nih.gov/cgi/wblast2.cgi>

FIG. 6

FIG. 6

File Name : ProtX(GG/SK seq)CDNA
 Range : 4 - 1566 Mode : Normal
 Codon Table : Universal

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	CCA	GGG	AGG	CCC	CGT	CTG	GCC	CCT	CCC	CAG	AAT	GTG	ACG	CTG	CTC	TCC	CAG	AAC	111
	P	G	R	P	R	L	A	P	P	Q	N	V	T	L	L	S	Q	N	
	TTC	AGC	GTG	TAC	CTG	ACA	TGG	CTC	CCA	GGG	CTT	GGC	AAC	CCC	CAG	GAT	GTG	ACC	165
	F	S	V	Y	L	T	W	L	P	G	L	G	N	P	Q	D	V	T	
	TAT	TTT	GTG	GCC	TAT	CAG	AGC	TCT	CCC	ACC	CGT	AGA	CGG	TGG	CGC	GAA	GTG	GAA	219
	Y	F	V	A	Y	Q	S	S	P	T	R	R	R	W	R	E	V	E	
	GAG	TGT	GCG	GGA	ACC	AAG	GAG	CTG	CTA	TGT	TCT	ATG	ATG	TGC	CTG	AAG	AAA	CAG	273
	E	C	A	G	T	K	E	L	L	C	S	M	N	C	L	K	K	Q	
	GAC	CTG	TAC	AAC	AAG	TTC	AAG	GGA	CGC	GTG	CGG	ACG	GTT	TCT	CCC	AGC	TCC	AAG	327
	D	L	Y	N	K	F	K	G	R	V	R	T	V	S	P	S	S	K	
	TCC	CCC	TGG	GTG	GAG	TCC	GAA	TAC	CTG	GAT	TAC	CTT	TTT	GAA	GTG	GAG	CCG	GCC	381
	S	P	W	V	E	S	E	Y	L	D	Y	L	F	E	V	E	P	A	
	CCA	CCT	GTC	CTG	GTG	CTC	ACC	CAG	ACG	GAG	GAG	ATC	CTG	AGT	GCC	AAT	GCC	ACG	435
	P	P	V	L	V	L	T	Q	T	E	E	I	L	S	A	N	A	T	
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	Y	Q	L	P	P	C	M	P	P	L	D	L	K	Y	E	V	A	F	
	TGG	AAG	GAG	GGG	GCC	GGA	AAC	AAG	ACC	CTA	TTT	CCA	GTC	ACT	CCC	CAT	GGC	CAG	543
	W	K	E	G	A	G	N	K	T	L	F	P	V	T	P	H	G	Q	
	CCA	GTC	ATC	ATC	ACT	CTC	CAG	CCA	GCT	GCC	AGC	GAA	CAC	CAC	TGC	CTC	AGT	GCC	597
	P	V	Q	I	T	L	Q	P	A	A	S	E	H	H	C	L	S	A	
	AGA	ACC	ATC	TAC	ACG	TTC	AGT	GTC	CCG	AAA	TAC	AGC	AAG	TTC	TCT	AAG	CCC	ACC	651
	R	T	I	Y	T	F	S	V	P	K	Y	S	K	F	S	K	P	T	

FIG. 7

ANALYSIS DNA Translation (Protein/SR sequence)

TGC	TTC	660	TTC	CTG	GAG	669	GTC	CCA	G	578	JCC	AAC	TGG	687	GCT	TTC	CTG	696	GTG	CT	705	A	TCC
C	F	L	L	E	V	P	E	A	N	W	A	F	L	V	L	P	S						
CTT	CTG	714	ATA	CTG	CTG	723	TTA	GTA	ATT	732	GCC	GCA	GGG	741	GGT	GTG	ATC	750	TGG	AAG	ACC	759	CTC
L	L	I	L	L	L	V	I	A	A	G	G	V	I	W	K	T	L						
ATG	GGG	768	AAC	CCC	TGG	777	TTT	CAG	CGG	786	GCA	AAG	ATG	795	CCA	CGG	GCC	804	CTG	GAC	TTT	813	TCT
M	G	N	P	W	F	Q	R	A	K	M	P	R	A	L	D	F	S						
GGA	CAC	822	ACA	CAC	CCT	831	GTG	GCA	ACC	840	TTT	CAG	CCC	849	AGC	AGA	CCA	858	GAG	TCC	867	GTG	AAT
G	H	T	H	P	V	A	T	F	Q	P	S	R	P	E	S	V	N						
GAC	TTG	876	TTC	CTC	TGT	885	CCC	CAA	AAG	894	GAA	CTG	ACC	903	AGA	GGG	GTG	912	AGG	CCG	921	ACG	CCT
D	L	F	L	C	P	Q	K	E	L	T	R	G	V	R	P	T	P						
CGA	GTC	930	AGG	GCC	CCA	939	GCC	ACC	CAA	948	CAG	ACA	AGA	957	TGG	AAG	AAG	966	GAC	CTT	975	GCA	GAG
R	V	R	A	P	A	T	Q	Q	T	R	W	K	K	D	L	A	E						
GAC	GAA	984	GAG	GAG	GAG	993	GAT	GAG	GAG	1002	GAC	ACA	GAA	1011	GAT	GGC	GTG	1020	AGC	TTC	1029	CAG	CCC
D	E	E	E	E	D	E	E	D	T	E	D	G	V	S	F	Q	P						
TAC	ATT	1038	GAA	CCA	CCT	1047	TCT	TTC	CTG	1056	GGG	CAA	GAG	1065	CAC	CAG	GCT	1074	CCA	GGG	1083	CAC	TCC
Y	I	E	P	P	S	F	L	G	Q	E	H	Q	A	P	G	H	S						
GAG	GCT	1092	GGT	GGG	GTG	1101	GAC	TCA	GGG	1110	AGG	CCC	AGG	1119	GCT	CCT	CTG	1128	GTC	CCA	1137	AGC	GAA
E	A	G	G	V	D	S	G	R	P	R	A	P	L	V	P	S	E						
GGC	TCC	1146	TCT	GCT	TGG	1155	GAT	TCT	TCA	1164	GAC	AGA	AGC	1173	TGG	GCC	AGC	1182	ACT	GTG	1191	GAC	TCC
G	S	S	A	W	D	S	S	D	R	S	W	A	S	T	V	D	S						
TCC	TGG	1200	GAC	AGG	GCT	1209	GGG	TCC	TCT	1218	GGC	TAT	TTG	1227	GCT	GAG	AAG	1236	GGG	CCA	1245	GGC	CAA
S	W	D	R	A	G	S	S	G	Y	L	A	E	K	G	P	G	Q						
GGG	CCG	1254	GGT	GGG	GAT	1263	GGG	CAC	CAA	1272	GAA	TCT	CTC	1281	CCA	CCA	CCT	1290	GAA	TTC	1299	TCC	AAG
G	P	G	G	D	G	H	Q	E	S	L	P	P	P	E	F	S	K						
GAC	TCG	1308	GGT	TTC	CTG	1317	GAA	GAG	CTC	1326	CCA	GAA	GAT	1335	AAC	CTC	TCC	1344	TCC	TGG	1353	GCC	ACC
D	S	G	F	L	E	E	L	P	E	D	N	L	S	S	W	A	T						

INAS15 DNA translation LPP02A(00.00) sequence

1362	1371	1380	1389	1398	1407
TGG GGC ACC	TTA CCA CCG GAG	AAT CTG GTC CCT	GGG GGA CCC C	TT TCT	
W	G T L P P E P	N L V P G G	P P V S		
1416	1425	1434	1443	1452	1461
CTT CAG ACA	CTG ACC TTC TGC TGG GAA AGC AGC CCT	GAG GAG GAA GAG GAG GCG			
L	Q T L T F C W E S S P E E E E A				
1470	1479	1488	1497	1506	1515
AGG GAA TCA GAA ATT GAG GAC AGC GAT GCG GGC AGC TGG GGG GCT GAG AGC ACC					
R E S E I E D S D A G S W G A E S T					
1524	1533	1542	1551	1560	
CAG AGG ACC GAG GAC AGG GGC CGG ACA TTG GGG CAT TAC ATG GCC AGG TGA 3'					
Q R T E D R G R T L G H Y M A R *					

DNASIS DNA Translation (ProtX(GG/SK seq)CDNA
 File Name : ProtX(GG/SK seq)CDNA
 Range : 4 - 1566 Mod Normal
 Codon Table : Universal

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5'  ATG GCG GGG CCC GAG CGC TGG GGC CCC CTG CTC CTG TGC CTG CTG CAG GCC GCT
    Met Ala Gly Pro Glu Arg Trp Gly Pro Leu Leu Leu Cys Leu Leu Gln Ala Ala

    CCA GGG AGG CCC CGT CTG GCC CCT CCC CAG AAT GTG ACG CTG CTC TCC CAG AAC
    Pro Gly Arg Pro Arg Leu Ala Pro Pro Gln Asn Val Thr Leu Leu Ser Gln Asn

    TTC AGC GTG TAC CTG ACA TGG CTC CCA GGG CTT GGC AAC CCC CAG GAT GTG ACC
    Phe Ser Val Tyr Leu Thr Trp Leu Pro Gly Leu Gly Asn Pro Gln Asp Val Thr

    TAT TTT GTG GCC TAT CAG AGC TCT CCC ACC CGT AGA CGG TGG CGC GAA GTG GAA
    Tyr Phe Val Ala Tyr Gln Ser Ser Pro Thr Arg Arg Arg Trp Arg Glu Val Glu

    GAG TGT GCG GGA ACC AAG GAG CTG CTA TGT TCT ATG ATG TGC CTG AAG AAA CAG
    Glu Cys Ala Gly Thr Lys Glu Leu Leu Cys Ser Met Met Cys Leu Lys Lys Gln

    GAC CTG TAC AAC AAG TTC AAG GGA CGC GTG CGG ACG GTT TCT CCC AGC TCC AAG
    Asp Leu Tyr Asn Lys Phe Lys Gly Arg Val Arg Thr Val Ser Pro Ser Ser Lys

    TCC CCC TGG GTG GAG TCC GAA TAC CTG GAT TAC CTT TTT GAA GTG GAG CCG GCC
    Ser Pro Trp Val Glu Ser Glu Tyr Leu Asp Tyr Leu Phe Glu Val Glu Pro Ala

    CCA CCT GTC CTG GTG CTC ACC CAG ACG GAG GAG ATC CTG AGT GCC AAT GCC ACG
    Pro Pro Val Leu Val Leu Thr Gln Thr Glu Glu Ile Leu Ser Ala Asn Ala Thr

    TAC CAG CTG CCC CCC TGC ATG CCC CCA CTG GAT CTG AAG TAT GAG GTG GCA TTC
    Tyr Gln Leu Pro Pro Cys Met Pro Pro Leu Asp Leu Lys Tyr Glu Val Ala Phe

    TGG AAG GAG GGG GCC GGA AAC AAG ACC CTA TTT CCA GTC ACT CCC CAT GGC CAG
    Trp Lys Glu Gly Ala Gly Asn Lys Thr Leu Phe Pro Val Thr Pro His Gly Gln

    CCA GTC CAG ATC ACT CTC CAG CCA GCT GCC AGC GAA CAC CAC TGC CTC AGT GCC
    Pro Val Gln Ile Thr Leu Gln Pro Ala Ala Ser Glu His His Cys Leu Ser Ala

