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Form Revision Date: February 8, 2006

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A SUBMISSION UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER JHU3260-4
INTERNATIONAL APPLICATION NO. PCT/US2011/027317		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) PRIORITY DATE CLAIMED March 5, 2010
INTERNATIONAL FILING DATE March 4, 2011		
TITLE OF INVENTION COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		
APPLICANT(S) FOR DO/EO/US Atul BEDI and Rajani RAVI		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information: <ol style="list-style-type: none"> 1. ☒ This is a FIRST submission of items concerning a submission under 35 U.S.C. 371. 2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a submission under 35 U.S.C. 371. 3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371 (f)). The submission must include items (5), (6), (9) and (21) indicated below. 4. ☒ The US has been elected (Article 31). 5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c)(2)) <ol style="list-style-type: none"> a. ☐ is attached hereto (required only if not communicated by the International Bureau). b. ☐ has been communicated by the International Bureau. c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US). 6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> a. ☐ is attached hereto. b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4). 7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> a. ☐ are attached hereto (required only if not communicated by the International Bureau). b. ☐ have been communicated by the International Bureau. c. ☐ have not been made; however, the time limit for making such amendments has NOT expired. d. ☒ have not been made and will not be made. 8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (unsigned) 10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). <p>Items 11 to 20 below concern documents) or information included:</p> <ol style="list-style-type: none"> 11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. ☒ A preliminary amendment. 14. ☐ An Application Data Sheet under 37 CFR 1.76. 15. ☐ A substitute specification. 16. ☐ A power of attorney and/or change of address letter. 17. ☒ A computer-readable form of the sequence listing in accordance with PCT Rule 13^{ter}.2 and 37 CFR 1.821- 1.825. 18. ☐ A second copy of the published International Application under 35 U.S.C. 154(d)(4). 19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). 		

This collection of information is required by 37 CFR 1.414 and 1.491-1.492. 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 15 minutes to complete, including gathering information, preparing, and submitting the completed 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: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)		INTERNATIONAL APPLICATION NO. PCT/US2011/027317		ATTORNEY'S DOCKET NUMBER JHU3260-4	
20. ☒ Other items or information: Notification of Transmittal of the International Search Report and the Written Opinion of the International Searching Authority, or the Declaration and PTO Form SB/08a.					
The following fees have been submitted				CALCULATIONS	PTO USE ONLY
21. ☒ Basic national fee (37 CFR 1.492(a)) \$380				\$ 380.00	
22. ☒ Examination fee (37 CFR 1.492(c))					
If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) \$0					
All other situations \$250				\$ 250.00	
23. ☒ Search fee (37 CFR 1.492(b))					
If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) \$0					
Search fee (37 CFR 1.445(a)(2)) has been paid on the international application to the USPTO as an International Searching Authority \$120					
International Search Report prepared by an ISA other than the US and provided to the Office or previously communicated to the USA by the IB \$490					
All other situations \$620				\$ 490.00	
TOTAL OF 21, 22 and 23 =				\$ 1,120.00	
☒ Additional fee for specification and drawings filed in paper over 100 sheets (excluding sequence listing in compliance with 37 CFR 1.821(c) or (e) in an electronic medium or computer program listing in an electronic medium) (37 CFR 1.492(j)). The fee is \$270 for each additional 50 sheets of paper or fraction thereof.					
Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof (round up to a whole number)		RATE	
154	-100 =	54 /50 =		2	x \$310
				\$ 620.00	
Surcharge of \$130.00 for furnishing any of the search fee, examination fee, or the oath or declaration after the date of commencement of the national stage (37 CFR 1.492(h)). ☒				\$ 130.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	53	-20 =	33	x \$60	\$ 1,980.00
Independent claims	2	-3 =	0	x \$250	\$ 0.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable) ☐				+ \$450	\$ 0.00
TOTAL OF ABOVE CALCULATIONS =				\$ 3,850.00	
☒ Applicant claims small entity status. See 37 CFR 1.27. Fees above are reduced by 1/2.				\$ 1,925.00	
SUBTOTAL =				\$ 1,925.00	
☐ Processing fee of \$130.00 for furnishing the English translation later than 30 months from the earliest claimed priority date (37 CFR 1.492(i)).				\$ 0.00	
TOTAL NATIONAL FEE =				\$ 1,925.00	
☐ Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				\$ 0.00	
TOTAL FEES ENCLOSED =				\$ 1,925.00	
				Amount to be refunded:	\$
				Amount to be charged:	\$

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

- a. ☐ A check in the amount of \$_____ to cover the above fees is enclosed.
- b. ☒ Please charge my Deposit Account No. **07-1896** in the amount of **\$1,925.00** to cover the above fees.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **07-1896**.
- d. ☐ Fees are to be charged to a credit card. **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. The PTO-2038 should only be mailed or faxed to the USPTO. However, when paying the basic national fee, the PTO-2038 may NOT be faxed to the USPTO.

ADVISORY: If filing by EFS-Web, do NOT attach the PTO-2038 form as a PDF along with your EFS-Web submission. Please be advised that this is not recommended and by doing so **your credit card information may be displayed via PAIR.** To protect your information, it is recommended paying fees online by using the electronic payment method.

NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the International Application to pending status.

SEND ALL CORRESPONDENCE TO:

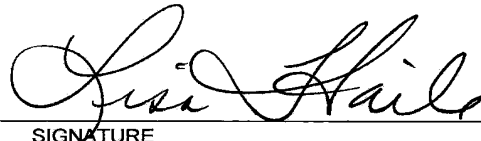
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FAX: (858) 677-1465

September 4, 2012



SIGNATURE

Lisa A. Haile, J.D., Ph.D.

NAME

38,347

REGISTRATION NUMBER

DECLARATION FOR PATENT APPLICATION

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS**, the specification of which

_____ is attached hereto.

 X was filed on September 4, 2012, with an International Application date of March 4, 2011 (Attorney Docket No.: JHU3260-4WO), as U.S. Application Serial No. 13/_____ and was amended on _____ if applicable (the "Application").

I hereby authorize and request insertion of the application serial number of the Application when officially known.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability of the subject matter of the Application as defined in Title 37, Code of Federal Regulations ("C.F.R."), § 1.56.

With respect to the Application, I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

<u>61/435,671</u> (Application Serial No.)	<u>January 24, 2011</u> (Filing Date)
<u>61/311,255</u> (Application Serial No.)	<u>March 5, 2010</u> (Filing Date)
<u> </u> (Application Serial No.)	<u> </u> (Filing Date)

With respect to the Application, I hereby claim the benefit under 35 U.S.C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of the application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability of the subject matter of the Application as defined in Title 37, C.F.R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of the Application:

PCT/US2011/027317 (Application Serial No.)	March 4, 2011 (Filing Date)	pending (Status) (pending, expired)
 (Application Serial No.)	 (Filing Date)	 (Status) (patented, pending, abandoned)
 (Application Serial No.)	 (Filing Date)	 (Status) (patented, pending, abandoned)

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

COUNTRY	APPLICATION NO.	FILING DATE	PRIORITY CLAIMED
_____	_____	_____	☐ Yes ☐ No

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first inventor: **Atul Bedi**

Inventor's signature: _____

Date: _____

Residence: Timonium, Maryland

Country of Citizenship: US

Post Office Address: 2211 Dalewood Road
Timonium, MD 21093

ATTORNEY DOCKET NO.:
JHU3260-4

Full name of second inventor: **Rajani Ravi**

Inventor's signature: _____

Date: _____

Residence: Ruxton, Maryland

Country of Citizenship: US

Post Office Address: 7810 Ballston Road
Ruxton, MD 21204

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Bedi and Ravi	Art Unit:	Not Yet Assigned
Application No.:	Not Yet Assigned	Examiner:	Not Yet Assigned
US Submission Date:	September 4, 2012	Conf. No.:	Not Yet Assigned
Based on Int'l Appl:	PCT/US2011/027317		
IA Filing Date:	March 4, 2011	Priority Date:	March 5, 2010
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

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

PRELIMINARY AMENDMENT

Sir:

Prior to the examination of the above-identified patent application, entry of the following amendments is respectfully requested.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 3 of this paper.

Remarks begin on page 12 of this paper.

In re Application of:
Bedi and Ravi

PATENT
Attorney Docket No.: JHU3260-4

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I. AMENDMENTS

A. In the Specification

On page 1 of the specification, please insert the following paragraph regarding priority after the title:

--CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a 35 USC § 371 National Stage application of International Application No. PCT/US2011/027317 filed March 4, 2011, now pending; 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.--

B. In the Claims

Please cancel claims 3, 9, 13, 17, 21, 53-54, 60-61 and 63-67. Please amend claims 4, 10, 14, 18, 22 and 55.

Upon entry of the present amendment, the claims will stand as follows in the present application:

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

4. (Currently amended) The molecule of claim [[3]]1, wherein the immunomodulatory moiety comprises an extracellular ligand-binding domain of Transforming growth factor-beta receptor TGF- β RII, TGF- β RIIb, or TGF- β RIII.

5. (Original) The molecule of claim 4, wherein the immunomodulatory moiety comprises an extracellular ligand-binding domain of TGF- β RII.

6. (Original) The molecule of claim 5, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, epidermal growth factor receptor (EGFR1), CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4.

7. (Original) The molecule of claim 5, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

8. (Original) The molecule of claim 5, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to Programmed Death-1 (PD-1), Programmed death-1 ligand 1 (PD-L1), or Programmed death-1 ligand 2 (PD-L2).

9. (Canceled).

10. (Currently amended) The molecule of claim [[9]]8, wherein the molecule comprises PD-1 ectodomain, immunoglobulin Fc region, and TGF β RII ectodomain.

11. (Original) The molecule of claim 10, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

12. (Original) The molecule of claim 5, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- κ B (RANK) or Receptor activator of nuclear factor- κ B ligand (RANKL).

13. (Canceled).
14. (Currently amended) The molecule of claim ~~[[13]]~~12, wherein the molecule comprises RANK ectodomain, immunoglobulin Fc region, and TGF β RII ectodomain.
15. (Original) The molecule of claim 14, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 13 or 14.
16. (Original) The molecule of claim 1, wherein the immunomodulatory moiety comprises a molecule that specifically binds to Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2).
17. (Canceled).
18. (Currently amended) The molecule of claim ~~[[17]]~~16, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, Epidermal growth factor receptor (EGFR1), CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4.
19. (Original) The molecule of claim 18, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.
20. (Original) The molecule of claim 16, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- κ B (RANK) or Receptor activator of nuclear factor- κ B ligand (RANKL).
21. (Canceled).
22. (Currently amended) The molecule of claim ~~[[21]]~~20, wherein the molecule comprises RANK ectodomain, immunoglobulin Fc region, and PD-1 ectodomain.

In re Application of:

Bedi and Ravi

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23. (Original) The molecule of claim 22, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 25 or 26.

24. (Original) The molecule of claim 1, wherein the immunomodulatory moiety comprises a molecule that specifically binds to Receptor activator of nuclear factor- κ B ligand (RANKL).

25. (Original) The molecule of claim 24, wherein the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK).

26. (Original) The molecule of claim 25, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, Epidermal growth factor receptor (EGFR1), CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4.

27. (Original) The molecule of claim 26, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36.

28. (Original) The molecule of claim 1, wherein the immunomodulatory moiety comprises an antibody or ligand that specifically binds to 4-1BB, OX40, or GITR/AITR.

29. (Original) The molecule of claim 28, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, Epidermal growth factor receptor (EGFR1), CD20, vascular endothelial growth factor (VEGF), CD25, CD4, cytotoxic T-lymphocyte antigen-4 (CTLA-4), Transforming growth factor-beta (TGF- 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), glucocorticoid-

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induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18), 4-1BB, OX40, or Interleukin-4 receptor (IL-4R).

30. (Original) The molecule of claim 1, wherein the targeting moiety specifically binds to Tumor Necrosis Factor- α (TNF- α), and the immunomodulatory moiety comprises a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2).

31. (Original) The molecule of claim 30, wherein the targeting moiety comprises an antibody that binds TNF- α or an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2), and the immunomodulatory moiety comprises a sequence from PD-1 ligand 1 (PD-L1 or B7-H1).

32. (Original) The molecule of claim 31, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 37, 38, or 39.

33. (Original) The molecule of claim 1, wherein the targeting moiety comprises an antibody, antibody fragment, or a peptide that specifically binds to CD20, CD25, or CD4, and the immunomodulatory moiety comprises a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2).

34. (Original) The molecule of claim 33, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 40, 41, 42, 43, or 44.

35. (Original) The molecule of claim 1, wherein the targeting moiety comprises an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety comprises a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2).

36. (Original) The molecule of claim 35, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 46 or 47.

37. (Original) The molecule of claim 1, wherein the targeting moiety comprises transforming growth factor- β (TGF- β) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety comprises a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2).

38. (Original) The molecule of claim 37, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 48 or 49.

39. (Original) The molecule of claim 1, wherein the immunomodulatory moiety comprises a sequence from transforming growth factor- β (TGF- β).

40. (Original) The molecule of claim 39, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor- α (TNF- α), CD20, CD25 (IL-2 α receptor; IL-2 α R), or CD4, and the immunomodulatory moiety comprises a sequence from transforming growth factor- β (TGF- β).

41. (Original) The molecule of claim 40, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 50, 51, 52, 53, 54, 55, 56, 57, or 58.

42. (Original) The molecule of claim 39, wherein the targeting moiety comprises an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety comprises a sequence from transforming growth factor- β (TGF- β).

43. (Original) The molecule of claim 42, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 59 or 60.

44. (Original) The molecule of claim 25, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor- α (TNF- α), and the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK).

45. (Original) The molecule of claim 44, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 61, 62, or 63.

46. (Original) The molecule of claim 25, wherein the targeting moiety comprises an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK).

47. (Original) The molecule of claim 46, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

48. (Original) The molecule of claim 25, wherein the targeting moiety comprises a sequence from transforming growth factor- β (TGF- β) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK).

49. (Original) The molecule of claim 48, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

50. (Original) The molecule of claim 25, wherein the targeting moiety comprises a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK).

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51. (Original) The molecule of claim 50, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

52. (Original) The molecule of claim 1, wherein the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

53-54. (Canceled).

55. (Currently amended) A method of preventing or treating a neoplastic disease comprising:

administration to a subject in need thereof one or more molecule of ~~claims 1-29~~claim 1.

56. (Original) The method of claim 55, wherein the subject is administered in combination with another anticancer therapy.

57. (Original) The method of claim 55, wherein the anticancer therapy comprises a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent, ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

58. (Original) The method of claim 55, wherein the subject is administered in combination with a vaccine.

59. (Original) The method of claim 55, wherein the subject is human.

60-61. (Canceled).

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62. (Original) A method of treatment of a neoplastic disease comprising:
administering to a subject in need thereof an antibody or Fc-containing fusion protein that targets CD4 in combination with another therapy, wherein the neoplastic disease is not a T cell malignancy, and the therapy comprises one or more of the following:

- (i) an immunostimulatory molecule;
- (ii) a tumor-targeted antibody; or
- (iii) a cytotoxic anticancer agent.