    AGA ACC ATC TAC ACG TTC AGT GTC CCG AAA TAC AGC AAG TTC TCT AAG CCC ACC
    Arg Thr Ile Tyr Thr Phe Ser Val Pro Lys Tyr Ser Lys Phe Ser Lys Pro Thr
  
```

FIG 8

INASIS DNA translation (Protein sequence)

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      660      669      678      687      696      705
TGC TTC TTG CTG GAG GTC CCA GCC AAC TGG GCT TTC CTG GTG CTA TCG
Cys Phe Leu Leu Glu Val Pro Glu Ala Asn Trp Ala Phe Leu Val Leu Pro Ser

      714      723      732      741      750      759
CTT CTG ATA CTG CTG TTA GTA ATT GCC GCA GGG GGT GTG ATC TGG AAG ACC CTC
Leu Leu Ile Leu Leu Leu Val Ile Ala Ala Gly Gly Val Ile Trp Lys Thr Leu

      768      777      786      795      804      813
ATG GGG AAC CCC TGG TTT CAG CGG GCA AAG ATG CCA CGG GCC CTG GAC TTT TCT
Met Gly Asn Pro Trp Phe Gln Arg Ala Lys Met Pro Arg Ala Leu Asp Phe Ser

      822      831      840      849      858      867
GGA CAC ACA CAC CCT GTG GCA ACC TTT CAG CCC AGC AGA CCA GAG TCC GTG AAT
Gly His Thr His Pro Val Ala Thr Phe Gln Pro Ser Arg Pro Glu Ser Val Asn

      876      885      894      903      912      921
GAC TTG TTC CTC TGT CCC CAA AAG GAA CTG ACC AGA GGG GTC AGG CCG ACG CCT
Asp Leu Phe Leu Cys Pro Gln Lys Glu Leu Thr Arg Gly Val Arg Pro Thr Pro

      930      939      948      957      966      975
CGA GTC AGG GCC CCA ACC CAA CAG ACA AGA TGG AAG AAG GAC CTT GCA GAG
Arg Val Arg Ala Pro Ala Thr Gln Gln Thr Arg Trp Lys Lys Asp Leu Ala Glu

      984      993      1002      1011      1020      1029
GAC GAA GAG GAG GAT GAG GAG GAC ACA GAA GAT GGC GTC AGC TTC GAC CCC
Asp Glu Glu Glu Glu Asp Glu Glu Asp Thr Glu Asp Gly Val Ser Phe Gln Pro

      1038      1047      1056      1065      1074      1083
TAC ATT GAA CCA CCT TCT TTC CTG GGG CAA GAG CAC CAG GCT CCA GGG CAC TCG
Tyr Ile Glu Pro Pro Ser Phe Leu Gly Gln Glu His Gln Ala Pro Gly His Ser

      1092      1101      1110      1119      1128      1137
GAG GCT GGT GGG GTG GAC TCA GGG AGG CCC AGG GCT CCT CTG GTC CCA AGC GAA
Glu Ala Gly Gly Val Asp Ser Gly Arg Pro Arg Ala Pro Leu Val Pro Ser Glu

      1146      1155      1164      1173      1182      1191
GGC TCC TCT GCT TGG GAT TCT TCA GAC AGA AGC TGG GCC AGC ACT GTG GAC TCC
Gly Ser Ser Ala Trp Asp Ser Ser Asp Arg Ser Trp Ala Ser Thr Val Asp Ser

      1200      1209      1218      1227      1236      1245
TCC TGG GAC AGG GCT GGG TCC TCT GGC TAT TTG GCT GAG AAG GGG CCA GGC CAA
Ser Trp Asp Arg Ala Gly Ser Ser Gly Tyr Leu Ala Glu Lys Gly Pro Gly Gln

      1254      1263      1272      1281      1290      1299
GGG CCG GGT GGG GAT GGG CAC CAA GAA TCT CTC CCA CCA CCT GAA TTC TCC AAG
Gly Pro Gly Gly Asp Gly His Gln Glu Ser Leu Pro Pro Pro Glu Phe Ser Lys

      1308      1317      1326      1335      1344      1353
GAC TCG GGT TTC CTG GAA GAG CTC CCA GAA GAT AAC CTC TCC TCC TGG GCC ACC
Asp Ser Gly Phe Leu Glu Glu Leu Pro Glu Asp Asn Leu Ser Ser Trp Ala Thr

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INASIS DNA Translation [Protein (66/58) sequence]

1362	1371	1380	1389	1398	1407
TGG GGC ACC TTA CCA CCG GAG C	AAT CTG GTC CCT GGG GGA CCC CC	T TCT			
Trp Gly Thr Leu Pro Pro Glu Pro Asn Leu Val Pro Gly Gly Pro Pro Val Ser					
1416	1425	1434	1443	1452	1461
CTT CAG ACA CTG ACC TTC TGC TGG GAA AGC AGC CCT GAG GAG GAA GAG GAG GCG					
Leu Gln Thr Leu Thr Phe Cys Trp Glu Ser Ser Pro Glu Glu Glu Glu Glu Ala					
1470	1479	1488	1497	1506	1515
AGG GAA TCA GAA ATT GAG GAC AGC GAT GCG GGC AGC TGG GGG GCT GAG AGC ACC					
Arg Glu Ser Glu Ile Glu Asp Ser Asp Ala Gly Ser Trp Gly Ala Glu Ser Thr					
1524	1533	1542	1551	1560	
CAG AGG ACC GAG GAC AGG GGC CGG ACA TTG GGG CAT TAC ATG GCC AGG TGA 3'					
Gln Arg Thr Glu Asp Arg Gly Arg Thr Leu Gly His Tyr Met Ala Arg ***					

UNK243
 Prot-X(GG/SK predn)prot.DNA

10	20	30	40	50	60
ATGGCNGGNC	CNGARMGNTG	GGGNCAYTN	YTNYTNTGYY	TNYTNCARGC	NGCNC
70	80	90	100	110	120
MGNCNCMGNY	TNGCNCNCC	NCARAAYGTN	ACHYTNNTNW	SNCARAAYTT	YWSNGTNTAY
130	140	150	160	170	180
YTHACNTGGY	TNCCNGGNYT	NGGNAAYCCN	CARGAYGTNA	CNTAYTTYGT	NGCNTAYCAR
190	200	210	220	230	240
WSNWSNCCNA	CNMGMNMG	NTGGMGNGAR	GTNGARGART	GYGCNGGNAC	NAARGARYTN
250	260	270	280	290	300
YTNTGYWSNA	TGATGTGYT	NAARAARCAR	GAYTNTTAYA	AYAARTTYAA	RGGMNGMGTN
310	320	330	340	350	360
MGNACNGTNW	SNCCNWSNWS	NAARWSNCCN	TGGGTNGARM	SNGARTAYYT	NGAYTAYTTH
370	380	390	400	410	420
TTYGARGTNG	ARCCNGCNC	NCCNGTNYTN	GTNYTNACNC	ARACNGARGA	RATHYTNWSN
430	440	450	460	470	480
GCNAAYGCNA	CNTAYCARYT	NCCNCNTGY	ATGCCNCNY	TNGAYYTNA	RTAYGARGTN
490	500	510	520	530	540
GCNTTYTGG	ARGARGGNGC	NGGNAAYAR	ACHYTNNTTYC	CNGTNACNCC	NCAYGGNCAR
550	560	570	580	590	600
CCNGTNCARA	THACHYTNCA	RCCNGCNGCN	WSHGARCAYC	AYTGYTNWS	NGCNGMGNACN
610	620	630	640	650	660
ATHYAYCNT	TYWSNGTNCC	NAARTAYWSN	AARTTYWSNA	ARCCNACNTG	YTTYTYTNYTN
670	680	690	700	710	720
GARGTNCCNG	ARGCNAAYTG	GGCNTTYTTH	GTNYTNCCNW	SNYTNAT	HYTNNTNYTN
730	740	750	760	770	780
GTNATHGCNG	CNGGNGGNGT	NATHTGGAAR	ACHYTNATGG	GNAAYCCNTG	GTTYCARGMN
790	800	810	820	830	840
GCNAARATGC	CNMGNCCNYT	NGAYTTYWSN	GGNCAYACNC	AYCCNGTNGC	NACNTTYCAR
850	860	870	880	890	900
CCNWSNMGNC	CNGARNSGT	NAAYGAYTN	TTYTNTGYC	CNCARAARGA	RYTNACHMGN
910	920	930	940	950	960
GGNGTNMGNC	CNACNCCNMG	NGTNMGHGCN	CCNGCNAACNC	ARCARACNMG	NTGGAARAAR
970	980	990	1000	1010	1020
GAYTNGCNG	ARGAYGARGA	RGARGARGAY	GARGARGAYA	CNGARGAYGG	NGTNWSHTTY
1030	1040	1050	1060	1070	1080
CARCCNTAYA	THGARCCNCC	NWSNTTYTTH	GGNCARGARC	AYCARGCNC	NGGNCAYWSN
1090	1100	1110	1120	1130	1140
GARGCNGGNG	GNGTNGAYWS	NGGNGMGNCCN	MGNCCNCNY	TNGTNCCNWS	NGARGGNWSN
1150	1160	1170	1180	1190	1200
WSNGCNTGGG	AYWSNWSNGA	YMGNWSNTGG	GCNWSNACNG	TNGAYWSNWS	NTGGGAYMGN
1210	1220	1230	1240	1250	1260
GCNGGNWSNW	SNGGNTAYYT	NGCNGARAAR	GGNCCNGGNC	ARGGNCNGG	NGGNGAYGNG
1270	1280	1290	1300	1310	1320

FIG 9

.....
 Prot-X(GG/SK predn)prot.DNA

CAYCARGARW	SNYTNCCNCC	NCCK	TY	WSNAARGAYW	SNGGHTTYT	NGAR	'N
1330	1340	1350	1360	1370	1380		
CCNGARGAYA	AYYTNWSNWS	NTGGGCNACN	TGGGGNACNY	TNCCNCNGA	RCCNAAYTN		
1390	1400	1410	1420	1430	1440		
GTNCCNGGNG	GNCCNCCNGT	NWSNYTNCAR	ACNYTNACNT	TYTGYTGGGA	RWSNWSNCCN		
1450	1460	1470	1480	1490	1500		
GARGARGARG	ARGARGCMNG	NGARWSNGAR	ATHGARGAYW	SNGAYGCNGG	NWSNTGGGGH		
1510	1520	1530	1540	1550	1560		
GCNGARWSNA	CNCARMGNAC	NGARGAYMGN	GGNMGNACNY	TNGGNCAYTA	YATGGCENNGN		
1570	1580	1590	1600	1610	1620		
.....							

INAS15 Amino Acid Conversion Table
 File Name: Prot-X(GG/SK predn)prot
 Range: 1 - 520
 Codon Table: Universal

										10										20									
Met	Ala	Gly	Pro	Glu	Arg	Trp	Gly	Pro	Leu	Leu	Cys	Leu	Leu	Gln	Ala	Ala	Pro	Gly											
ATG	GCN	GGN	CCN	GAR	MGN	TGG	GGN	CCN	YTN	YTN	TGY	YTN	YTN	CAR	GCN	GCN	CCN	GGN											
ATG	GCT	GGT	CCT	GAA	CGT	TGG	GGT	CCT	TTA	TTA	TGT	TTA	TTA	CAA	GCT	GCT	CCT	GGT											
	GCC	GGC	CCC	GAG	CGC		GGC	CCC	TTG	TTG	TGC	TTG	TTG	CAG	GCC	GCC	CCC	GGC											
	GCA	GGA	CCA		CGA		GGA	CCA	CTT	CTT	CTT	CTT	CTT		GCA	GCA	CCA	GGA											
	GCG	GGG	CCG		CGG		GGG	CCG	CTC	CTC	CTC	CTC	CTC		GCG	GCG	CCG	GGG											
					AGA				CTA	CTA	CTA	CTA	CTA																
					AGG				CTG	CTG	CTG	CTG	CTG																
Arg	Pro	Arg	Leu	Ala	Pro	Pro	Gln	Asn	Val	Thr	Leu	Leu	Ser	Gln	Asn	Phe	Ser	Val	Tyr										
MGN	CCN	MGN	YTN	GCN	CCN	CCN	CAR	AAY	GTN	ACN	YTN	YTN	WSN	CAR	AAY	TTY	WSN	GTN	TAY										
CGT	CCT	CGT	TTA	GCT	CCT	CCT	CAA	AAT	GTT	ACT	TTA	TTA	TCT	CAA	AAT	TTT	TCT	GTT	TAT										
CGC	CCC	CGC	TTG	GCC	CCC	CCC	CAG	AAC	GTC	ACC	TTG	TTG	TCC	CAG	AAC	TTT	TCC	GTC	TAC										
CGA	CCA	CGA	CTT	GCA	CCA	CCA			GTA	ACA	CTT	CTT	TCA				TCA	GTA											
CGG	CCG	CGG	CTC	GCG	CCG	CCG			GTG	ACG	CTC	CTC	TCG				TCG	GTG											
AGA			CTA						CTA	CTA	AGT						AGT												
AGG			CTG						CTG	CTG	AGC						AGC												
Leu	Thr	Trp	Leu	Pro	Gly	Leu	Gly	Asn	Pro	Gln	Asp	Val	Thr	Tyr	Phe	Val	Ala	Tyr	Gln										
YTN	ACN	TGG	YTN	CCN	GGN	YTN	GGN	AAY	CCN	CAR	GAY	GTN	ACN	TAY	TTY	GTN	GCN	TAY	CAR										
TTA	ACT	TGG	TTA	CCT	GGT	TTA	GGT	AAT	CCT	CAA	GAT	GTT	ACT	TAT	TTT	GTT	GCT	TAT	CAA										
TTG	ACC		TTG	CCC	GGC	TTG	GGC	AAC	CCC	CAG	GAC	GTC	ACC	TAC	TTT	GTC	GCC	TAC	CAG										
CTT	ACA		CTT	CCA	GGA	CTT	GGA	CCA				GTA	ACA			GTA	GCA												
CTC	ACG		CTC	CCG	GGG	CTC	GGG	CCG				GTG	ACG			GTG	GCG												
CTA			CTA			CTA																							
CTG			CTG			CTG																							
Ser	Ser	Pro	Thr	Arg	Arg	Arg	Trp	Arg	Glu	Val	Glu	Glu	Cys	Ala	Gly	Thr	Lys	Glu	Leu										
WSN	WSN	CCN	ACN	MGN	MGN	MGN	TGG	MGN	GAR	GTN	GAR	GAR	TGY	GCN	GGN	ACN	AAR	GAR	YTN										
TCT	TCT	CCT	ACT	CGT	CGT	CGT	TGG	CGT	GAA	GTT	GAA	GAA	TGT	GCT	GGT	ACT	AAA	GAA	TTA										
TCC	TCC	CCC	ACC	CGC	CGC	CGC		CGC	GAG	GTC	GAG	GAG	TGC	GCC	GGC	ACC	AAG	GAG	TTG										
TCA	TCA	CCA	ACA	CGA	CGA	CGA		CGA						GCA	GGA	ACA		CTT											
TCG	TCG	CCG	ACG	CGG	CGG	CGG		CGG		GTG				GCG	GGG	ACG		CTC											
AGT	AGT			AGA	AGA	AGA		AGA										CTA											
AGC	AGC			AGG	AGG	AGG		AGG										CTG											
Leu	Cys	Ser	Met	Met	Cys	Leu	Lys	Lys	Gln	Asp	Leu	Tyr	Asn	Lys	Phe	Lys	Gly	Arg	Val										
YTN	TGY	WSN	ATG	ATG	TGY	YTN	AAR	AAR	CAR	GAY	YTN	TAY	AAY	AAR	TTY	AAR	GGN	MGN	GTN										
TTA	TGT	TCT	ATG	ATG	TGT	TTA	AAA	AAA	CAA	GAT	TTA	TAT	AAT	AAA	TTT	AAA	GGT	CGT	GTT										
TTG	TGC	TCC			TGC	TTG	AAG	AAG	CAG	GAC	TTG	TAC	AAC	AAG	TTT	AAG	GGC	CGC	GTC										
CTT		TCA				CTT					CTT						GGA	CGA	GTA										
CTC		TCG				CTC					CTC						GGG	CGG	GTG										
CTA		AGT				CTA					CTA																		
CTG		AGC				CTG					CTG																		
Arg	Thr	Val	Ser	Pro	Ser	Ser	Lys	Ser	Pro	Trp	Val	Glu	Ser	Glu	Tyr	Leu	Asp	Tyr	Leu										
MGN	ACN	GTN	WSN	CCN	WSN	WSN	AAR	WSN	CCN	TGG	GTN	GAR	WSN	GAR	TAY	YTN	GAY	TAY	YTN										
CGT	ACT	GTT	TCT	CCT	TCT	TCT	AAA	TCT	CCT	TGG	GTT	GAA	TCT	GAA	TAT	TTA	GAT	TAT	TTA										
CGC	ACC	GTC	TCC	CCC	TCC	TCC	AAG	TCC	CCC		GTC	GAG	TCC	GAG	TAC	TTG	GAC	TAC	TTG										
CGA	ACA	GTA	TCA	CCA	TCA	TCA		TCA	CCA		GTA	TCA	TCA			CTT													
CGG	ACG	GTG	TCG	CCG	TCG	TCG		TCG	CCG		GTG	TCG	TCG			CTC													

FIG. 10

DNASIS	Amino	Acid	Conversion	[Pre-X(GG/SK prean)prot]	CTA	CTA
AGA	AGT	AGT	AGT	AGT	CTA	CTA
AGG	AGC	AGC	AGC	AGC	CTG	CTG
130						
Phe	Glu	Val	Glu	Pro	Ala	Pro
TTY	GAR	GTN	GAR	CCN	GCN	CCN
TTT	GAA	GTT	GAA	CCT	GCT	CCT
TTC	GAG	GTC	GAG	CCC	GCC	CCC
		GTA		CCA	GCA	CCA
		GTG		CCG	GCG	CCG
				CTC	CTC	CTC
				CTA	CTA	CTA
				CTG	CTG	CTG
140						
Val	Leu	Thr	Gln	Thr	Glu	Glu
YTN	GTN	YTN	ACN	CAR	GAR	GAR
TTA	TTA	TTA	ACT	CAA	ACT	GAA
TTG	TTG	TTG	ACC	CAG	ACC	GAG
CTT	CTT	CTT	ACA	ACA	ATA	CTT
GTG	GTG	GTG	ACG	ACG	CTC	TCG
CTA	CTA	CTA	CTG	CTG	CTA	AGT
CTG	CTG	CTG	CTG	CTG	CTG	AGC
150						
Ala	Asn	Ala	Thr	Tyr	Gln	Leu
GCN	AAY	GCN	ACN	TAY	CAR	YTN
GCT	AAT	GCT	ACT	TAT	CAA	TTA
GCC	AAC	GCC	ACC	TAC	CAG	TTG
GCA	GCA	ACA			CTT	CCA
GCG	GCG	ACG			CTC	CCG
					CTA	CTG
					CTG	CTG
160						
Pro	Pro	Leu	Asp	Leu	Lys	Tyr
CCN	CCN	YTN	GAY	YTN	AAR	TAY
CTT	CTT	TTA	GAT	TTA	AAA	TAT
CCC	CCC	TTG	GAC	TTG	AAG	TAC
CCA	CCA	CTT		CTT		GAA
CCG	CCG	CTC		CTC		GAG
CTA	CTA	CTA		CTA		GTA
CTG	CTG	CTG		CTG		GTG
170						
Ala	Phe	Trp	Lys	Glu	Gly	Ala
GCN	TTY	TGG	AAR	GAR	GGN	GCN
GCT	TTT	TGG	AAA	GAA	GGT	GCT
GCC	TTT	TGG	AAA	GAG	GGC	GCC
GCA				GGA	GCA	GGA
GCG				GGG	GCG	GGG
					CTC	CTC
					CTA	CTA
					CTG	CTG
180						
Pro	Val	Thr	Pro	His	Gly	Gln
CCN	GTN	ACN	CCN	CAY	GGN	CAR
CTT	CTT	TTA	CTT	CTT	CTT	CAA
CCC	CCC	TTG	CCC	CTC	CTC	GAG
CCA	GTA	ACA	CCA	CTC	CTC	GTA
CCG	GTG	ACG	CCG	CTG	CTG	GTG
						CTA
						CTG
190						
Pro	Val	Gln	Ile	Thr	Leu	Gln
CCN	GTN	CAR	ATH	ACN	YTN	CAR
CCT	GTT	CAA	ATT	ACT	TTA	CAA
CCC	GTC	CAG	ATC	ACC	TTG	CAG
CCA	GTA	ATA	ACA	CTT		CCA
CCG	GTG		ACG	CTC		CCG
				CTA		CTA
				CTG		CTG
200						
Pro	Val	Gln	Ile	Thr	Leu	Gln
CCN	GTN	CAR	ATH	ACN	YTN	CAR
CCT	GTT	CAA	ATT	ACT	TTA	CAA
CCC	GTC	CAG	ATC	ACC	TTG	CAG
CCA	GTA	ATA	ACA	CTT		CCA
CCG	GTG		ACG	CTC		CCG
				CTA		CTA
				CTG		CTG
210						
Ile	Tyr	Thr	Phe	Ser	Val	Pro
ATH	TAY	ACN	TTY	WSN	GTN	CCN
ATT	TAT	ACT	TTT	TCT	GTT	CCT
ATC	TAC	ACC	TTT	TCC	GTC	CCC
ATA		ACA		TCA	GTA	CCA
		ACG		TCG	GTG	CCG
				AGT		AGT
				AGC		AGC
220						
Pro	Val	Gln	Ile	Thr	Leu	Gln
CCN	GTN	CAR	ATH	ACN	YTN	CAR
CCT	GTT	CAA	ATT	ACT	TTA	CAA
CCC	GTC	CAG	ATC	ACC	TTG	CAG
CCA	GTA	ATA	ACA	CTT		CCA
CCG	GTG		ACG	CTC		CCG
				CTA		CTA
				CTG		CTG
230						
Glu	Val	Pro	Glu	Ala	Asn	Trp
GAR	GTN	CCN	GAR	GCN	AAY	TGG
GAA	GTT	CCT	GAA	GCT	AAT	TGG
GAG	GTC	CCC	GAG	GCC	AAC	GCC
	GTA	CCA		GCA		GCA
	GTG	CCG		GCG		GCG
				CTC		CTC
				CTG		CTG
240						
Val	Leu	Pro	Ser	Leu	Leu	Ile
GTN	YTN	CCN	WSN	YTN	YTN	ATH
GTT	TTA	CCT	TCT	TTA	ATT	TTA
GTC	TTG	CCC	TCC	TTG	ATC	TTG
GTA	GTA	CTT	CCA	CTT	CTT	CTT
GTG	GTG	CTC	CCG	CTC	CTC	CTC
						CTA
						CTG

NASIS Amino Acid Conversion										L-Prote-Ala-Gly-Ser-Phe-Lys-Arg-Val-Ile-Trp										CTA CTA CTA CTA CTA CTA CTA CTA CTA CTA									
										250										260									
Val	Ile	Ala	Ala	Gly	Gly	Val	Ile	Trp	Lys	Thr	Leu	Met	Gly	Asn	Pro	Trp	Phe	Gln	Arg										
GTN	ATH	GCN	GCN	GGN	GGN	GTN	ATH	TGG	AAR	ACN	YTN	ATG	GGN	AAY	CCN	TGG	TTY	CAR	MGN										
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---										
GTT	ATT	GCT	GCT	GGT	GGT	GTT	ATT	TGG	AAA	ACT	TTA	ATG	GGT	AAT	CCT	TGG	TTT	CAA	CGT										
GTC	ATC	GCC	GCC	GGC	GGC	GTC	ATC	---	AAG	ACC	TTG	---	GGC	AAC	CCC	---	TTC	CAG	CGC										
GTA	ATA	GCA	GCA	GGA	GGA	GTA	ATA	---	---	ACA	CTT	---	GGA	---	CCA	---	---	---	CGA										
GTG	---	GCG	GCG	GGG	GGG	GTG	---	---	---	ACG	CTC	CTA	GGG	---	CCG	---	---	---	CGG										
---	---	---	---	---	---	---	---	---	---	---	CTG	---	---	---	---	---	---	---	AGG										
										270										280									
Ala	Lys	Met	Pro	Arg	Ala	Leu	Asp	Phe	Ser	Gly	His	Thr	His	Pro	Val	Ala	Thr	Phe	Gln										
GCN	AAR	ATG	CCN	MGN	GCN	YTN	GAY	TTY	WSN	GGN	CAY	ACN	CAY	CCN	GTN	GCN	ACN	TTY	ACN										
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---										
GCT	AAA	ATG	CCT	CGT	GCT	TTA	GAT	TTT	TCT	GGT	CAT	ACT	CAT	CCT	GTT	GCT	ACT	---	---										
GCC	AAG	---	CCC	CGC	GCC	TTG	GAC	TTC	TCC	GGC	CAC	ACC	CAC	CCA	GTC	GCC	ACC	TTT	CAA										
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GCG	---	---	CCG	AGG	GCG	CTC	---	---	TCG	GGG	---	ACG	---	---	GTG	GCG	ACG	---	---										
---	---	---	---	---	---	CTG	---	---	AGT	---	---	---	---	---	---	---	---	---	---										
---	---	---	---	---	---	---	---	---	AGC	---	---	---	---	---	---	---	---	---	---										
										290										300									
Pro	Ser	Arg	Pro	Glu	Ser	Val	Asn	Asp	Leu	Phe	Leu	Cys	Pro	Gln	Lys	Glu	Leu	Thr	Arg										
CCN	WSN	MGN	CCN	GAR	WSN	GTN	AAY	GAY	YTN	TTY	YTN	TGY	CCN	CAR	AAR	GAR	YTN	ACN	MGN										
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---										
CCT	TCT	CGT	CCT	GAA	TCT	GTT	AAT	GAT	TTA	TTT	TTA	TGT	CCT	CAA	AAA	GAA	TTA	ACT	CGT										
CCC	TCC	CGC	CCC	GAG	TCC	GTC	AAC	GAC	TTG	TTC	TTG	TGC	CCA	CAG	AAG	GAG	TTG	ACC	CGC										
CCA	TCA	CGA	CCA	---	TCA	GTA	---	---	CTT	---	CTT	---	CCA	---	---	---	CTT	ACA	CGA										
CCG	TCG	CGG	CCG	---	TCG	GTG	---	---	CTC	---	CTC	---	CCG	---	---	---	CTC	ACG	CGG										
---	AGT	AGG	---	---	AGT	---	---	---	CTA	---	CTA	---	---	---	---	---	CTA	---	AGG										
---	AGC	---	---	---	AGC	---	---	---	CTG	---	CTG	---	---	---	---	---	CTG	---	---										
										310										320									
Gly	Val	Arg	Pro	Thr	Pro	Arg	Val	Arg	Ala	Pro	Ala	Thr	Gln	Gln	Thr	Arg	Trp	Lys	Lys										
GGN	GTN	MGN	CCN	ACN	CCN	MGN	GTN	MGN	GCN	CCN	GCN	ACN	CAR	CAR	ACN	MGN	TGG	AAR	AAR										
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---										
GGT	GTT	CGT	CCT	ACT	CCT	CGT	GTT	CGT	GCT	CCT	GCT	ACT	CAA	CAA	ACT	CGT	---	AAA	AAA										
GGC	GTC	CGC	CCC	ACC	CCC	CGC	GTC	CGC	CCC	CCC	GCC	ACC	CAG	CAG	ACC	CGC	TGG	AAG	AAG										
GGA	GTA	CGA	CCA	ACA	CCA	CGA	GTA	CGA	GCA	CCA	GCA	ACA	---	---	ACA	CGA	---	---	---										
GGG	GTG	CGG	CCG	ACG	CCG	CGG	GTG	CGG	CGG	CCG	GCG	ACG	---	---	ACG	CGG	---	---	---										
---	---	AGG	---	---	---	AGG	---	AGG	---	---	---	---	---	---	---	AGG	---	---	---										
										330										340									
Asp	Leu	Ala	Glu	Asp	Glu	Glu	Glu	Glu	Asp	Glu	Glu	Asp	Thr	Glu	Asp	Gly	Val	Ser	Phe										
GAY	YTN	GCN	GAR	GAY	GAR	GAR	GAR	GAR	GAY	GAR	GAR	GAY	ACN	GAR	GAY	GGN	GTN	WSN	TTY										
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---										
GAT	TTA	GCT	GAA	GAT	GAA	GAA	GAA	GAA	GAT	GAA	GAA	GAT	ACT	GAA	GAT	GGT	GTT	TCT	TTT										
GAC	TTG	GCC	GAG	GAC	GAG	GAG	GAG	GAG	GAC	GAG	GAG	GAC	ACC	GAG	GAC	GGC	GTC	TCC	TTC										
---	---	GCA	---	---	---	---	---	---	---	---	---	---	ACA	---	---	GGA	GTA	TCA	---										
---	---	GCG	---	---	---	---	---	---	---	---	---	---	ACG	---	---	GGG	GTG	TCG	---										
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	AGT	---	---										
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	AGC	---	---										
										350										360									
Gln	Pro	Tyr	Ile	Glu	Pro	Pro	Ser	Phe	Leu	Gly	Gln	Glu	His	Gln	Ala	Pro	Gly	His	Ser										
CAR	CCN	TAY	ATH	GAR	CCN	CCN	WSN	TTY	YTN	GGN	CAR	GAR	CAY	CAR	GCN	CCN	GGN	CAY	WSN										
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---										
CAA	CCT	TAT	ATT	GAA	CCT	CCT	TCT	TTT	TTA	GGT	CAA	GAA	CAT	CAA	GCT	CCT	GGT	CAT	TCT										
CAG	CCC	TAC	ATC	GAG	CCC	CCC	TCC	TTC	TTG	GGC	CAG	GAG	CAC	CAG	GCC	CCC	GGA	CAC	TCC										
---	CCA	---	ATA	---	CCA	CCA	TCA	---	CTT	GGA	---	---	---	---	GCA	CCA	GGA	---	TCA										
---	CCG	---	---	---	CCG	CCG	TCG	---	TTG	GGG	---	---	---	---	CGC	CCG	GGG	---	TCG										
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---										

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DNASIS.
ProtX(GG/SK seq)3'UTR

```

      10      20      30      40      50
GCTGTCCCC GACATCCAC CGAATCTGAT GCTGCTGCTG CCTTTGCAAG GACTACUGG

      70      80      90     100     110     120
CTTCCCAAGA AACTCAAGAG CCTCCGTACC TCCCCTGGGC GCGGAGGGG CATTGCACTT

     130     140     150     160     170     180
CCGGGAAGTC CACCTAGCGG CTGTTTGCTT GTCGGGCTGA GCAACAAGAT GCCCTCCCT

     190     200     210     220     230     240
CCTGTGACCC GCCCTCTTTA GGCTGAGCTA TAAGAGGGGT GGACACAGGG TGGGCTGAGG

     250     260     270     280     290     300
TCAGAGGTTG GTGGGGTGTG ATCACCCECA TTGTCCTAG GTTGACAGGC CAGGGGAAA

     310     320     330     340     350     360
AATTATCCCC GGACAACATG AAACAGGTGA GGTGAGTCA CTGCGGACAT CAAGGGCGGA

     370     380     390     400     410     420
CACCACCAAG GGGCCCTCTG GAACTTGAGA CCACTGGAGG CACACTGCT ATACCTCATG

     430     440     450     460     470     480
CCTTTCCAG CAGCCACTGA ACTCCCCAT CCCAGGGCTC AGCCTCCTGA TTCTGGGTC

     490     500     510     520     530     540
CCCTAGTTAG GCCCAGATAA AAATCCAGTT GGCTGAGGGT TTTGGATGGG AAGGGAAGGG

     550     560     570     580     590     600
TGGCTGTCTT CAAATCCTGG TCTTTGGAGT CATGGCACTG TACGGTTTGA GTGTCAGACA

     610     620     630     640     650     660
GACCGGGGTT CAAATCCAG CTCTGCTGTT CACTGGTGT ATGATCTGG GGAAGACATC

     670     680     690     700     710     720
TTCTTCTCT GCCTCGGCTT CCTCATCTGC AGCTACGCTT GGTGTGGTG AGGGTTCTAG

     730     740     750     760     770     780
GGGATCTCAG ATGTGTGTAG CACGGAGCCT GCTGTGCTT GGTGCTCTC TACGTGGTGG

     790     800     810     820     830     840
CCGGTAGAAT TCTCATCTA TCCAGGCTCC AGGAGACCCC TGGGCATCTC CCACCTGTGG

     850     860     870     880     890     900
CCCCTAACCC CAGAGTGACT GAGAGCACTT AACATTGAGC TTGTCTCATC CCCAGTCTAC

     910     920     930     940     950     960
CTCCTTCCTT CTACCCTCAC TGCTCCAG TCAGGAGAGT GAGCTCTCAG AAGCCAGAGC

     970     980     990    1000    1010    1020
CCCCCAAG GGGACCTGG TCTCTCGCC TTACCTAGC AATGGGAACC CTGCTTCCA

    1030    1040    1050    1060    1070    1080
GGGGAGGAAC CAACTGCTCC ACCTTCTAGG GACCCAGTTT GTTGAGTAG GACAGTAACA

    1090    1100    1110    1120    1130    1140
TGGCAGGAAT CGGACTTCTG GGCTGTAAT CCCAGTTTGG ATGGCACGTT AGACTCTTGG

    1150    1160    1170    1180    1190    1200
TTGACGTTG TGGTCCTTAG AAGTCCATT CTCCCTTCCA GTTATGAGAA ACCAATGCCT

    1210    1220    1230    1240    1250    1260
TCTAGATTCA GGTGACTATC CTTACCTGGG GGTGCTGATG CATCTCAGT TAACCTACAC

    1270    1280    1290    1300    1310    1320

```

FIG. 11

DNASIS
PfoTX(GG/SK seq)3'UTR