63-67. (Canceled).

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Application No.: Not Yet Assigned
US Submission Date: September 4, 2012
Based on Intl Appl: PCT/US2011/027317
IA Filing Date: March 4, 2011
Page 12

PATENT
Attorney Docket No.: JHU3260-4

II. REMARKS

The application filed herewith is a 35 USC § 371 National Stage application of International Application No. PCT/US2011/027317 filed March 4, 2011. Upon entry of the present amendment, claims 1-2, 4-8, 10-12, 14-16, 18-20, 22-52, 55-59 and 62 will be pending.

The amendment to the specification updates the priority information and, therefore, does not add new matter. Claims 3, 9, 13, 17, 21, 53-54, 60-61 and 63-67 have been canceled and, therefore, do not add new matter. Claims 4, 10, 14, 18, 22 and 55 are amended herein and are supported by the specification as originally filed. There are no new claims. Accordingly, entry of the amendments is respectfully requested.

The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to this application. 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.

Respectfully submitted,

Date: September 4, 2012



Lisa A. Haile, J.D., Ph.D.

Reg. No. 38,347

Telephone: (858) 677-1456

Facsimile: (858) 677-1465

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

PATENT COOPERATION TREATY

PCT/US2011/027317

RECEIVED

From the INTERNATIONAL SEARCHING AUTHORITY

To:

HAILE LISA A.

DLA PIPER LLP (US) 4365 EXECUTIVE DRIVE, SUITE
1100 SAN DIEGO CA 92121-2133 USA

PCT

FEB 16 2012

DLA PIPER

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing
(day/month/year) 08 FEBRUARY 2012 (08.02.2012)

Applicant's or agent's file reference

JHU3260-4WO

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.

PCT/US2011/027317

International filing date
(day/month/year)

04 MARCH 2011 (04.03.2011)

Applicant

THE JOHNS HOPKINS UNIVERSITY et al

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes
1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 . 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.
3. ☐ With regard to any protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
- ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.
- ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Reminders

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90bis.1 and 90bis.3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the PCT Applicant's Guide, National Chapters.

Name and mailing address of the ISA/KR

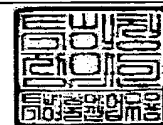
Korean Intellectual Property Office
Government Complex-Daejeon, 189 Cheongsu-ro,
Seo-gu, Daejeon 302-701, Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

COMMISSIONER

Telephone No. 82-42-481-8754



Form PCT/ISA/220 (July 2010)

* Attention

Copies of the documents cited in the international search report can be searched in the following Korean Intellectual Property Office English website for three months from the date of mailing of the international search report.

<http://www.kipo.go.kr/en/> => PCT Services => PCT Services

ID : PCT international application number
PW : **3XTYDGJR**

Inquiries related to PCT International Search Report or Written Opinion prepared by KIPO as an International Searching Authority can be answered not only by KIPO but also through IPKC (Intellectual Property Korea Center), located in Vienna, VA, which functions as a PCT Help Desk for PCT applicants.

Homepage: <http://www.ipkcenter.com>
Email: ipkc@ipkcenter.com
Phone: +1 703 388 1066
Fax: +1 703 388 1084

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference JHU3260-4WO	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No. PCT/US2011/027317	International filing date (day/month/year) 04 MARCH 2011 (04.03.2011)	(Earliest) Priority Date (day/month/year) 05 MARCH 2010 (05.03.2010)
Applicant THE JOHNS HOPKINS UNIVERSITY et al		

This International search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the language, the international search was carried out on the basis of:

- ☒ the international application in the language in which it was filed
☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))

b. ☐ This international search report has been established taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☒ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. ☐ Certain claims were found unsearchable (See Box No. II)

3. ☒ Unity of invention is lacking (See Box No. III)

4. With regard to the title,

- ☒ the text is approved as submitted by the applicant.
☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

- ☒ the text is approved as submitted by the applicant.
☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the drawings,

- a. the figure of the drawings to be published with the abstract is Figure No. 53G
☒ as suggested by the applicant.
☐ as selected by this Authority, because the applicant failed to suggest a figure.
☐ as selected by this Authority, because this figure better characterizes the invention.
- b. ☐ none of the figure is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2011/027317

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of:

a. a sequence listing filed or furnished

- ☐ on paper
☒ in electronic form

b. time of filing or furnishing

- ☐ contained in the international application as filed
☒ filed together with the international application in electronic form
☐ furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2011/027317

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.:
because they relate to subject matter not required to be searched by this Authority, namely:
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:

See extra sheet

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 fee, 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.: 1-6,60
Invention 1: a molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the immunomodulatory moiety comprises TGF- β RII, the targeting moiety comprises an antibody that binds to HER2/neu (claims 1-6,60 partially)
Invention 3: a molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the immunomodulatory moiety comprises TGF- β RII, the targeting moiety comprises an antibody that binds to CD20 (claims 1-6,60 partially)
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.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2011/027317**A. CLASSIFICATION OF SUBJECT MATTER***C07K 19/00(2006.01)i, C07K 14/495(2006.01)i, C07K 16/18(2006.01)i, A61K 38/16(2006.01)i, A61P 35/00(2006.01)i*

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)

C07K 19/00; A61K 39/395; A61K 39/04; C12N 5/08; A61K 48/00; C07K 16/46; C07K 16/30

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility modelsElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubMed**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2006/0263368 A1 (ROSENBLUM, M. G. et al.) 23 November 2006 See abstract; claims 1, 3, 4, 6, 10, 15.	1-6, 60
A	US 2009/0226435 A1 (KHARE, S.) 10 September 2009 See abstract, paragraphs 34, 36; claims 1, 2.	1-6, 60
A	US 2008/0075717 A1 (TRANCHAND-BUNEL, D.) 27 March 2008 See abstract; claims 1, 9.	1-6, 60
A	US 2007/0212337 A1 (BEDI, A. et al.) 13 September 2007 See abstract; paragraphs 6, 51; claims 1, 5, 6.	1-6, 60
A	US 2006/0135459 A1 (EPSTEIN, A. L. et al.) 22 June 2006 See abstract; claims 1, 3, 5, 13.	1-6, 60

☐ 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 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

03 FEBRUARY 2012 (03.02.2012)

Date of mailing of the international search report

08 FEBRUARY 2012 (08.02.2012)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
Government Complex-Daejeon, 189 Cheongsu-ro,
Seo-gu, Daejeon 302-701, Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

Lee Hyun Ji

Telephone No. 82-42-481-8745



Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2011/027317

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2006-0263368 A1	23.11.2006	AU 2006-203850 A1	13.07.2006
		CA 2593648 A1	13.07.2006
		CN 101203247 A	18.06.2008
		EP 1846039 A2	24.10.2007
		JP 2008-526889 A	24.07.2008
		JP 2008-526889 T	24.07.2008
		RU 2007130552 A	20.02.2009
		WO 2006-074451 A2	13.07.2006
		WO 2006-074451 A3	08.03.2007
US 2009-0226435 A1	10.09.2009	WO 2006-074451 A9	26.04.2007
		AU 2009-223784 A1	17.09.2009
		EP 2262531 A1	22.12.2010
		WO 2009-114110 A1	17.09.2009
US 2008-0075717 A1	27.03.2008	EP 1587825 A2	26.10.2005
		FR 2850384 A1	30.07.2004
		US 7803378 B2	28.09.2010
		WO 2004-069140 A2	19.08.2004
		WO 2004-069140 A3	19.08.2004
		WO 2004-069140 A3	16.09.2004
US 2007-0212337 A1	13.09.2007		
		AU 2007-211334 A1	09.08.2007
		BR P10707679A2	10.05.2011
		CA 2641026 A1	09.08.2007
		EP 1986698 A2	05.11.2008
		IL193106D0	11.02.2009
		JP 2009-525048 A	09.07.2009
		JP 2009-525048 T	09.07.2009
		KR20080100353A	17.11.2008
		MX 2008009970 A	19.11.2008
		RU 2008135318 A	10.03.2010
		WO 2007-089871 A2	09.08.2007
		WO 2007-089871 A3	30.10.2008
		WO 2007-089871 A8	21.12.2007
US 2006-0135459 A1	22.06.2006		
		AU 2005-304771 A1	18.05.2006
		CA 2586913 A1	18.05.2006
		EP 1809671 A2	25.07.2007
		EP 1809671 A4	25.02.2009
		WO 2006-052900 A2	18.05.2006
		WO 2006-052900 A3	20.07.2006