```

CCACCTGAAT ATAGATGAGC GTAGCT TTTACCCGT AGGACCGAAG TGTTTT
1330      1340      1350      1360      1370      1380
TGGAGTATCT GAACAACCTT GGCTCTGTGG CCATTCAACC TGCCAGGACT AACATTCTG
1390      1400      1410      1420      1430      1440
GATTTGTGAA GAAGGGATCT TCAAAGCCAT TGAACCCACA GAGCTGTGTT GCTTTAAAGC
1450      1460      1470      1480      1490      1500
CACCACAAGG GTACAGCATT AAATGGCAGA ACTGGAAAAG CTTCTTAGGG CATCTCATCC
1510      1520      1530      1540      1550      1560
AGGGATTCTC AAACCATGTC CCCAGAGGGC CTTGGGCTGC AGTTGCAGGG GCGGCCATGG
1570      1580      1590      1600      1610      1620
GGCTATAGGA GCCTCCCACT TTCACCAGAG CAGCCTCACT GTGCCCTGAT TCACACACTG
1630      1640      1650      1660      1670      1680
TGGCTTTCCA CGTGAGGTTT TGTTTAGAGG GATCCACTAC TCAAGAAAAA GTTAGCAAAC
1690      1700      1710      1720      1730      1740
CACTCCTTTT GTTGCAAGG AGCTGAGGTC AAGGGTGGCA AAGGCACTTG TCCAAGGTCTG
1750      1760      1770      1780      1790      1800
CCCAGCAGTG CTGCTCTGAT GACTTGTGCA CATCCCAAG GGTAAAGCT TCGATCTCTG
1810      1820      1830      1840      1850      1860
CACAGCCGGG CCAACCTCTG ACCCCTTGTC CATGTCAGTA AAATATGAAG GTCACAGCCA
1870      1880      1890      1900      1910      1920
GGATTCTCTA GGGTCAGGAG GCCTTCACCG CTGCTGGGGC ACACACACAC ACATGCATAC
1930      1940      1950      1960      1970      1980
ACACATACGA CACACACCTG TGTCTCCCA GGGGTTTTCC CTGCAGTGAG GCTGTGCCAG
1990      2000      2010      2020      2030      2040
ATGATTGAGC CCAGGAGAGG AAGAACAAAC AAATACGGA GCTGGGGAGG GCTGTGGCTT
2050      2060      2070      2080      2090      2100
GGGGCCAGCT CCCAGGGAAA TTCCAGAGCC TGTACCGATG TTCTCTCTGG CACCAGCCGA
2110      2120      2130      2140      2150      2160
GCTGCTTCGT GGAGGTAACT TCAAAAAAGT AAAAGCTATC ATCAGCATCA TCTTAGACTT
2170      2180      2190      2200      2210      2220
GTATGAAATA ACCAETCCGT TTCTATTCTT AAACCTTACC ATTTTGTGTT TGTGTTGTTT
2230      2240      2250      2260      2270      2280
TTTTGAGTCG GAGTTTTGTT CTTTTGCCT AGGCTGGAGT GCAGTGGTAC AATCTCGGCT
2290      2300      2310      2320      2330      2340
CACTGCAACG TCCACCTCCC GGGTTCAAGT GATTCTCCTG CCTCAGCCTC CCAAGTAGCT
2350      2360      2370      2380      2390      2400
GGGATTACAG GCACCCGCCA CCACACCTGG CTAATTTTTT TGTATTTTGA GTAGAGACGG
2410      2420      2430      2440      2450      2460
GGTTTCACCA TGTTGGCCAG GCTGGTCTCG AACTCCTGAC CTCAGGTGAT CCGCCCGCCT
2470      2480      2490      2500      2510      2520
CGGCCTCCCA AAGTGCTGGG ATTACAGGCG TGAGCCACCG CGCCAGCCA AACCTTAETA
2530      2540      2550      2560      2570      2580
TTTTTTTAAA GAATTTTTTC CAGAGTTTAA TTTCTGACAT AGCTTAAGTT TTCCAGTAAC

```


UNAS15
P7otX(GG/SK seq)3'UTR

```

      2590      2600      2610      2620      2630      2640
TCTAACTCC ATCTCCTTTA TCGTCATTAA GTCATTCACA AAAAGCCAGG AGAAGCATT

      2650      2660      2670      2680      2690      2700
GGAAAGGGCA TGATAATCAG TATAATAATT TGCCTTGTGT GGTGAGCACT TAACTGTTTA

      2710      2720      2730      2740      2750      2760
CAAAGCCCTT TCACGTGCAC AGCAGGTGGG AACTGCGCGG TGTGGGCTGG GCCTGCGCTG

      2770      2780      2790      2800      2810      2820
GAAGCATATC CCGTGAAAAG TGTTAGTGCC TTAGGTGAAA GCAACATGTA TCCCTTTAGA

      2830      2840      2850      2860      2870      2880
CTACTAACGG TATATGTTGT TCTTATGTAT TTGTATTTAT TTCTATTTTT TCTATGTTTA

      2890      2900      2910      2920      2930      2940
TGTCATATTT AAACGATATC CTA CTGCTTG TTGGTATTAC CCTAACTGT TTAAATAAAG

      2950      2960      2970      2980      2990      3000
AGCTCTATTT TTAAAGAAAA AAGGTACAAT TGA.....

```

Fig. 12
Mouse CRF2-12 cDNA

```

51  TTGCAGAGCG CCCTAGGAAG GCCCCGTCTA GCCCCACCCA GAAACGTGAC
101 ACTCTTCTCC CAGAACTTCA CTGTTTACCT GACATGGCTT CCGGGGCTTG
151 GGAGCCCCCC AAATGTGACC TATTTCGTGA CCTACCAAAG CTATATCAAA
201 ACCGGTTGGC GACCAAGTGA GCATGTGCA GGTATCAAGG CTCTGGTGTG
251 TCCCCTGATG TGCCTGAAGA AACTGAACCT GTACTCCAAG TTCAAAGGAC
301 GAGTACAGGC AGCTTCCGCA CACGGCAGGT CCCCACGGGT GGAGTCCCGG
351 TACCTGGAAT ACCTTTTGA CGTGGAGCTA GCCCCGCCCA CCCTGGTGCT
401 CACCCAGATG GAGAAGATCC TAAGGGTCAA CGCTACCTAC CAGCTGCCAC
451 CTTGCATGCC GTCGCTGGAA CTGAAGTACC AGGTGGAATT CTGGAAGGAG
501 GGTCTGGGAA GCAAGACCCT GTTTCCTGAC ACTCCCTATG GCCAGCCAGT
551 GCAGATTCCCT CTCCAGCAAG GTGCTAGCCG ACGCCACTGC CTCAGCGCCA
601 GAACCGTCTA TACCTTAATT GACATTAAGT ACAGCCAGTT CTCTGAGCCC
651 AGCTGCATCT TCCTAGAGGC TCCAGGGGAC AAAAGAGCTG TCCTGGCAAT
701 GCCCTCACTC TTGCTTCTAC TGATAGCAGC CGTTGCAGCA GGTGTGGCAT
751 GGAAGATAAT GAAAGGAAAC CCCTGGTTTC AGGGGGTGAA GACGCCCCGG
801 GCACTGGACT TTTCTGAATA CAGATACCCA GTGGCAACCT TTCAGCCCAG
851 TGGACCTGAG TTCTCTGATG ACTTGATCCT TTGTCCCCAG AAGGAACTGA
901 CCATAAGGAA CAGGCCAGCC CCTCAGGTCA GAAACCCAGC CACGCTACAG
951 GCAGGACCAG AAAGAGACAG TACTGAGGAT GAAGACGAGG ACACAGACTA
1001 CGATGACGAC GGTGACAGCG TCCAGCCCTA CCTGGAACGG CCCCTCTTCA
1051 TCAGCGAGAA GCCCCGGGTT ATGGAACACT CGGAGACAGA CGAGTCTGGG
1101 GTGGATTGAG GGGGGCCTTG GACATCCCCA GTTGGGAGTG ACGGCTCCTC
1051 TCAGCGAGAA GCCCCGGGTT ATGGAACACT CGGAGACAGA CGAGTCTGGG
1101 GTGGATTGAG GGGGGCCTTG GACATCCCCA GTTGGGAGTG ACGGCTCCTC
1151 TGCATGGGAC TCTTCAGACA GGAGCTGGTC CAGCACAGGG GACTCCTCAT
1201 ATAAGGATGA AGTTGGGTCT TCCAGCTGTT TGGACCGAAA GGAGCCGGAC

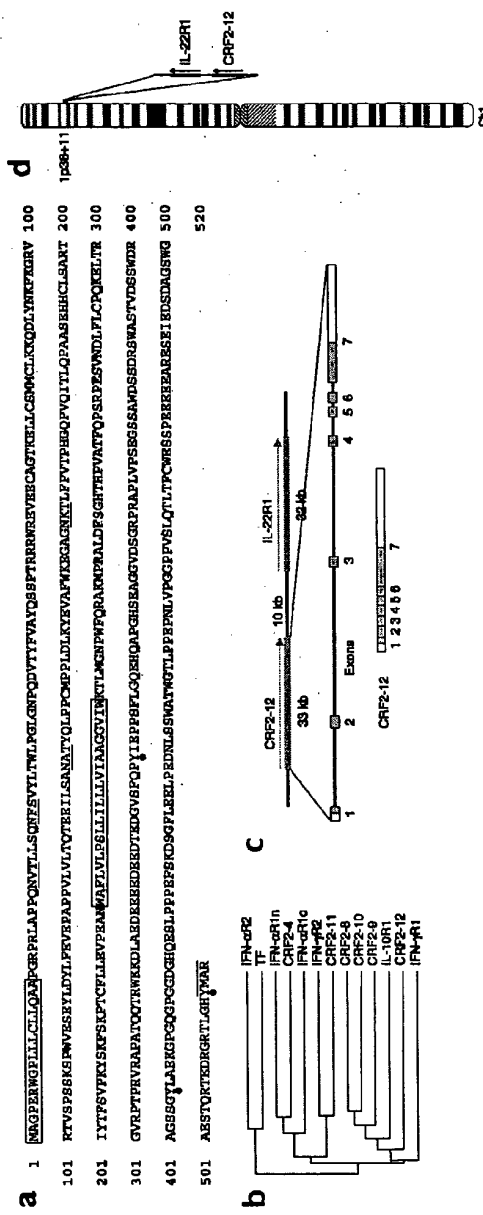
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1251 CAGGCGCCCT GTGGGGATTG GCTCCAGGAG GCTCTCCCAT GCCTGGAATT
1301 TTCTGAGGAC TTGGGCACCG TGGAAGAGCC TCTGAAGGAT GGCCTCTCCG
1351 GGTGGAGGAT TTCTGGTTCC TTATCCTCAA AGAGAGATCT GGCTCCTGTG
1401 GAGCCCCCAG TTTCTCTTCA GACACTGACT TTCTGCTGGG TCAACAATCC
1451 TGAGGGGGAG GAGGAACAGG AGGACGAGGA GGAAGAGGAG GAGGAGGAGG
1501 AGGAGGAAGA CTGGGAATCA GAACCTAAGG GCAGCAATGC CGGCTGTTGG
1551 GGCACCTTCAA GCGTGACAGAG GACGGAGGTC AGGGGCCGGA TGCTGGGAGA
1601 TTACTTGGTC AGGTGAgctg gccctggtgg tccatctaca cattctctac
1651 tgcacttccc ctggaccaca gacacctcgg agagtcattgt ccccttgagt
1701 ggtagccaag gtcctggcca ttcgtaatgg atctttatgg cactttgtcg
1751 ttgggattga gttgtaagat gtcaccattt ccaatgaccc accagctata
1801 ggtgacctgt gagcgggaca gagctgtgat cagagactag ttgagttcca
1851 atctgccccat tgtccctggg atgacgggcc tgatggaaaa atcatcctgt
1901 gataaaatga aacaggtgaa gccaggtgac tgtggacacc aagggtagat
1951 acgccctagt ccataagtca cacacagcat tgcgcaaac agacctcgg
2001 ccaaactcact actctgctgt ttctgatagt cactgagcaa gcgactctta
2051 acctctcaac ctccagcttcc ccttctgcag cagtctctgtg ggtatgggga
2101 ggtaacaggt gacacagggc caccaatgtc acatgtactg actaggcact
2151 agaaccctcc atc

Fig. 13

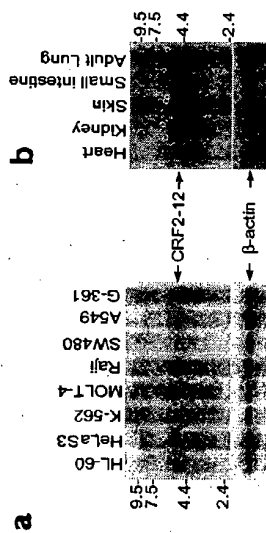
Mouse CRF2-12

```
1  MWRADRWAPL LLFLLQSALG RPRLAPPRNV TLFSQNFTVY LTWLPGLGSP
51  PNVTYFVTYQ SYIKTGWRPV EHCAGIKALV CPLMCLKKLN LYSKFKGRVQ
101 AASAHGRSPR VESRYLEYLF DVELAPPTLV LTQMEKILRV NATYQLPPCM
151 PSLELKYQVE FWKEGLGSKT LFPDTPYGQP VQIPLQQGAS RRHCLSARTV
201 YTLIDIKYSQ FSEFSCIFLE APGDKRAVLA MPSLLLLLLIA AVAAGVAWKI
251 MKGNPWFQGV KTPRALDFSE YRYPVATFQP SGPEFSDDLI LCPQKELTIR
301 NRPAPQVRNP ATLQAGPERD STEDEDEDTD YDDGDSVQF YLERPLFISE
351 KPRVMEHSET DMSGVDSGGP WTSPVGS DGS SAWDSSDRSW SSTGDSSYKD
401 EVGSSSCILDR KEPDQAPCGD WLQEALPCLE FSEDLGTVEE PLKDGLSGWR
451 ISGSLSSKRD LAPVEPPVSL QTLTFCWVNN PEGEEEQEDE EEEEEEEEEE
501 DWESEPKGSN AGCWGTSSVQ RTEVRGRMLG DYLVK
```



CRF2-12. (a) The deduced amino acid sequence of CRF2-12. Amino acid residues of the predicted signal peptide and transmembrane domain of CRF2-12 are boxed. Potential glycosylation sites are underlined and the C-terminal intracellular tyrosine-based motif, which is also similar to that within the IFN-αR2c intracellular domain and is likely to participate in STAT activation, is overlined. Three tyrosine residues within the intracellular domain, which are potential targets for phosphorylation, are marked with dots. (b) A phylogenetic tree was generated by alignment of amino acid sequences of the CRF2-12 extracellular domain and extracellular domains of other members of the class II cytokine receptor family. (c) Schematic exon-intron structure of the gene encoding CRF2-12 is shown. Coding regions of exons are shaded and the segments corresponding to 5' and 3' untranslated regions are left open. (d) Chromosomal localization of the gene encoding CRF2-12 (IFNLR1) and its close neighbor, the gene encoding IL-22R1 (IL22R), as well as the ideogram of human chromosome 1 was generated from the NCBI database. The genes are transcribed in the direction indicated by the arrows.

Fig. 14



Expression pattern of CRF2-12 mRNA. Northern blotting was performed on two blots containing mRNA isolated from (a) human cancer cell lines (promyelocytic leukemia HL-60, epitheloid carcinoma HeLaS3, lymphoblastic leukemia MOLT-4, Burkitt's lymphoma Raji, colorectal adenocarcinoma SW480, lung carcinoma A549 and melanoma G361) and (b) normal human fetal tissues (heart, kidney, skin and small intestine) and adult lung. Arrows point to the CRF2-12 and the β -actin transcripts. Equal RNA loading was assessed by evaluating the expression of the gene encoding β -actin.

Fig. 15

IFN- λ 1**A**

1 atggctgcag ctggaccgt ggtgctggtg actttggtgc taggttggc cgtggcaggc
 61 cctgtcccca cttccaagcc caccacaact gggaagggtt gccacattgg caggttcaaa
 121 tctctgtcac cacaggagct agcgagcttc aagaaggcca gggacgcctt ggaagagtca
 181 ctcaagctga aaaactggag ttgcagctct cctgtcttcc ccgggaattg ggacctgagg
 241 cttctccagg tgagggagcg ccctgtggcc ttggaggctg agctggccct gacgctgaag
 301 gtectggagg ccgctgctgg ccagccctg gaggacgtcc tagaccagcc ccttcacacc
 361 ctgcaccaca tcctctccca gctccaggcc tgtatccagc ctcagcccac agcaggggccc
 421 agggcccggg gccgcctcca ccactggctg caccggctcc aggaggcccc caaaaaggag
 481 tccgctggct gcctggaggc atctgtcacc ttcaacctct tccgcctct caccgcgagac
 541 ctcaaatatg tggccgatgg gaacctgtgt ctgagaacgt caaccacccc tgagtccacc
 601 tga

B

MAAAWTVVLVTLVGLAVAGPVPTSKPTPTGKGCHIGRFKSLSPQELASFKKARDALEESLKLKNWSCSSPVFPGNWDLRLQLQVRE
 RPVALEAEALATLKVLEAAAGPALEDVLDQPLHTLHHILSQLQACIQPQTAGPRPRGRLLHHLRLQEAAPKKESAGCLEASVTFN
 LFRLLTRDLKYVADGNLCLRTSTHPEST

Fig. 16

IFN- λ 2**A**

1 atgactggg actgcacgcc agtgctggtg ctgatggccg cagtctgac cgtgactgga
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Fig. 17

IFN- λ 3**A**

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Fig. 18

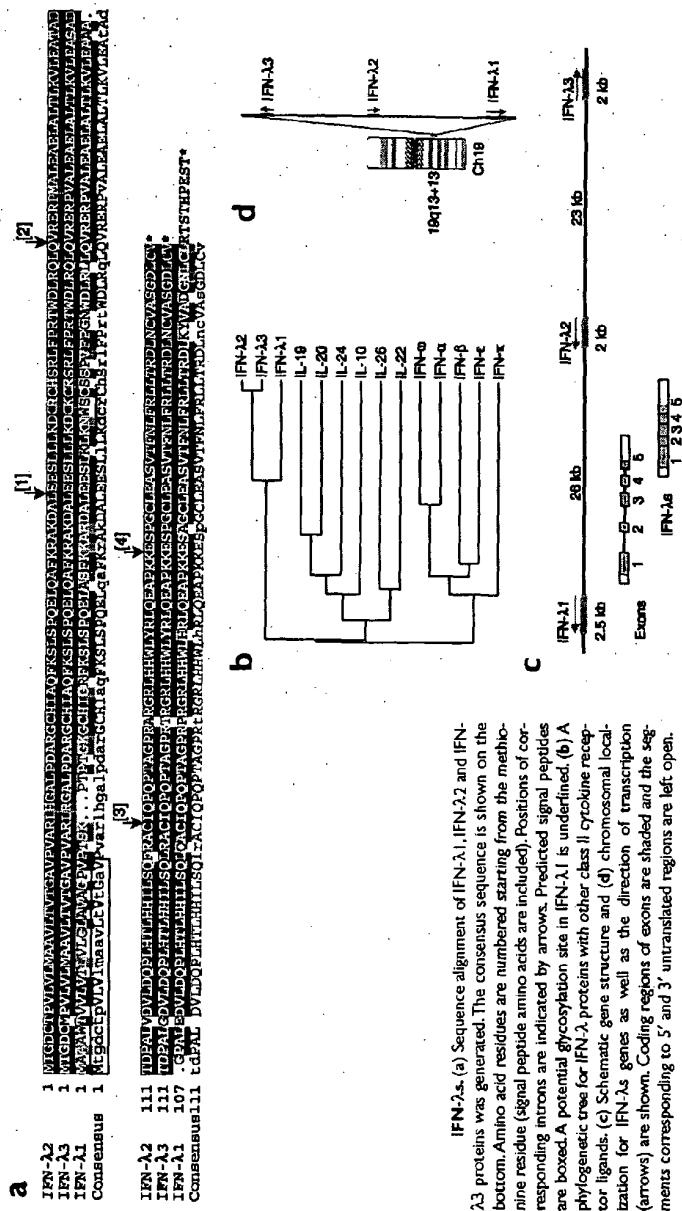
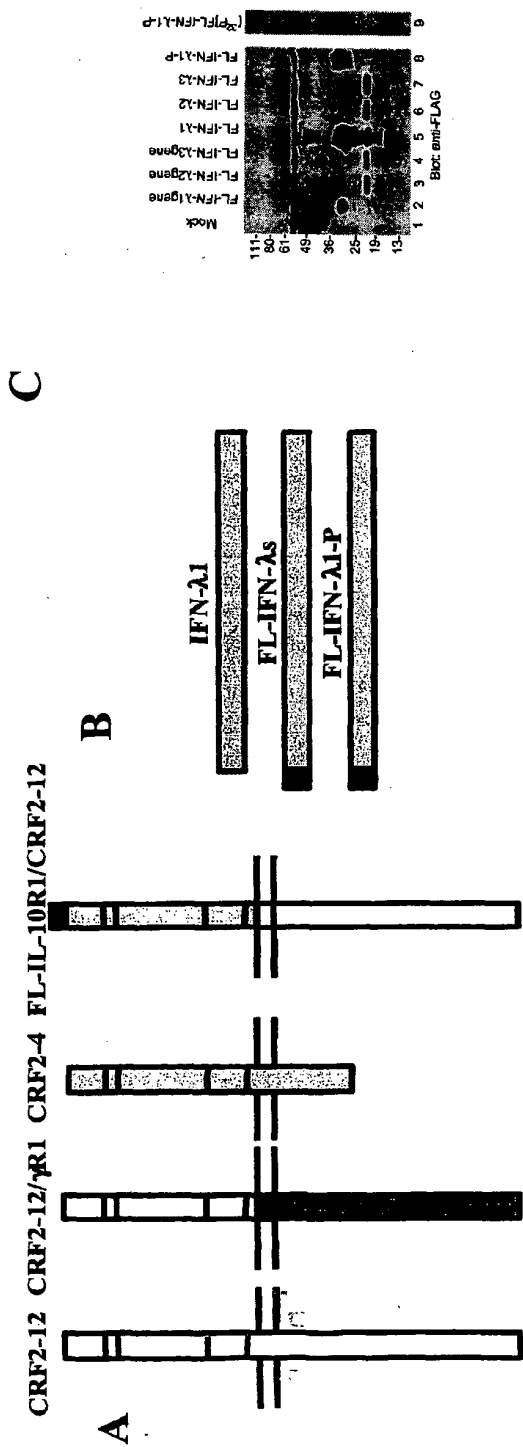
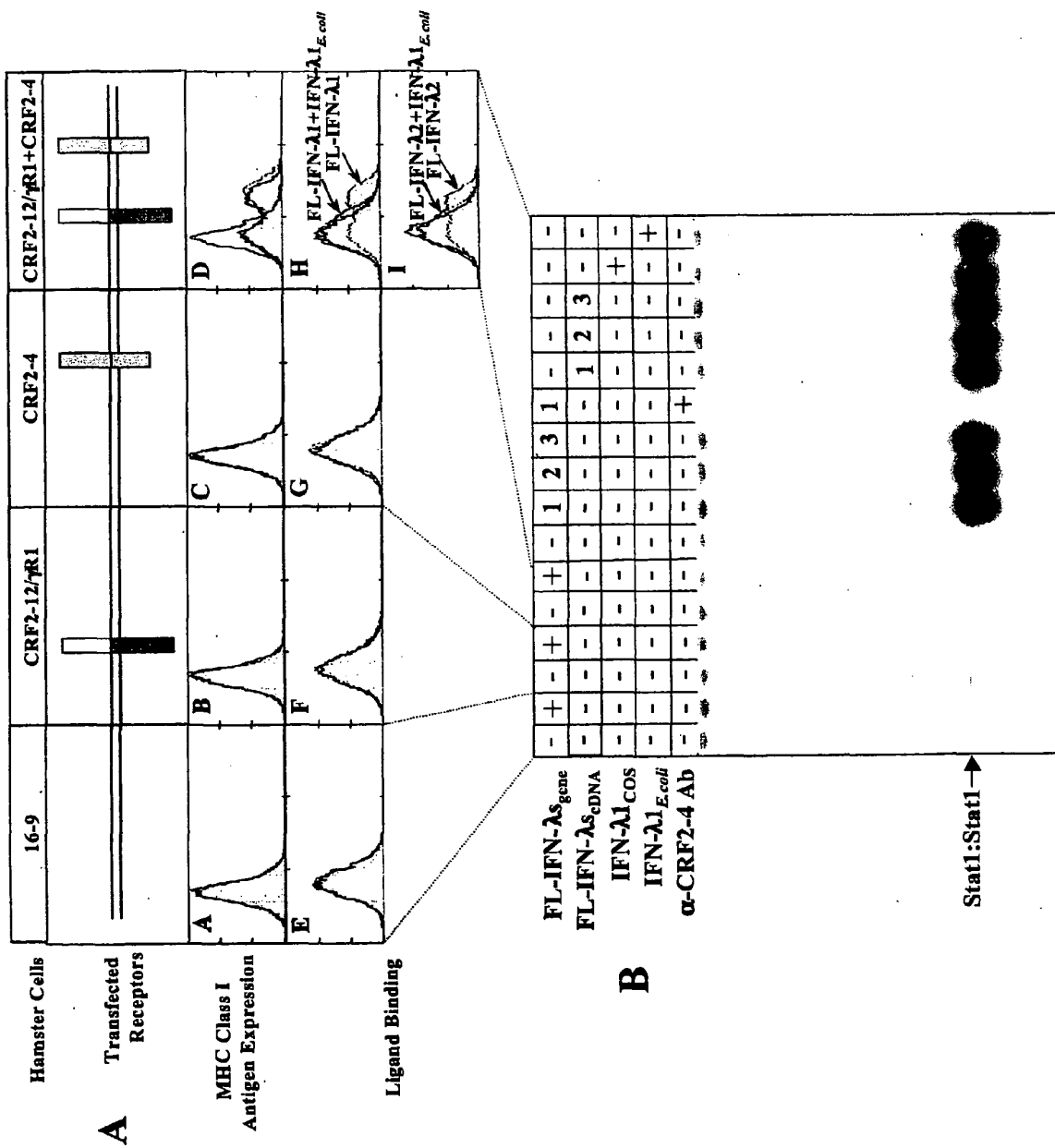


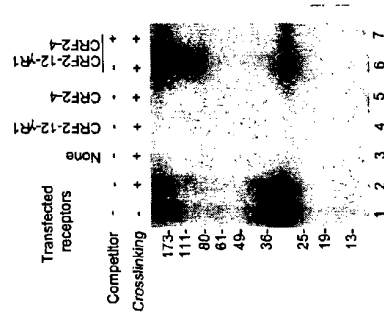
Fig. 19



IFN-αs expression. Conditioned media from COS-1 cells transfected with the control plasmid pEF-SPFL (lane 1); plasmids encoding IFN-α genes, pEF-FL-IFN-α1 gene (lane 2), pEF-FL-IFN-α2 gene (lane 3) and pEF-FL-IFN-α3 gene (lane 4); plasmids encoding IFN-α cDNAs, pEF-FL-IFN-α1 (lane 5), pEF-FL-IFN-α2 (lane 6) and pEF-FL-IFN-α3 (lane 7); and FL-IFN-α1-P purified from conditioned media by affinity chromatography (lane 8) were evaluated by immunoblotting with FLAG mAb. Lane 9 represents an autoradiograph of the SDS-PAGE gel containing radiolabeled FL-IFN-α1-P. The molecular weight markers in kDa are shown on the left.

Fig. 20





Ligand cross-linking. Untreated [¹²⁵I]FL-IFN-λ1-P was loaded as a control (lane 1). [¹²⁵I]FL-IFN-λ1-P was also cross-linked in solution as described" (lane 2). The untransfected and transfected cells with indicated receptor chains were incubated with [¹²⁵I]FL-IFN-λ1-P with or without addition of a 100-fold excess of unlabeled IFN-λ1 (competitor), washed, harvested and cross-linked. The cross-linked complexes were analyzed on 10% SDS-PAGE. The molecular weight markers in kDa are shown on the left.