Form PCT/ISA/210 (patent family annex) (July 2009)

Continuation of Box III

Invention 1: a molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the immunomodulatory moiety comprises TGF- β RII, the targeting moiety comprises an antibody that binds to HER2/neu (claims 1-6,60 partially)

Invention 2-7: a molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the immunomodulatory moiety comprises TGF- β RII, the targeting moiety comprises an antibody that binds to EGFR1, CD20, VEGF, CTLA-4, CD25, or CD40 (claims 1-6,60 partially)

Invention 8-55: a molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the immunomodulatory moiety comprises an antibody binds to 4-1BB, OX40, or GITR, the targeting moiety comprises an antibody binds to HER2/neu, EGFR1, CD20, VEGF, CTLA-4, CD25, CD40, TGF- β , PD-1, PD-L1, PD-L2, RANKL, GITR, 4-1BB, OX-40, or IL-4R (claims 1,28,29,52,53,60 partially)

Invention 56-65: one selecting from SEQ ID No. 1-10 (claims 1-5,7,52,53,60 partially)

Invention 66-67: one selecting from SEQ ID No. 11 and 12 (claims 1-5,8-11,52,53,60 partially)

Invention 68-69: one selecting from SEQ ID No. 13 and 14 (claims 1-5,12-15,52,53,60 partially)

Invention 70-79: one selecting from SEQ ID No. 15 and 24 (claims 1,16-19,52,53,60 partially)

Invention 80-81: one selecting from SEQ ID No. 25 and 26 (claims 1,16,20-23,52,53,60 partially)

Invention 82-91: one selecting from SEQ ID No. 27 and 36 (claims 1,24-27,52,53,60 partially)

Invention 92-94: one selecting from SEQ ID No. 37 and 39 (claims 1,30-32,52,53,60 partially)

Invention 95-99: one selecting from SEQ ID No. 40 and 44 (claims 1,33,34,52,53,60 partially)

Invention 100-101: one selecting from SEQ ID No. 46 and 47 (claims 1,35,36,52,53,60 partially)

Invention 102-103: one selecting from SEQ ID No. 48 and 49 (claims 1,37,38,60 partially)

Invention 104-112: one selecting from SEQ ID No. 50 and 58 (claims 1,39-41,52,53,60 partially)

Invention 113-114: one selecting from SEQ ID No. 59 and 60 (claims 1,39,42,43,52,53,60 partially)

Invention 115-117: one selecting from SEQ ID No. 61 and 63 (claims 1,24, 25, 44, 45,52,53,60 partially)

Invention 118-119: one selecting from SEQ ID No. 64 and 65 (claims 1,24,25,46,47,52,53,60 partially)

Invention 120-121: one selecting from SEQ ID No. 66 and 67 (claims 1,24,25,48,49,52,53,60 partially)

Invention 122-123: one selecting from SEQ ID No. 68 and 69 (claims 1,24,25,50,51,52,53,60 partially)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

To:

HAILE LISA A.

DLA PIPER LLP (US) 4365 EXECUTIVE DRIVE, SUITE
1100 SAN DIEGO CA 92121-2133 USA

Date of mailing
(day/month/year) 08 FEBRUARY 2012 (08.02.2012)

Applicant's or agent's file reference

JHU3260-4WO

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US2011/027317

International filing date (day/month/year)

04 MARCH 2011 (04.03.2011)

Priority date(day/month/year)

05 MARCH 2010 (05.03.2010)

International Patent Classification (IPC) or both national classification and IPC

C07K 19/00(2006.01)i, C07K 14/495(2006.01)i, C07K 16/18(2006.01)i, A61K 38/16(2006.01)i, A61P 35/00(2006.01)i

Applicant

THE JOHNS HOPKINS UNIVERSITY et al

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
Government Complex-Daejeon, 189
Cheongsu-ro, Seo-gu, Daejeon 302-
701, Republic of Korea
Facsimile No. 82-42-472-7140



Date of completion of this opinion

03 FEBRUARY 2012 (03.02.2012)

Authorized officer

Lee Hyun Ji

Telephone No. 82-42-481-8745



Form PCT/ISA/237 (cover sheet) (July 2011)

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US2011/027317

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

- ☒ the international application in the language in which it was filed
- ☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of:

a. a sequence listing filed or furnished

- ☐ on paper
- ☒ in electronic form

b. time of filing or furnishing

- ☐ contained in the international application as filed.
- ☒ filed together with the international application in electronic form.
- ☐ furnished subsequently to this Authority for the purposes of search.

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2011/027317

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
- ☒ paid additional fees
 - ☐ paid additional fees under protest and, where applicable, the protest fee
 - ☐ paid additional fees under protest but the applicable protest fee was not paid
 - ☐ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

- ☐ complied with
- ☒ not complied with for the following reasons:

See Supplemental Box.

4. Consequently, this opinion has been established in respect of the following parts of the international application :

- ☐ all parts.
- ☒ the parts relating to claims Nos. 1-6, 60

Form PCT/ISA/237 (Box No. IV) (July 2011)

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US2011/027317

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-6,60	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	1-6,60	YES
	Claims	NONE	NO
Industrial applicability (IA)	Claims	1-6,60	YES
	Claims	NONE	NO

2. Citations and explanations :

1. Prior Arts

The following documents are referred to in this written opinion:

D1: US2006/0263368 A1 (ROSENBLUM, M. G. et al.) 23 November 2006
D2: US2009/0226435 A1 (KHARE, S.) 10 September 2009

2. Novelty and inventive step[PCT article 33(2) and 33(3)]

2.1

In case of Invention 1, claims 1-6 are directed to a molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the immunomodulatory moiety comprises TGF- β R11, the targeting moiety comprises an antibody that binds to HER2/neu.

In case of Invention 2, claims 1-6 are directed to a molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the immunomodulatory moiety comprises TGF- β R11, the targeting moiety comprises an antibody that binds to CD20.

D1 disclosed a chimeric cancer therapeutic molecules comprising a cell-specific targeting moiety and an anti-cell proliferation moiety, wherein the targeting moiety is an anti-HER2/neu antibody, the anti-cell proliferation moiety is TGF- β .

D2 disclosed fusion molecules consisting of a tumor targeting moiety fused with cytokines, wherein the a tumor targeting moiety is anti-Her2/neu antibody or anti-CD20 antibody.

There is no prior art, which directly indicates or fairly suggests the molecules comprising TGF- β R11, and anti-HER2/neu or anti-CD20 antibody. According to the description, the said molecules provide effective compositions and methods for cancer treatment by counteracting tumor-induced immune tolerance. The advantage achieved is also unforeseen over prior art without exercising undue experimentation. And also, it is non-obvious that a skilled person in the art could arrived at the claimed invention.