Fig. 22

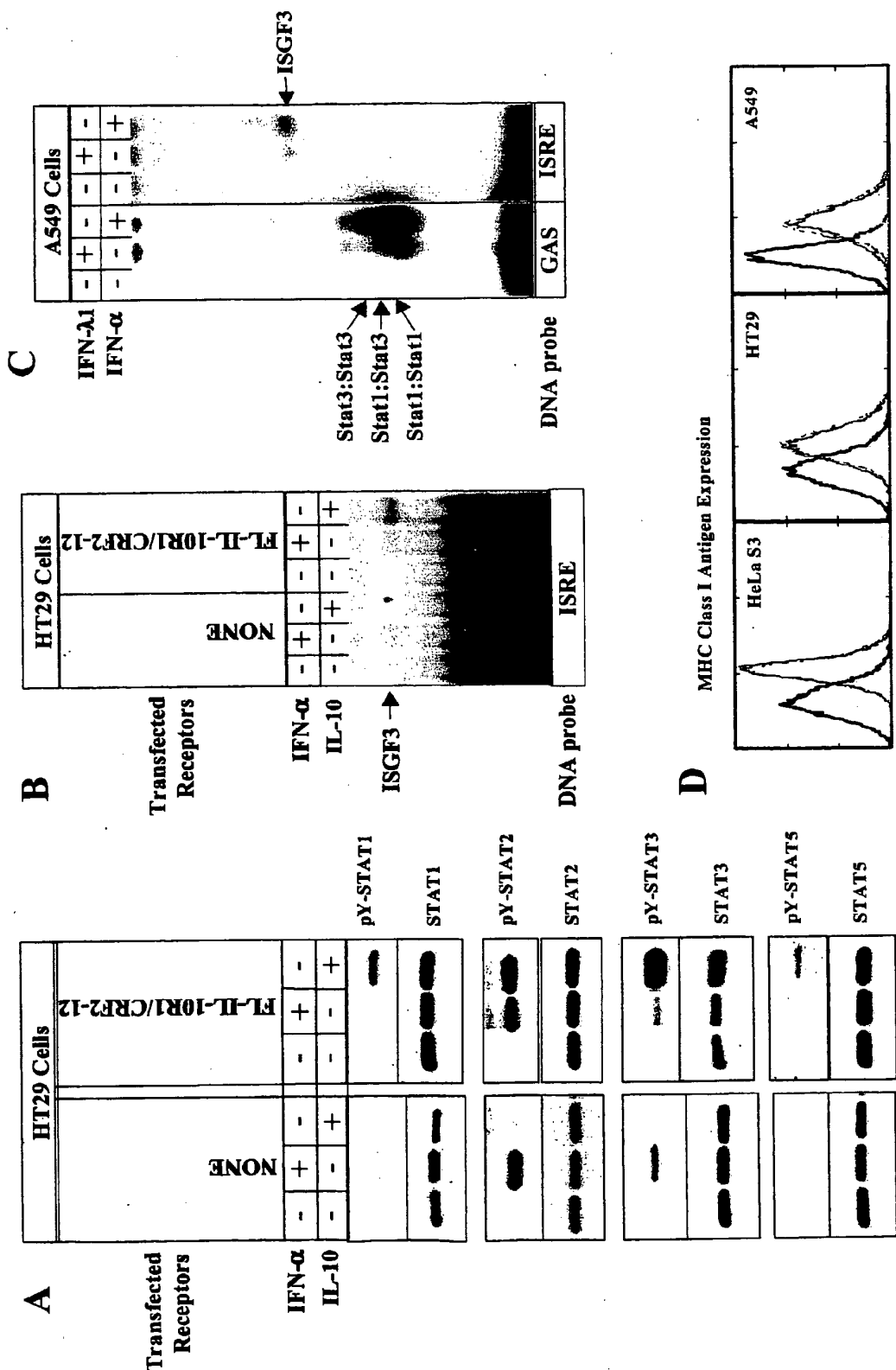


Fig. 23

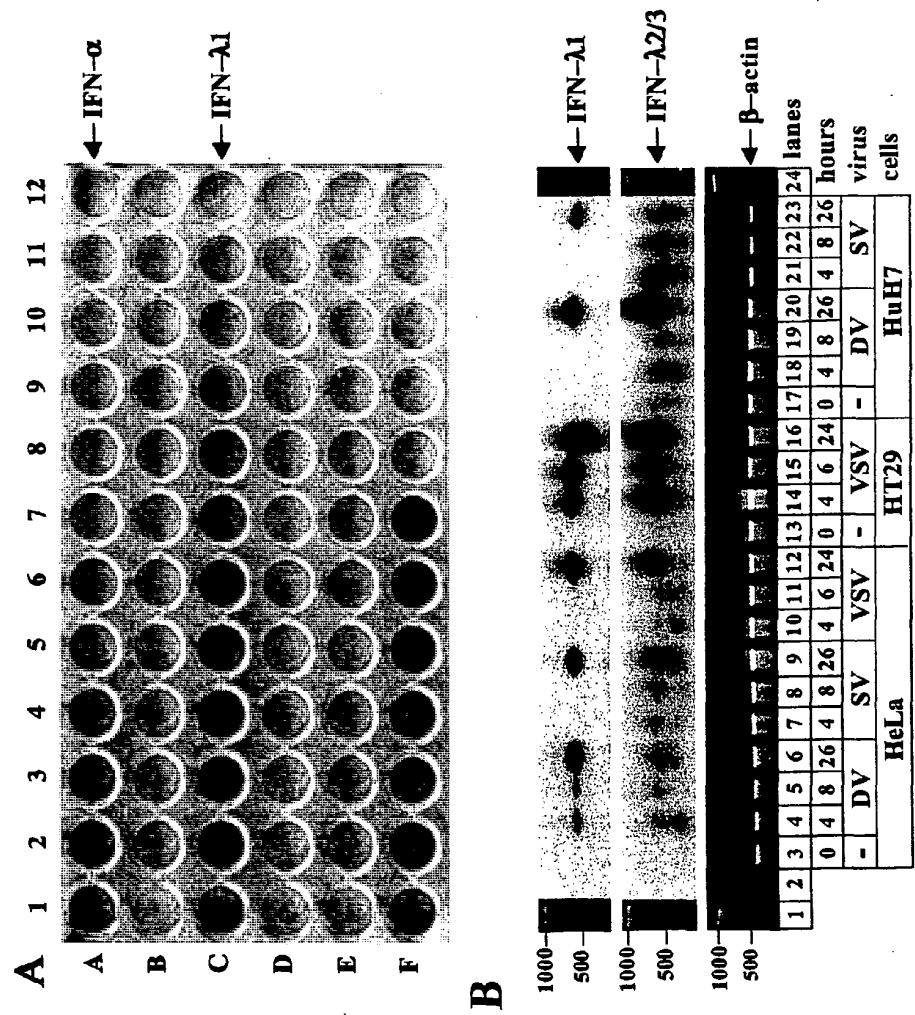


Fig. 24

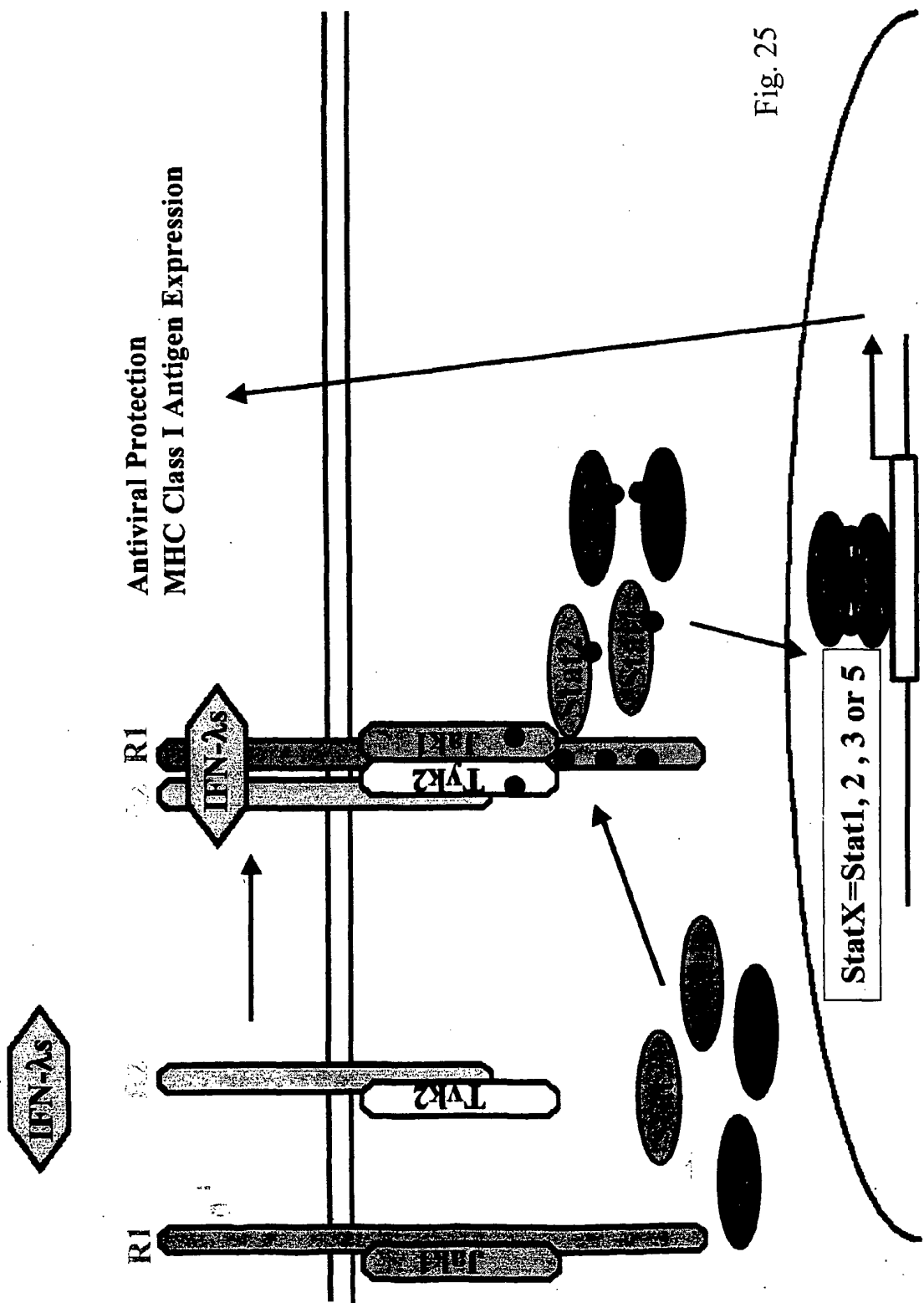


Fig. 25

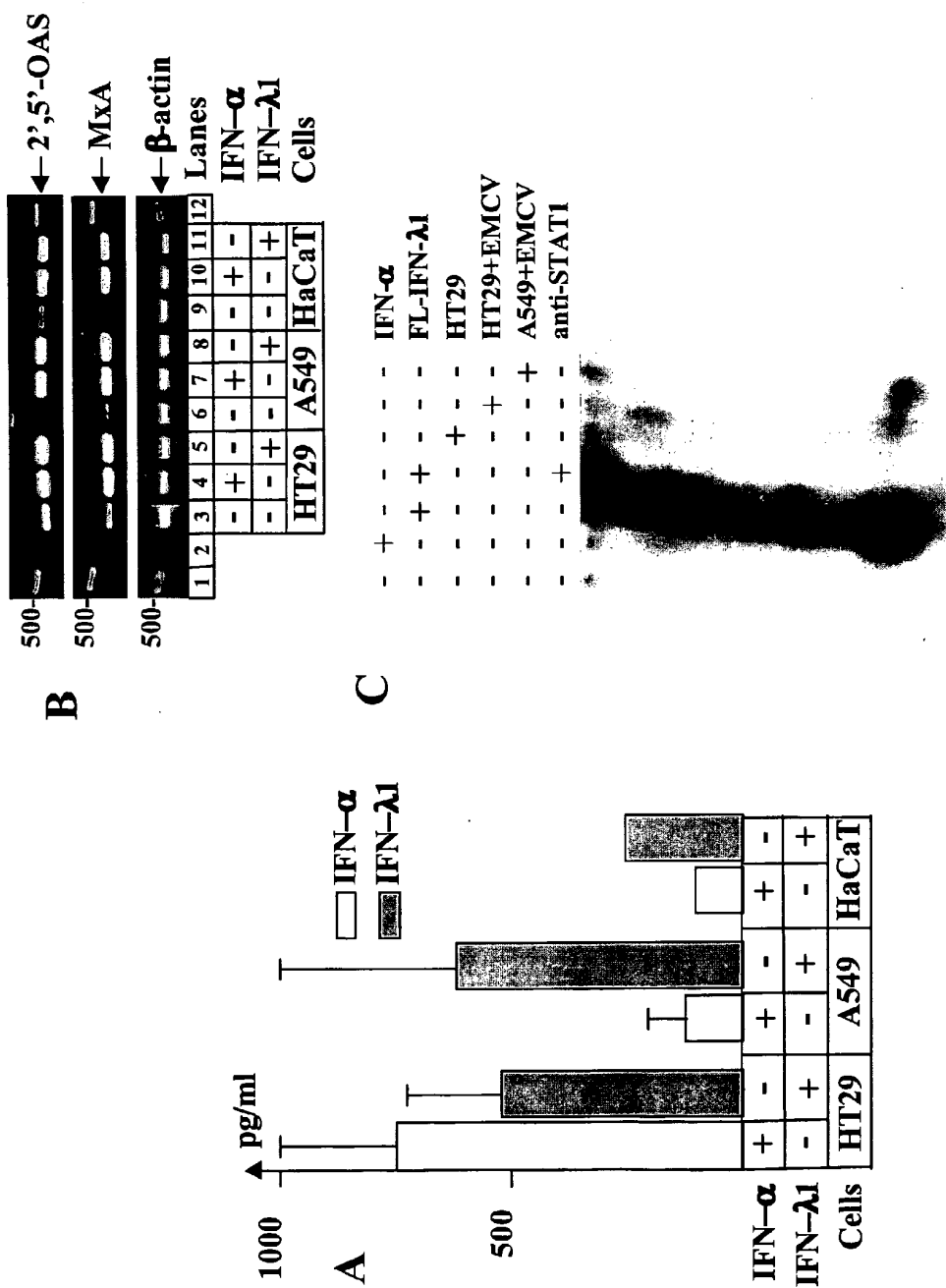


Fig. 26

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IFN- λ 1	101	VLEAAAGPALEDVLDQPLHTLHHILSQLQACIQOPTAGPRGRGLHHLRLQEAAPKESAGCCLEASVTNLFRLLTRDLKYVADGDLCLRTSTHPEST*	200
IFN- λ 2	1	MTGDCPTVLVLMMAAVLTVTGAVPVARLHGALPDARGCHIAQFKSLSPQELQAFKRAKDALEESLLKDCRCHSRLEFRTWDLRQLQVRRPMALEAEIAL	100
IFN- λ 2	101	TLKVLEATADTDEPALVDVLDQPLHTLHHILSQLQACIQOPTAGPRGRGLHHLRLQEAAPKESAGCCLEASVTNLFRLLTRDLNCVASGDLCV*	196
IFN- λ 3	1	MTGDCMPVLVLMMAAVLTVTGAVPVARLHGALPDARGCHIAQFKSLSPQELQAFKRAKDALEESLLKDCRCHSRLEFRTWDLRQLQVRRPVALEAEIAL	100
IFN- λ 3	101	TLKVLEASADTDEPALGDVLDQPLHTLHHILSQLRACIQOPTAGPRGRGLHHLRLQEAAPKESAGCCLEASVTNLFRLLTRDLNCVASGDLCV*	196

Fig. 27

SEQUENCE LISTING

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THROUGH A NOVEL LIGAND-RECEPTOR PAIR: IFN-lambda LIGANDS ENGAGE
A
NOVEL RECEPTOR IFN-lambdaR1 (CRF2-12) AND IL-10R2 (CRF2-4) FOR
SIGNALING AND INDUCTION OF BIOLOGICAL ACTIVITIES