Accordingly, **Claims 1-6** are considered to be novel and inventive under PCT Article 33(2) and 33(3).

Continued on Supplemental Box

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US2011/027317

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Box V

Therefore **claim 60** referring to claim 1 is also considered to be novel and inventive under PCT Article 33(2) and 33(3).

3. Industrial Applicability

Claims 1-6, 60 of the present invention are considered industrially applicable under Article 33(4) PCT.

※ Claim 60 is directed to a method of treatment of the human body. The search has been carried out and statement on novelty, inventive step, and industrial applicability has been established, based on the alleged effects of the composition.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US2011/027317

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

BOX IV

Invention 1: a molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the immunomodulatory moiety comprises TGF- β RII, the targeting moiety comprises an antibody that binds to HER2/neu (claims 1-6,60 partially)

Invention 2-7: a molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the immunomodulatory moiety comprises TGF- β RII, the targeting moiety comprises an antibody that binds to EGFR1, CD20, VEGF, CTLA-4, CD25, or CD40 (claims 1-6,60 partially)

Invention 8-55: a molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the immunomodulatory moiety comprises an antibody binds to 4-1BB, OX40, or GITR, the targeting moiety comprises an antibody binds to HER2/neu, EGFR1, CD20, VEGF, CTLA-4, CD25, CD40, TGF- β , PD-1, PD-L1, PD-L2, RANKL, GITR, 4-1BB, OX-40, or IL-4R (claims 1,28,29,52,53,60 partially)

Invention 56-65: one selecting from SEQ ID No. 1-10 (claims 1-5,7,52,53,60 partially)

Invention 66-67: one selecting from SEQ ID No. 11 and 12 (claims 1-5,8-11,52,53,60 partially)

Invention 68-69: one selecting from SEQ ID No. 13 and 14 (claims 1-5,12-15,52,53,60 partially)

Invention 70-79: one selecting from SEQ ID No. 15 and 24 (claims 1,16-19,52,53,60 partially)

Invention 80-81: one selecting from SEQ ID No. 25 and 26 (claims 1,16,20-23,52,53,60 partially)

Invention 82-91: one selecting from SEQ ID No. 27 and 36 (claims 1,24-27,52,53,60 partially)

Invention 92-94: one selecting from SEQ ID No. 37 and 39 (claims 1,30-32,52,53,60 partially)

Invention 95-99: one selecting from SEQ ID No. 40 and 44 (claims 1,33,34,52,53,60 partially)

Invention 100-101: one selecting from SEQ ID No. 46 and 47 (claims 1,35,36,52,53,60 partially)

Invention 102-103: one selecting from SEQ ID No. 48 and 49 (claims 1,37,38,60 partially)

Invention 104-112: one selecting from SEQ ID No. 50 and 58 (claims 1,39-41,52,53,60 partially)

Invention 113-114: one selecting from SEQ ID No. 59 and 60 (claims 1,39,42,43,52,53,60 partially)

Invention 115-117: one selecting from SEQ ID No. 61 and 63 (claims 1,24, 25, 44, 45,52,53,60 partially)

Invention 118-119: one selecting from SEQ ID No. 64 and 65 (claims 1,24,25,46,47,52,53,60 partially)

Invention 120-121: one selecting from SEQ ID No. 66 and 67 (claims 1,24,25,48,49,52,53,60 partially)

Invention 122-123: one selecting from SEQ ID No. 68 and 69 (claims 1,24,25,50,51,52,53,60 partially)

Continued on Supplemental Box.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US2011/027317

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

BoX IV

The common technical feature linking inventions 1-123 is a molecule comprising a targeting moiety fused with an immunomodulatory moiety. However, this concept common to all claims of the present invention is described in D1, so the feature does not define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art under PCT Rule 13.2.

Thus the inventions 1-123 do not relate to a single general inventive concept under PCT Rule 13.1.

D1: US 2009/0226435 A1 (KHARE, S.) 10 September 2009

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Bedi and Ravi	Art Unit:	Not Yet Assigned
Application No.:	Not Yet Assigned	Examiner:	Not Yet Assigned
US Submission Date:	September 4, 2012	Conf. No.:	Not Yet Assigned
Based on Int'l Appl:	PCT/US2011/027317		
IA Filing Date:	March 4, 2011	Priority Date:	March 5, 2010
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

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

INFORMATION DISCLOSURE STATEMENT

Sir:

In accordance with 37 C.F.R. § 1.97, enclosed are references relating to the above-identified application. For the convenience of the Examiner, these 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 within three months of the date of entry of the national stage as set forth in 37 C.F.R. §§ 1.97 and 1.491.

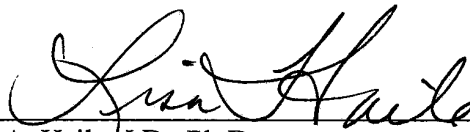
In re Application of:
Bedi and Ravi
Application No.: Not Yet Assigned
US Submission Date: September 4, 2012
Based on Intl Appl: PCT/US2011/027317
IA Filing Date: March 4, 2011
Page 2

PATENT
Attorney Docket No.: JHU3260-4

No fee is deemed necessary in connection with the filing of this Information Disclosure Statement because it is being submitted within three months of the date of entry of the national stage filing. However, if any fee is required, authorization is hereby given to charge any fees associated with the filings submitted herewith, or credit any overpayment to Deposit Account No. 07-1896.

Respectfully submitted,

Date: September 4, 2012



Lisa A. Haile, J.D., Ph.D.
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4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

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**

(Use as many sheets as necessary)

Complete if Known

Application Number	
Filing Date	
First Named Inventor	Atul Bedi
Art Unit	
Examiner Name	
Attorney Docket Number	JHU3260-4

Sheet

1

of

1

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)			
	AA	US-7,803,378 B2	09-28-2010	Tranchand-Bunel	
	AB	US-2006/0135459 A1	06-22-2006	Epstein et al.	
	AC	US-2006/0263368 A1	11-23-2006	Rosenblum et al.	
	AD	US-2007/0212337 A1	09-13-2007	Bedi et al.	
	AE	US-2008/0075717 A1	03-27-2008	Tranchand-Bunel	
	AF	US-2009/0226435 A1	09-10-2009	Khare	
		US-			
		US-			
		US-			
		US-			
		US-			
		US-			
		US-			
		US-			
		US-			

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
SignatureDate
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.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Electronic Patent Application Fee Transmittal				
Application Number:				
Filing Date:				
Title of Invention:		Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins		
First Named Inventor/Applicant Name:		Atul Bedi		
Filer:		Lisa A. Haile/Aldon Griffis		
Attorney Docket Number:		JHU3260-4		
Filed as Small Entity				
U.S. National Stage under 35 USC 371 Filing Fees				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Basic National Stage Fee	2631	1	190	190
Natl Stage Search Fee - Report provided	2642	1	245	245
Natl Stage Exam Fee - all other cases	2633	1	125	125
Pages:				
Natl Stage Appl Sz fee per 50 pgs >100	2681	2	155	310
Claims:				
Claims in excess of 20	2615	33	30	990
Miscellaneous-Filing:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Oath/decl > 30 mo. from priority date	2617	1	65	65
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				1925