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Leu Ala



Search Strategy from Dialog

February 06 2025 17:07

Search Strategy

Databases: ABI/INFORM® Professional Advanced, Abstracts in New Technology & Engineering, AdisInsight: Drugs, AdisInsight: Trials, Adis Pharmacoeconomics & Outcomes News, AGRICOLA, AGRIS, Allied & Complementary Medicine™, Analytical Abstracts, APA PsycInfo®, Aqualine, Aquatic Science & Fisheries Abstracts (ASFA), Australian Education Index, BIOSIS® Toxicology, BIOSIS Previews®, British Library Inside Conferences, British Nursing Index, Business & Industry, CAB ABSTRACTS, Chemical Business Newbase, Chemical Engineering & Biotechnology Abstracts, Chemical Safety Newbase, Civil Engineering Abstracts, ClinicalTrials.gov, Current Contents® Search, Derwent Drug File, Derwent Drug Registry, DH-DATA: Health Administration, Medical Toxicology & Environmental Health, DIOGENES® FDA Regulatory Updates, Drug Information Fulltext, Earthquake Engineering Abstracts, EconLit, Ei Compendex®, Embase®, Embase® French Local Literature, Embase Preprints, EMCare®, ERIC, ESPICOM Pharmaceutical & Medical Device News, FDAnews, FLUIDEX (Fluid Engineering Abstracts), Foodline®: MARKET, Foodline®: PRODUCT, Foodline®: SCIENCE, FSTA®, Gale Group Computer Database™, Gale Group Health Periodicals Database, Gale Group New Product Announcements / Plus®, Gale Group Newsletter Database™, Gale Group PharmaBiomed Business Journals, Gale Group PROMT®, Gale Group Trade & Industry Database™, GEOBASE®, GeoRef, Global Health, HSELINE: Health and Safety, ICONDA - International Construction Database, IMS Company Profiles, IMS New Product Focus, IMS Pharma Trademarks, IMS R&D Focus, IMS R&D Focus Drug News, Inspec®, International Pharmaceutical Abstracts, Jane's Defense & Aerospace News, King's Fund, KOSMET: Cosmetic Science, Lancet Titles, Mechanical & Transportation Engineering Abstracts, MEDLINE®, Meteorological & Geostrophysical Abstracts, Morressier Life Science Conference Abstracts and Posters, New England Journal of Medicine, Northern Light Life Sciences Conference Abstracts, NTIS: National Technical Information Service, Oceanic Abstracts, PAIS International, Paperbase, PAPERCHEM, ProQuest Advanced Tech & Aerospace Professional, ProQuest Biological & Health Science Professional, ProQuest Dissertations and Theses Professional, ProQuest Environmental Science Professional, ProQuest Materials Research Professional, ProQuest Newsstand Professional, ProQuest Technology Research Professional, Prous Science Daily Essentials, Prous Science Drug Data Report, Prous Science Drugs Of The Future™, Publicly Available Content, Registry of Toxic Effects of Chemical Substances (RTECS®), SciSearch®: a Cited Reference Science Database, Social SciSearch®, ToxFile®, Transport Research International Documentation, TULSA™ (Petroleum Abstracts), UBM Computer Full Text, Weldasearch®, Zoological Record

Set#	Searched for	Results
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S3	IL-10R OR IL10R OR CD210 OR IL-10R1 OR IL-10RA OR IL-10R2 OR IL-10RB OR IL10R1 OR IL10RA OR IL10R2 OR IL-10RB OR CRF2-4 OR (interleukin pre/2 10 pre/2 receptor)	23616*
S4	s3 and (s1 or s2)	0°
S5	s3 and (John pre/3 Hopkins)	33°
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	OR link OR linked OR linkage)	
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S13	s3 pre/5 (trap or traps)	1°
S14	cytokine pre/5 receptor pre/5 fusion pre/5 protein*	214°
S15	(cytokine pre/5 receptor pre/5 fusion pre/5 protein*) and (pd(1998-2011))	31°
S16	s14 and s3	5°
S17	s3 pre/150 (fusion OR fuse OR fused OR join OR joined) pre/50 (CD19 or EGFR or EGFR1 or Erbb1 or CD20 or HER2 or VEGF or IGFIR or IGF1R or IGF-IR or IGF-1R or TRAIL or mucin or CD30 or CD33 or CD40 or PDL1 or IL2 or IL-2 or CTLA4 or CTLA-4 or CD25 or PD-1 or RANKL or OX40)	11°

* Duplicates are removed from the search, but included in the result count.

° Duplicates are removed from the search and from the result count.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/694,541	03/14/2022	Atul Bedi	JHU3260-9	4182
100899	7590	02/12/2025		
The John Hopkins University c/o DLA Piper LLP (US) 4365 Executive Drive, Suite 1100 San Diego, CA 92121-2133			EXAMINER BUNNER, BRIDGET E	
			ART UNIT 1647	PAPER NUMBER
			NOTIFICATION DATE 02/12/2025	DELIVERY MODE 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

DETAILED ACTION

Notice of Pre-AIA or AIA Status

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

Status of Application, Amendments and/or Claims

Claims 1-11 are pending and under consideration in the instant application.

Information Disclosure Statement

The information disclosure statements (IDS) submitted on 14 March 2022; 01 June 2022; 25 October 2022; and 03 January 2025 are in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statements are being considered by the examiner.

Nucleotide and/or Amino Acid Sequence Disclosures

REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Items 1) and 2) provide general guidance related to requirements for sequence disclosures.

- 1) 37 CFR 1.821(c) requires that patent applications which contain disclosures of nucleotide and/or amino acid sequences that fall within the definitions of 37 CFR 1.821(a) must contain a "Sequence Listing," as a separate part of the disclosure, which presents the nucleotide and/or amino acid sequences and associated information using the symbols and format in accordance with the requirements of 37 CFR 1.821 - 1.825. This "Sequence Listing" part of the disclosure may be submitted:
 - a) In accordance with 37 CFR 1.821(c)(1) via the USPTO patent electronic filing system (see Section I.1 of the Legal Framework for Patent Electronic System (<https://www.uspto.gov/PatentLegalFramework>), hereinafter "Legal Framework") as an ASCII text file, together with an incorporation-by-reference of the material in the ASCII

text file in a separate paragraph of the specification as required by 37 CFR 1.823(b)(1) identifying:

- i) the name of the ASCII text file;
 - ii) the date of creation; and
 - iii) the size of the ASCII text file in bytes;
- b) In accordance with 37 CFR 1.821(c)(1) on read-only optical disc(s) as permitted by 37 CFR 1.52(e)(1)(ii), labeled according to 37 CFR 1.52(e)(5), with an incorporation-by-reference of the material in the ASCII text file according to 37 CFR 1.52(e)(8) and 37 CFR 1.823(b)(1) in a separate paragraph of the specification identifying:
- i) the name of the ASCII text file;
 - ii) the date of creation; and
 - iii) the size of the ASCII text file in bytes;
- c) In accordance with 37 CFR 1.821(c)(2) via the USPTO patent electronic filing system as a PDF file (not recommended); or
- d) In accordance with 37 CFR 1.821(c)(3) on physical sheets of paper (not recommended).
- 2) When a "Sequence Listing" has been submitted as a PDF file as in 1(c) above (37 CFR 1.821(c)(2)) or on physical sheets of paper as in 1(d) above (37 CFR 1.821(c)(3)), 37 CFR 1.821(e)(1) requires a computer readable form (CRF) of the "Sequence Listing" in accordance with the requirements of 37 CFR 1.824.
- a) If the "Sequence Listing" required by 37 CFR 1.821(c) is filed via the USPTO patent electronic filing system as a PDF, then 37 CFR 1.821(e)(1)(ii) or 1.821(e)(2)(ii) requires submission of a statement that the "Sequence Listing" content of the PDF copy and the CRF copy (the ASCII text file copy) are identical.
 - b) If the "Sequence Listing" required by 37 CFR 1.821(c) is filed on paper or read-only optical disc, then 37 CFR 1.821(e)(1)(ii) or 1.821(e)(2)(ii) requires submission of a statement that the "Sequence Listing" content of the paper or read-only optical disc copy and the CRF are identical.

1. Specific deficiencies and the required response to this Office Action are as follows:

Specific deficiency - The Incorporation by Reference paragraph required by 37 CFR 1.821(c)(1) is missing or incomplete. See item 1) a) or 1) b) above. Specifically, the incorporation by reference paragraph is defective because the file size is in kilobytes (KB). The size of the file must be in bytes.

Required response – Applicant must provide:

- A substitute specification in compliance with 37 CFR 1.52, 1.121(b)(3) and 1.125 inserting the required incorporation-by-reference paragraph, consisting of:
 - A copy of the previously-submitted specification, with deletions shown with strikethrough or brackets and insertions shown with underlining (marked-up version);
 - A copy of the amended specification without markings (clean version); and
 - A statement that the substitute specification contains no new matter.

2. **Specific deficiency** – Nucleotide and/or amino acid sequences appearing in the specification/claims are not identified by sequence identifiers in accordance with 37 CFR 1.821(d). Specifically, the linker sequence recited in claim 6 is not accompanied by the required reference to the relevant sequence identifier (SEQ ID NO: 123) (see specification page 56, [00184]).

Required response – Applicant must provide:

- Amend claim 6 to refer to SEQ ID NO: 123

Specification

3. The disclosure is objected to because of the following informalities:
- 3a. At page 1, [0002], in the last line of the paragraph, the word “than” should be amended to recite “that”.
- 3b. The specification identifies conflicting identity information for the amino acid sequence of SEQ ID NO: 93 that should be updated for accuracy/consistency. For example, at page 14, [0054], SEQ ID NO: 93 is listed as the PD-1 extracellular domain (ectodomain). Meanwhile, at page 15, [0054], it is listed as RANK.

Appropriate correction is required.

Claim Objections

4. Claim 8 is objected to because of the following informalities:
- 4a. In claim 8, line 1, the word “that” is extraneous and should be deleted.

Appropriate correction is required.

Claim Rejections - 35 USC § 112(a)

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.

5. Claims 10 and 11 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), 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 or a joint inventor, or for applications subject to pre-AIA 35 U.S.C. 112, the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 10 is directed to an isolated fusion protein comprising a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety comprises a ligand binding amino acid sequence of the extracellular domain of PD-1. Claim 11 recites that the targeting moiety comprises the amino acid sequence corresponding to SEQ ID NO: 93 or a ligand binding fragment thereof.

The specification of the instant application teaches that the present invention is based on the discovery that targeting immunomodulatory antibodies and fusion proteins can counteract or reverse immune tolerance of cancer cells (page 2, [0005]). The specification discloses the invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of IL-10R (pages 50-51, [0166-0167]). The specification continues to disclose that the targeting moiety comprises an extracellular ligand-binding domain of PD-1 (page 4, [0012]). The specification indicates that the amino acid sequence of SEQ ID NO: 93 is the PD-1 extracellular domain and that the amino acid sequence of SEQ ID NO: 94 is the ligand-binding region of PD-1 (pages 13-14, [0054]). The specification defines the term “fragment” as referring to any subject polypeptide having an amino acid residue sequence shorter than that of a polypeptide whose amino acid sequence residue sequence is disclosed (pages 58-59, [0192]).

Instant claim 10 is broadly interpreted by the Examiner as being directed to a fusion molecule comprising any ligand binding amino acid sequence of the extracellular domain of PD-1 (including fragments). Instant claim 11 recites "the amino acid sequence corresponding to SEQ ID NO: 93 or a ligand binding fragment thereof" (emphasis added by Examiner) and thus, is broadly interpreted by the Examiner as reading upon any size fragment of SEQ ID NO: 93, as long as the fragment retains binding to the ligand, PD-L1 and/or PD-L2.

The first paragraph of 35 U.S.C. § 112 "requires a 'written description of the invention' which is separate and distinct from the enablement requirement." *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563 (Fed. Cir. 1991). An adequate written description of a chemical invention "requires a precise definition, such as by structure, formula, chemical name, or physical properties." *University of Rochester v. G.D. Searle & Co., Inc.*, 358 F.3d 916, 927 (Fed. Cir. 2004); *Regents of the Univ. of Cal. v. Eli Lilly & Co., Inc.*, 119 F.3d 1559, 1566 (Fed. Cir. 1997); *Fiers v. Revel*, 984 F.2d 1164, 1171 (Fed. Cir. 1993). "A description of what a material does, rather than of what it is, usually does not suffice." *Rochester*, 358 F.3d at 923; *Eli Lilly*, 119 F.3d at 1568. Instead, the "disclosure must allow one skilled in the art to visualize or recognize the identity of the subject matter purportedly described." *Id.* In addition, possession of a genus "may be achieved by means of a recitation of a representative number of [compounds]... falling within the scope of the genus." *Eli Lilly*, 119 F.3d at 1569. Possession may not be shown by merely describing how to obtain possession of members of the claimed genus. See *Rochester*, 358 F.3d at 927.

Thus, case law dictates that to provide evidence of possession of a claimed genus, the specification must provide sufficient distinguishing identifying characteristics of the genus. The factors to be considered include actual reduction to practice, disclosure of drawings or structure

chemical formulas, sufficient relevant identifying characteristics (such as, complete or partial structure, physical and/or chemical properties, and functional characteristics when coupled with a known or disclosed structure/function correlation), methods of making the claimed product, level of skill and knowledge in the art, predictability in the art, or any combination thereof. In the instant case, the only factors present in the fusion molecule claims are (i) a structural characteristic of a targeting moiety comprising the amino acid sequence corresponding to SEQ ID NO: 93 or a fragment thereof and (ii) a functional characteristic of the fragment binding a ligand (i.e., PD-L1 and/or PD-L2). There is no identification of any particular sequence or structure of the fragment of SEQ ID NO: 93, other than the amino acid sequence of SEQ ID NO: 94, that must be conserved in order to provide the required function of binding ligand (i.e., PD-L1 and/or PD-L2). Thus, the claims are drawn to a genus of ligand binding fragments of the extracellular domain of PD-1 (SEQ ID NO: 93).

The instant specification fails to disclose and there is no art-recognized correlation between the structure of the genus of fragments of the amino acid sequence of SEQ ID NO: 93 and the function of binding ligand. In other words, the specification does not teach the structure which results in a fragment of the amino acid sequence of SEQ ID NO: 93 with the claimed required characteristic of binding ligand. The description of a single ligand-binding fragment of extracellular domain of PD-1 comprising the amino acid sequence of SEQ ID NO: 94 is not adequate written description of an entire genus of ligand binding fragments of the amino acid sequence of SEQ ID NO: 93. The art recognizes that protein function cannot be predicted from structure alone (Bork, 2000, *Genome Research* 10:398-400; Skolnick et al., 2000, *Trends in Biotech.* 18(1):34-39, especially p. 36 at Box 2; Doerks et al., 1998, *Trends in Genetics* 14:248-250; Smith et al., 1997, *Nature Biotechnology* 15:1222-1223; Brenner, 1999, *Trends in Genetics*

15:132-133; Bork et al., 1996, Trends in Genetics 12:425-427). See also Tokuriki et al. (Current Opinion in Structural Biology 19: 596-604, 2009), who teach that mutations are generally destabilizing. For instance, Tokuriki et al. teach at page 596, right column, last paragraph, that “as mutations accumulate, protein fitness declines exponentially...or even more than exponentially...So by the time an average protein accumulates, on average, five mutations, its fitness will decline to <20%.” Further, at page 598, left column, last paragraph, Tokuriki et al. note that 50% of mutations are destabilizing, and >15% of mutations are highly destabilizing, and of the about 5% of mutations that are stabilizing values...many of these mutations result in inactive protein. Fenton et al. (Medicinal Chemistry Research 29:1133-1146, 2020) also state that while it is well known that most substitutions at conserved amino acid positions (which they call “toggle” switches) abolish function, it is also true that substitutions at nonconserved positions (which they call “rheostat” positions) are equally capable of affecting protein function. They conclude that substitutions at rheostat positions have highly unpredictable outcomes on the activities and specificities of protein-based drugs. Bhattacharya et al. (PLOS ONE 12(3): e0171355, 2017) state that the range of possible effects of even single nucleotide variations at the protein level are significantly greater than currently assumed by existing software prediction methods, and that correct prediction of consequences remains a significant challenge (p. 18).