Electronic Acknowledgement Receipt

EFS ID:	13655976
Application Number:	13582717
International Application Number:	PCT/US11/27317
Confirmation Number:	3707
Title of Invention:	Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Lisa A. Haile/Aldon Griffis
Filer Authorized By:	Lisa A. Haile
Attorney Docket Number:	JHU3260-4
Receipt Date:	04-SEP-2012
Filing Date:	
Time Stamp:	19:09:59
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$1925
RAM confirmation Number	7096
Deposit Account	071896
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. 1.492 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		C11123_P11123-03_20120904_APP_DLAP_USNationalEFS.pdf	1468782 07f56e01af1930c53b5669b2c588f7a90355b370	yes	34
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Transmittal of New Application		1	3	
	Oath or Declaration filed		4	7	
	Preliminary Amendment		8	19	
	Documents submitted with 371 Applications		20	34	
Warnings:					
Information:					
2		C11123_P11123-03_20120904_APP_DLAP_USNationalIDS_EFS.pdf	127497 56c6579084e6359e8f647b7b4d480ff548fd1e9b	yes	3
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Transmittal Letter		1	2	
	Information Disclosure Statement (IDS) Form (SB08)		3	3	
Warnings:					
Information:					
3	Sequence Listing (Text File)	JHU3260-4_ST25.txt	469696	no	0
Warnings:					
Information:					
4	Fee Worksheet (SB06)	fee-info.pdf	40149 82730fa7ff32890232ca32ac10dd5437a766360f	no	2
Warnings:					
Information:					
Total Files Size (in bytes):			2106124		

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.

341908

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

March 17, 2011

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: 61/435,671

FILING DATE: *January 24, 2011*

RELATED PCT APPLICATION NUMBER: *PCT/US11/27317*

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US61/435,671*



Certified by

David J. Kappas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

APPLICATION DATA SHEET

INVENTOR INFORMATION

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Country of Residence:: USA
Citizenship Country:: USA

CORRESPONDENCE ADDRESS: DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

CORRESPONDENCE CUSTOMER NUMBER :: 28213

APPLICATION INFORMATION

Title Line One:: TARGETED IMMUNOMODULATORY ANTIBODIES AND
Title Line Two:: FUSION PROTEINS
Total Drawing Sheets:: Zero (0)
Formal Drawings?: N/A
Application Type:: Provisional
Docket Number:: JHU3260-3

CONTINUITY INFORMATION

The instant application is a ::
Which claims the benefit of priority under 35 U.S.C. § 119(e) of:
Application One::
Filing Date::

ASSIGNEE INFORMATION

Assignee Name:: THE JOHNS HOPKINS UNIVERSITY
Street of Mailing Address:: 3400 N. Charles Street
City of Mailing Address:: Baltimore
State or Province:: Maryland
Country:: USA
Postal or Zip Code:: 21218

PROVISIONAL APPLICATION

for

UNITED STATES LETTERS PATENT

on

TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

by

Atul Bedi

Rajani Ravi

Sheets of Drawings: Zero (0)

Docket No.: JHU3260-3

Lisa A. Haile, J.D., Ph.D.
DLA PIPER LLP (US)
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133

WEST\223023163.1
331323-000890

Invention Disclosure Form

CONFIDENTIAL

JHTT USE ONLY:

JHU Ref. _____

TLA _____

Please submit Disclosures to:

Johns Hopkins University, Johns Hopkins Technology Transfer, 100 North Charles Street, 5th Floor, Baltimore, MD 21201
(410) 516-8300 Fax (410) 516-5113 JHTT-Communications@jhu.edu

This form is to be completed and submitted to the JHU office of Johns Hopkins Technology Transfer (JHTT) by anyone who believes they have developed a new Invention. The purpose of this form is to enable JHTT to evaluate whether legal protection to the Invention will be sought and/or commercialization pursued. Please submit this form with all Inventor(s) and Department Director(s) signatures. Visit the JHTT web site at <http://www.techtransfer.jhu.edu/faculty/disclosure.html> for .pdf and Word downloadable formats of this form.

INVENTION INFORMATION

- 1. Title of Invention:** [Title should be sufficiently descriptive to identify the Invention yet not reveal unique unpublished details.]

Targeted Immunomodulatory Antibodies and Fusion Proteins

- 2. Abstract of Invention** [Please provide a non-confidential summary of the Invention that can be used for marketing purposes..]

(I) The invention describes novel 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: (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); (iii) Increasing activation and proliferation of antitumor CD4+ and CD8+ T cells by negating immune suppression mediated by regulatory T cells and myeloid suppressor cells. These antitumor immune responses are 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 novelty of tumor-targeted immunostimulatory mAbs is 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. As such, the described targeted immunostimulatory antibodies and fusion proteins 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.

(II) The invention describes novel targeted immunosuppressive antibodies and fusion proteins that induce or promote immune tolerance by: (i) Inhibiting the activation of dendritic cells, T cells, and/or B cells; (ii) Promoting the development and/or suppressor function of regulatory T cells and immunosuppressive myeloid DCs. These targeted immunosuppressive molecules 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.

- 3. Problem Solved** [Describe the problem solved by this Invention]

(I) Targeted immunostimulatory antibodies and fusion proteins:

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, our studies demonstrate that these agents have immuno-adjuvant effects which activate innate and adaptive antitumor immune responses that are crucial for their antitumor efficacy *in vivo*. We find 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. Our studies indicate 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.

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 described tumor-targeted immunostimulatory antibodies and fusion proteins 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. As such, the described tumor-targeted immunostimulatory antibodies and fusion proteins have broad clinical relevance for advancing the treatment of many types of human cancers.

(II) Targeted immunosuppressive antibodies and 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 described targeted immunosuppressive antibodies and fusion proteins are designed to suppress

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unwanted or excessive immune or inflammatory responses and restore or promote immune tolerance. As such, they 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.

4. Novel Features [Identify those elements of the Invention that are new when compared to the current state of the art]

(I) The novelty of tumor-targeted immunostimulatory mAbs or fusion proteins is 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. As such, the described tumor-targeted immunostimulatory antibodies and fusion proteins 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 is distinguished from and superior to conventional tumor antigen-, allogeneic tumor cell- or DC-based vaccines in 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.

(II) The novelty of the targeted immunosuppressive antibodies or fusion proteins is 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 are distinguished from and superior to existing therapeutic molecules in the following aspects:

- (i) They enable targeted delivery of immunosuppressive molecules to immune cells or pro-inflammatory molecules in the milieu of the affected cell, tissue or organ.
- (ii) They 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.
- (iii) They provide a mechanism of simultaneously engaging two independent or synergistic mechanisms of immune tolerance or immune suppression.

5. Detailed Description of the Invention - On a separate page(s), attach a detailed description of how to make and use the Invention. The description must contain sufficient detail so that one skilled in the same discipline could reproduce the Invention. Include the following as necessary:

- | | |
|--|---|
| 1- data pertaining to the Invention; | 4- procedural steps if a process; |
| 2- drawings or photographs illustrating the Invention; | 5- a description of any prototype or working model; |
| 3- structural formulae if a chemical; | |

In general, a manuscript that has been prepared for submission to a journal will satisfy this requirement.

Please see the following documents:

- (i) Provisional Patent Application: 35575-709-1.101 Provisional SN61311255.pdf (Appendix 1)
- (ii) Provisional Patent Application: Targeted Immunomodulatory Antibodies and Fusion Proteins – 010311 (Appendix 2)

6. Workable Extent/Scope [Describe the future course of related work, and possible variations of the present Invention in terms of the broadest scope expected to be operable; if a compound, describe substitutions, breadth of substituents, derivatives, salts etc., if DNA or other biological material, describe modifications that are expected to be operable, if a machine or device, describe operational parameters of the device or a component thereof, including alternative structures for performing the various functions of the machine or device]

Please see the following documents:

- (i) Provisional Patent Application: 35575-709-1.101 Provisional SN61311255.pdf (Appendix 1)
- (ii) Provisional Patent Application: Targeted Immunomodulatory Antibodies and Fusion Proteins – 010311 (Appendix 2)

7. References [Please cite relevant journal citations, patents, general knowledge or other public information related to the Invention and distinguish between references that (A) contain a description of the current Invention from those that (B) contain background information.] ☐ No references available at this time.

(A) Description of current invention – No references available at this time.

(B) Background Information: Journal citations;

- Zitvogel L, Apetoh L, Ghiringhelli F and Kroemer G. Immunological aspects of cancer chemotherapy. *Nat Rev Immunol.* 8:59-73, 2008.
- Lake RA and Robinson WS. Immunotherapy and chemotherapy – a practical partnership. *Nat Rev Cancer* 5: 397-405, 2005.
- Sakaguchi S, Yamaguchi T, Nomura T, and Ono M. Regulatory T cells and immune tolerance. *Cell* 133(5):775-787, 2008.
- von Boehmer H. Mechanisms of suppression by suppressor T cells, *Nat. Immunol.* 6 (2005), pp. 338–344
- Tang Q and Bluestone JA. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation, *Nat. Immunol.* 9 (2008), pp. 239–244.
- Zou W. Regulatory T cells, tumor immunity, and immunotherapy. *Nat Rev Immunol* 6:295-307, 2006.
- Zou W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat Rev Cancer* 5:263-274, 2005.
- Brierie B and Moses HL. Tumor microenvironment: TGF β : the molecular Jekyll and Hyde of cancer. *Nat Rev Cancer* 6:506-520, 2006.

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- Wan YY and Flavell RA. 'Yin-Yang' functions of transforming growth factor-beta and T regulatory cells in immune regulation. *Immunol Rev* 220:199-213, 2007.
- Li MO, Wan YY, Sanjabi S, Robertson A-KL, Flavell RA. Transforming growth factor- β regulation of immune responses. *Annu Rev Immunol* 24:99-146, 2006.
- Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. *Immunol Rev* 236:219-242, 2010.

8. **Commercialization** - List any companies that you feel may be interested in this technology or are doing similar research. Indicate how the Invention complements the company's existing technology. If known, provide the names of any companies (and a contact person) that have contacted you regarding your research related to the Invention. ☐ No company interest known at this time.

IATRICa, Inc.

9. **Potential Commercial Use** - What products can be produced with this Invention?

- (I) Novel targeted immunostimulatory antibodies and fusion proteins:
- (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.
 - (iii) To serve as immune adjuvants or vaccines for prophylaxis of diverse cancers or infectious diseases.
- (II) Novel targeted immunosuppressive antibodies and fusion proteins:
- (i) To suppress unwanted or excessive immune or inflammatory responses in order to treat autoimmune or inflammatory diseases.
 - (ii) To prevent the rejection of a transplanted cell, tissue, or organ grafts.

Please see the following documents:

(iii) Provisional Patent Application: 35575-709-1.101 Provisional SN61311255.pdf (Appendix 1)

(iv) Provisional Patent Application: Targeted Immunomodulatory Antibodies and Fusion Proteins – 010311 (Appendix 2)

10. **Biological Material** – Does this disclosure include biological material? ☐ Yes ☒ No If yes,
- A. Please attach a list of materials for reference.
- B. Was the material derived from human materials? ☐ Yes ☐ No
- C. If 'YES', can the material be traced to its human donor(s) through identifiers or some other means? ☐ Yes ☐ No
- D. If 'YES', please provide IRB Protocol identifier: _____

11. **Software** – Does this disclosure include a software element or is software implemented in the Invention? ☐ Yes ☒ No

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12. Research Support Information

Indicate ALL contributions to the development of the Invention in terms of personnel, money, materials and facilities etc. Check each funding source that applies to this Invention:

☒ None ☐ Federal Sponsor(s) ☐ University Funding ☐ Commercial Funding ☐ Other

For each funding source, provide the: grant/funding source; award/contract number; title of grant; The name of the principal investigator on each grant/award*, and the percent of support from each funding source. Additionally, if "Commercial" or "Other" funding was used, please attach a copy of each such award notice.

* if the principal investigator is not included as an Inventor on this Invention Disclosure, JHTT will provide a copy of this completed form to the PI so that he or she may comply with agency reporting obligations

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If yes, please provide the: source of material(s) and the name of the material(s) for each item, and attach a copy of the agreement.

Was the "TET-System" used in the development of the Invention? ☐ Yes ☒ No

14. Disclosures of the Invention

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16. **INVENTOR(S) CONTACT INFORMATION**

[It is not necessary to obtain all signatures before submitting this report to JHTT.
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[Please copy this page for additional Inventors as necessary]

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Section A JHU SCHOOL and DEPARTMENT DIRECTOR'S ACKNOWLEDGEMENT		
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JHU Inventor Signature	<u>Atul Bedi</u> Typed or Printed Name	Date: % of Contribution <u>75</u>
JHU Inventor Signature	<u>Rajani Ravi</u> Typed or Printed Name	Date: % of Contribution <u>25</u>
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Inventor Signature	Typed or Printed Name	Date: % of Contribution
Inventor Signature	Typed or Printed Name	Date: % of Contribution

Targeted Immunomodulatory Antibodies and Fusion Proteins:

CLAIMS:

COMPOSITIONS:

1. A molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:
 - (a) said targeting moiety binds a specific target molecule, and
 - (b) said immunomodulatory moiety binds one of the following molecules:
 - (i) Transforming growth factor-beta (TGF- β);
 - (ii) Programmed death-1 ligand [PD-L; PD-L1 (B7-H1), PD-L2 (B7-DC)];
 - (iii) Transforming growth factor-beta receptor (TGF- β R);
 - (iv) Programmed death-1 (PD-1).
2. A molecule of claim 1, wherein said targeting moiety is an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell.
3. A molecule of claim 2, wherein said immunomodulatory moiety is a molecule that binds TGF- β .
4. A molecule of claim 3, wherein said immunomodulatory moiety is a sequence derived from the extracellular ligand-binding domain of Transforming growth factor-beta receptor (TGF- β RII, TGF- β RIIb, TGF- β RIII).
5. A molecule of claim 4, wherein said immunomodulatory moiety is a sequence from the extracellular ligand-binding domain of TGF- β RII.
6. A molecule of claim 5, wherein said targeting moiety is an antibody or antibody fragment that specifically binds the HER2/neu receptor.
7. A molecule of claim 6, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 1.
8. A molecule of claim 5, wherein said targeting moiety is an antibody or antibody fragment that specifically binds the EGFR1 receptor.
9. A molecule of claim 8, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 2.
10. A molecule of claim 5, wherein said targeting moiety is an antibody or antibody fragment that specifically binds CD20.
11. A molecule of claim 10, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 3.
12. A molecule of claim 5, wherein said targeting moiety is an antibody or antibody fragment that specifically binds vascular endothelial growth factor (VEGF).
13. A molecule of claim 12, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 4.
14. A molecule of claim 5, wherein said targeting moiety is an antibody or antibody fragment that specifically binds cytotoxic T-lymphocyte antigen-4 (CTLA-4).
15. A molecule of claim 14, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 5.