Applicant is reminded that generally, 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 (*Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956 (Fed. Cir. 2002); *Noelle v. Lederman*, 355 F.3d 1343 (Fed. Cir. 2004); *Regents of the University of California v. Eli Lilly Co.*, 119 F.3d 1559 (Fed. Cir. 1997)). A patentee must disclose “a representative

number of species within the scope of the genus of structural features common to the members of the genus so that one of skill in the art can visualize or recognize the member of the genus” (see *Amgen Inc. v. Sanofi*, 124 USPQ2d 1354 (Fed. Cir. 2017) at page 1358). An adequate written description must contain enough information about the actual makeup of the claimed products – “a precise definition, such as structure, formula, chemic name, physical properties of other properties, of species falling with the genus sufficient to distinguish the gene from other materials”, which may be present in “functional terminology when the art has established a correlation between structure and function” (*Amgen* page 1361).

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111, clearly 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 is, for purposes of the ‘written description’ inquiry, whatever is now claimed” (See page 1117). See also, *Amgen Inc. v. Sanofi*, 124 USPQ2d 1354 (Fed. Cir. 2017), relying upon *Ariad Pharms., Inc. v. Eli Lilly & Co.*, 94 USPQ2d 1161 (Fed Cir. 2010). 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). A “mere wish or plan” to obtain the claimed invention is not sufficient (*Centocor Orth Biotech, Inc. v. Abbott Labs*, 636 F.3d 1341 (Fed. Cir. 2011); *Regents of the Univ. of California*, 119 F.3d at 1566). In the instant application, the skilled artisan cannot envision the detailed chemical structure of the fusion molecules that comprise a genus of ligand binding fragments of the extracellular domain of PD-1 (SEQ ID NO: 93), and therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method of isolation. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method of isolating it. The specific fragment is required. See *Fiers v. Revel*, 25

USPQ2d 1601 at 1606 (CAFC 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016.

One cannot describe what one has not conceived. See *Fiddes v. Baird*, 30 USPQ2d 1481 at 1483. In *Fiddes*, claims directed to mammalian FGF's were found to be unpatentable due to lack of written description for that broad class. The specification provided only the bovine sequence.

Therefore, only a fusion molecule of claim 9, wherein the targeting moiety comprises the amino acid sequence of SEQ ID NO: 93 or a ligand binding fragment thereof comprising the amino acid sequence of SEQ ID NO: 94, but not the full breadth of the claims meets the written description provision of 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph. 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). See also *Ariad Pharm., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1355 (Fed. Cir. 2010).

6. Claims 1-11 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claim 1 of the instant application is directed to an isolated fusion molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:

(a) the targeting moiety comprises a polypeptide that specifically binds a component of a tumor cell that is a tumor cell surface molecule; and

(b) the immunomodulatory moiety comprises a ligand binding amino acid sequence of the extracellular domain of interleukin-10 receptor (IL-10R).

The specification of the instant application teaches that the present invention is based on the discovery that targeting immunomodulatory antibodies and fusion proteins can counteract or reverse immune tolerance of cancer cells (page 2, [0005]). The specification discloses the invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of IL-10R (pages 50-51, [0166-0167]).

The instant specification does not teach any methods or working examples that indicate the generation of a functional fusion protein comprising (i) a targeting moiety comprising a polypeptide that specifically binds a tumor cell surface molecule and (ii) a ligand binding amino acid sequence of the extracellular domain of interleukin-10 receptor (IL-10R). The state of the art at the time of filing only discloses the generation of a fusion protein comprising the extracellular domain of human IL-10R1 and the Fc region of human IgG1 (see Terai et al., Cancer Immunol Immunother 58: 1307-1317, 2009; abstract, Figure 1;; see also Ohtsuka et al., US 2009/0111146 and Priest et al., US 2009/0175819). Terai et al. suggest that the IL-10R-Fc fusion protein (immunoadhesion) may be useful as a therapeutic agent for cancer (page 1313, bottom of column 1 through page 1314, column 1; page 1315, column 2, last paragraph). However, a large quantity of experimentation would be required of the skilled artisan to generate a functional fusion protein comprising (i) one of a plethora of targeting moieties comprising a polypeptide that specifically binds a tumor cell surface molecule and (ii) a ligand binding amino

acid sequence of the extracellular domain of interleukin-10 receptor (IL-10R) and then screen such for activity. Such experimentation is considered undue. Furthermore, one skilled in the art would not be able to predict that any of the fusion proteins encompassed by the claims would be biologically active or treat cancer. For instance, relevant literature teaches that combining two different proteins in one molecule can lead to instability of the fusion protein (Challener, C.A. BioPharm Internat 30(5): 20-31, 37, 2017; page 30, column 2). Challener continues to indicate that achieving and maintaining proper folding of each of the components in fusion proteins is a major challenge in the molecular design because combining different elements of the fusion protein that do not naturally occur together can lead to folding problems and/or instability (page 31, column 2, first paragraph). Challener also teaches that some fusion proteins are prone to proteolytic degradation during production and/or after therapeutic administration (page 31, column 2, 3rd full paragraph). Additionally, the design of fusion proteins can create novel epitopes that may lead to undesired hypersensitivity or immune responses (page 31, bottom of column 2 through the top of column 3). Yu et al. (Biotechnol Adv 33: 155-164, 2015) even teach that current design strategies of fusion proteins are mostly empirical and intuitive with high uncertainty (page 162, column 2). Yu et al. echo the conclusions of Challener by stating that unexpected consequences such as domain misfolding, low protein yield and impaired bioactivity can occur and are hardly foreseeable (page 163, top column 1). Yu et al. disclose that:

“This is mostly because of our limited understanding of the structure–function relationships in these fusion proteins: the spatial orientation and interaction between fusion partners and the flexibility of inter-domain anchoring regions are largely unknown, poorly studied and often neglected during the design of new fusion proteins. Thus, more scientific endeavors are highly required to advance our knowledge about the structure–function relationships in artificially designed fusion proteins” (page 163, top of column 1).

Therefore, due to the large quantity of experimentation necessary to generate a fusion protein comprising (i) one of a multitude of targeting moieties comprising a polypeptide that specifically binds a tumor cell surface molecule and (ii) a ligand binding amino acid sequence of the extracellular domain of interleukin-10 receptor (IL-10R) and screen such for activity; the lack of direction/guidance presented in the specification regarding the same; the absence of working examples directed to the same; the complex nature of the invention (see Challener and Yu et al.); the unpredictability of the activity of all possible fusion proteins encompassed by the claims; and the breadth of the claims, undue experimentation would be required of the skilled artisan to make and/or use the claimed invention.

Conclusion

No claims are allowable.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure:

Renauld et al. US 2003/0158100 (teach the generation of a fusion protein comprising the extracellular domain of IL-10R and the *intracellular* domain of the receptor, LICR-2 (page 3, [0058])

Kotenko et al. WO 03/066002 (teach the generation of a fusion protein comprising the extracellular domain of IL-10R and the *intracellular* domain of IFN- λ R1 (page 228, 1st paragraph)

Review of IL-10R

Josephson et al. Immunity 14: 35-46, 2001

Liu et al. Immunity 152(4): 1821-1829, 1994

Moore et al. Annu Rev Immunol 19: 683-765, 2001

Review of the structure/binding between PD1 and ligands, PD-L1 and PD-L2

Lin et al. Proc Natl Acad Sci USA 105(8): 3011-3016, 2008

Wang et al. J Exp Med 197(9): 1083-1091, 2003

Any inquiry concerning this communication or earlier communications from the examiner should be directed to BRIDGET E BUNNER whose telephone number is (571)272-0881. The examiner can normally be reached Monday-Friday 9:00 am-6:00 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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BEB
Art Unit 1647
07 February 2025

/BRIDGET E BUNNER/
Primary Examiner, Art Unit 1647

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) (NOT for Provisional Applications)		Docket Number (Optional) JHU3260-9	
Application Number 17/694,541		Filed March 14, 2022	
For Compositions And Methods For Targeted Immunomodulatory Antibodies And Fusion Proteins			
Art Unit 1647		Examiner Bridget E. Bunner	
This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.			
The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):			
	<u>Fee</u>	<u>Small Entity Fee</u>	<u>Micro Entity Fee</u>
☐ One month (37 CFR 1.17(a)(1))	\$235	\$94	\$47
☐ Two months (37 CFR 1.17(a)(2))	\$690	\$276	\$138
☒ Three months (37 CFR 1.17(a)(3))	\$1,590	\$636	\$318
☐ Four months (37 CFR 1.17(a)(4))	\$2,495	\$998	\$499
☐ Five months (37 CFR 1.17(a)(5))	\$3,395	\$1,358	\$679
☐ Applicant asserts small entity status. See 37 CFR 1.27. ☐ Applicant certifies micro entity status. See 37 CFR 1.29. Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously. ☐ A check in the amount of the fee is enclosed. ☐ Payment by credit card. Form PTO-2038 is attached. ☐ The Director has already been authorized to charge fees in this application to a Deposit Account. ☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number. <u>07-1896</u> . ☒ Payment made via USPTO patent electronic filing system.			
WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.			
I am the			
☐ applicant/inventor. ☐ assignee of record of the entire interest. See 37 CFR 3.71. 37 CFR 3.73(b) statement is enclosed (Form PTO/SB/96). ☒ attorney or agent of record. Registration number <u>54984</u> . ☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.			
/ <u>Pamela Hwang /</u>		<u>August 12, 2025</u>	
Signature		Date	
<u>Pamela Hwang</u>		<u>+1 858.677.1473</u>	
Typed or printed name		Telephone Number	
NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.			
☒ * Total of <u>one</u> forms are submitted.			

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- 8) 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));
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- 10) the Office of Management and Budget (OMB) for legislative coordination and clearance.

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
17/694,541

RECEIPT DATE / TIME
08/12/2025 04:55:34 PM Z ET

ATTORNEY DOCKET #
JHU3260-9

Title of Invention

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	4182	FILED BY	Karen LePari
PATENT CENTER #	71821164	FILING DATE	03/14/2022
CUSTOMER #	100899	FIRST NAMED INVENTOR	Atul Bedi
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Lisa Haile

Documents

TOTAL DOCUMENTS: 1

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
JHU3260-9 Three-Month Extension of Time 12AUG2025 PH.pdf	2	Extension of Time	199 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
JHU3260-9 Three-Month Extension of Time 12AUG2025 PH.pdf	BE100FBB65A97183FD6A57BCD5E0D35BDC8C969D66644BD7D5DB1BDD1CB8D2AA44DB6B0D0F98A89C07460C7306E84F555CABB405E5C94910778837FA5B771875

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New Applications Under 35 U.S.C. 111

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

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New International Application Filed with the USPTO as a Receiving Office

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ELECTRONIC PAYMENT RECEIPT

APPLICATION #
17/694,541

RECEIPT DATE / TIME
08/12/2025 04:55:34 PM Z ET

ATTORNEY DOCKET #
JHU3260-9

Title of Invention

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	4182	FILED BY	Karen LePari
PATENT CENTER #	71821164	AUTHORIZED BY	Lisa Haile
CUSTOMER #	100899	FILING DATE	03/14/2022
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Atul Bedi

Payment Information

PAYMENT METHOD DA / 071896	PAYMENT TRANSACTION ID E20258BG56175179	PAYMENT AUTHORIZED BY Karen LePari
PRE-AUTHORIZED ACCOUNT 071896	PRE-AUTHORIZED CATEGORY 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)	

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New Applications Under 35 U.S.C. 111

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/694,541	03/14/2022	Atul Bedi	JHU3260-9	4182
100899	7590	09/22/2025		
The John Hopkins University c/o DLA Piper LLP (US) 4365 Executive Drive, Suite 1100 San Diego, CA 92121-2133			EXAMINER BUNNER, BRIDGET E	
			ART UNIT 1647	PAPER NUMBER
			NOTIFICATION DATE 09/22/2025	DELIVERY MODE 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

Notice of Abandonment	Application No.	Applicant(s)
	17/694,541	Bedi et al.
	Examiner	Art Unit
	BRIDGET E BUNNER	1647

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

The most recent incoming or outgoing communication in the prosecution history of this application is dated 12 February 2025.

This application is abandoned in view of:

Office Actions and Notices

1. ☒ Applicant's failure to timely file a proper reply to the Office letter mailed on February 12, 2025.
 - (a) ☐ A reply was received on _____ (with a Certificate of Mailing or Transmission dated _____), which is after the expiration of the period for reply (including a total extension of time of _____ month(s)) which expired on _____.
 - (b) ☐ A proposed reply was received on _____, but it does not constitute a proper reply under 37 CFR 1.113 to the final rejection. (A proper reply under 37 CFR 1.113 to a final rejection consists only of: (1) a timely filed amendment which places the application in condition for allowance; (2) a timely filed Notice of Appeal (with appeal fee); or (3) if this is utility or plant application, a timely filed Request for Continued Examination (RCE) in compliance with 37 CFR 1.114. Note that RCEs are not permitted in design applications or national stage applications that do not comply with 35 U.S.C 371.)
 - (c) ☐ A reply was received on _____ but it does not constitute a proper reply, or a *bona fide* attempt at a proper reply, to the non-final rejection. *See* 37 CFR 1.85(a) and 1.111. (See explanation in box 6 below).
 - (d) ☒ No reply has been received.

Prior to Decision on Appeal

2. ☐ The dismissal of the appeal in an application having no allowed claims.
 - (a) ☐ A notice of appeal was received on _____. No appeal brief has been received within the time prescribed by 37 CFR 41.37.
 - (b) ☐ A pre-appeal brief conference decision was mailed on _____. No appeal brief has been received within the time period set forth in MPEP § 1204.02.
 - (c) ☐ An appeal brief was received on _____, but was held non-compliant in a notice issued on _____. *See* 37 CFR 41.37(d).
 - (i) ☐ No reply to the notice has been received.
 - (ii) ☐ A reply to the notice was received on _____, but does not overcome all of the reasons for noncompliance.
 - (d) ☐ An examiner's answer was issued on _____. The appeal forwarding fee has not been paid within the period set forth in 37 CFR 41.45(a).
 - (e) ☐ Other (See explanation in box 6 below).

Following Decision on Appeal

3. ☐ The decision by the Patent Trial and Appeal Board rendered on _____ and because the period for requesting a rehearing or seeking court review of the decision has expired and there are no allowed claims. *See* 37 CFR 90.3 and 37 CFR 41.52.

Express Abandonment

4. ☐ The letter of express abandonment received on _____ which is signed by the attorney or agent of record or other party authorized under 37 CFR 1.33(b). *See* 37 CFR 1.138(b).
5. ☐ The letter of express abandonment received on _____ which is signed by an attorney or agent (acting in a representative capacity under 37 CFR 1.34) upon the filing of a continuing application.

Other reason(s)

6. ☐ The following reason(s): _____

Communication with Applicant/Applicant's Representative*

*Examiner Note: Completion of section (7) is optional, but recommended if this form is issued within one month following the maximum period for reply.

7. ☐ A telephone call was made to _____ on _____.
 - (a) ☐ The call confirmed that no response has been filed to the Office letter mailed on _____.
 - (b) ☐ A message was left with the party identified above, inquiring about the status of any response to the Office letter mailed on _____. No response to this message has been received.
 - (c) ☐ Additional comments (as needed): _____
8. ☒ No telephone call was made.

	/BRIDGET E BUNNER/ Primary Examiner, Art Unit 1647
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Petitions to revive under 37 CFR 1.137, or requests to withdraw the holding of abandonment under 37 CFR 1.181, should be promptly filed to minimize any negative effects on patent term.