16. A molecule of claim 5, wherein said targeting moiety is an antibody, antibody fragment, or polypeptide that specifically binds CD4.
17. A molecule of claim 5, wherein said targeting moiety is an antibody, antibody fragment, or polypeptide that specifically binds CD25 (IL-2 α R).
18. A molecule of claim 17, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 6.
19. A molecule of claim 17, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 7.
20. A molecule of claim 5, wherein said targeting moiety is an antibody, antibody fragment, or polypeptide that specifically binds Programmed Death-1.
21. A molecule of claim 5, wherein said targeting moiety is an antibody, antibody fragment, or polypeptide that specifically binds Programmed Death-1 ligand (PD-L1 or PD-L2).
22. A molecule of claim 21, wherein said targeting moiety is a polypeptide comprising the extracellular ligand-binding domain (ectodomain) of Programmed Death-1 (PD-1).
23. A molecule of claim 22, wherein said molecule comprises PD-1 ectodomain, Immunoglobulin Fc, and TGF β RII ectodomain.
24. A molecule of claim 23, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 9.
25. A molecule of claim 2, wherein said immunomodulatory moiety is a molecule that binds Programmed Death-1 ligand (PD-L1 or PD-L2).
26. A molecule of claim 25, wherein said immunomodulatory moiety is a sequence derived from the extracellular ligand-binding domain of Programmed Death-1 (PD-1 ectodomain).
27. A molecule of claim 26, wherein said targeting moiety is an antibody or antibody fragment that specifically binds the HER2/neu receptor.
28. A molecule of claim 27, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 10.
29. A molecule of claim 26, wherein said targeting moiety is an antibody or antibody fragment that specifically binds the EGFR1 receptor.
30. A molecule of claim 29, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 11.
31. A molecule of claim 26, wherein said targeting moiety is an antibody or antibody fragment that specifically binds CD20.
32. A molecule of claim 31, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 12.
33. A molecule of claim 26, wherein said targeting moiety is an antibody or antibody fragment that specifically binds vascular endothelial growth factor (VEGF).
34. A molecule of claim 33, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 13.
35. A molecule of claim 26, wherein said targeting moiety is an antibody or antibody fragment that specifically binds cytotoxic T-lymphocyte antigen-4 (CTLA-4).
36. A molecule of claim 35, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 14.
37. A molecule of claim 26, wherein said targeting moiety is an antibody, antibody fragment, or polypeptide that specifically binds CD4.

38. A molecule of claim 26, wherein said targeting moiety is an antibody, antibody fragment, or polypeptide that specifically binds CD25 (IL-2 α R).
39. A molecule of claim 38, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 15.
40. A molecule of claim 38, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 16.
41. A molecule of claim 1, wherein said targeting moiety binds Tumor Necrosis Factor- α (TNF- α), and said immunomodulatory moiety comprises a sequence from PD-1 ligand (PD-L1 or PD-L2).
42. A molecule of claim 41, wherein said targeting moiety is an antibody that binds TNF- α , and said immunomodulatory moiety comprises a sequence from PD-1 ligand 1 (PD-L1; B7-H1).
43. A molecule of claim 42, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 17.
44. A molecule of claim 41, wherein said targeting moiety is a polypeptide comprising the extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2), and said immunomodulatory moiety comprises a sequence from PD-1 ligand 1 (PD-L1; B7-H1).
45. A molecule of claim 44, comprising a sequence from TNFR2 Extracellular ligand binding domain, Immunoglobulin Fc, and a sequence from PD-L1.
46. A molecule claim 45, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 18.
47. A molecule claim 45, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 19.
48. A molecule of claim 1, wherein said targeting moiety is an antibody or antibody fragment that binds CD20, and said immunomodulatory moiety comprises a sequence from PD-1 ligand (PD-L1 or PD-L2).
49. A molecule of claim 48, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 20.
50. A molecule of claim 1, wherein said targeting moiety is an antibody, antibody fragment, or polypeptide that specifically binds CD4, and said immunomodulatory moiety comprises a sequence from PD-1 ligand (PD-L1 or PD-L2).
51. A molecule of claim 1, wherein said targeting moiety is an antibody, antibody fragment, or polypeptide that specifically binds CD25 (IL-2 α R), and said immunomodulatory moiety comprises a sequence from PD-1 ligand (PD-L1 or PD-L2).
52. A molecule of claim 51, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 21.
53. A molecule of claim 52, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 22.
54. A molecule of claim 1, wherein said targeting moiety comprises the extracellular domain of CTLA-4 and Immunoglobulin Fc (IgG Cy1), and said immunomodulatory moiety comprises a sequence from PD-1 ligand (PD-L1 or PD-L2).
55. A molecule of claim 54, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 23.
56. A molecule of claim 54, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 24.
57. A molecule of claim 1, wherein said targeting moiety comprises transforming growth

- factor- β (TGF- β) and Immunoglobulin Fc (IgG C γ 1), and said immunomodulatory moiety comprises a sequence from PD-1 ligand (PD-L1 or PD-L2).
58. A molecule of claim 57, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 25.
 59. A molecule of claim 57, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 26.
 60. A molecule of claim 1, wherein said targeting moiety binds Tumor Necrosis Factor- α (TNF- α), and said immunomodulatory moiety comprises a sequence from transforming growth factor- β (TGF- β).
 61. A molecule of claim 60, wherein said targeting moiety is an antibody that binds TNF- α , and said immunomodulatory moiety comprises a sequence from transforming growth factor- β (TGF- β).
 62. A molecule of claim 61, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 27.
 63. A molecule of claim 60, wherein said targeting moiety is a polypeptide comprising the extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2), and said immunomodulatory moiety comprises a sequence from transforming growth factor- β (TGF- β).
 64. A molecule of claim 63 comprising a sequence from TNFR2 Extracellular ligand binding domain, Immunoglobulin Fc, and a sequence from transforming growth factor- β (TGF- β).
 65. A molecule claim 64, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 28.
 66. A molecule claim 64, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 29.
 67. A molecule of claim 1, wherein said targeting moiety is an antibody or antibody fragment that binds CD20, and said immunomodulatory moiety comprises a sequence from transforming growth factor- β (TGF- β).
 68. A molecule of claim 67, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 30.
 69. A molecule of claim 1, wherein said targeting moiety is an antibody, antibody fragment, or polypeptide that specifically binds CD4, and said immunomodulatory moiety comprises a sequence from transforming growth factor- β (TGF- β).
 70. A molecule of claim 1, wherein said targeting moiety is an antibody, antibody fragment, or polypeptide that specifically binds CD25 (IL-2 α R), and said immunomodulatory moiety comprises a sequence from transforming growth factor- β (TGF- β).
 71. A molecule of claim 70, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 31.
 72. A molecule of claim 70, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 32.
 73. A molecule of claim 1, wherein said targeting moiety comprises the extracellular domain of CTLA-4 and Immunoglobulin Fc (IgG C γ 1), and said immunomodulatory moiety comprises a sequence from transforming growth factor- β (TGF- β).
 74. A molecule of claim 73, wherein said molecule has an amino acid sequence corresponding to SEQ. ID 33.
 75. A molecule of claim 73, wherein said molecule has an amino acid sequence

corresponding to SEQ. ID 34.

- 76. A molecule of claim 1, wherein said molecule is fused to one or more antigen, antigenic determinant, or epitope.
- 77. A composition comprising a molecule of claim 1 and a cell, wherein said cell is a tumor cell, immune cell, or dendritic cell.

METHODS OF USE:

- 78. A method of counteracting or overcoming immune tolerance comprising administration to a subject in need thereof one or more compositions of claims 1-40.
- 79. A method of preventing or treating a neoplastic disease comprising administration to a subject in need thereof one or more compositions of claims 1-40.
- 80. A method of claim 79, wherein said subject is administered one or more compositions in combination with any anticancer therapy.
- 81. A method of claim 80, wherein said anticancer therapy comprises a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent or cytotoxic agent.
- 82. A method of claim 80, wherein said anticancer therapy comprises ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, radiofrequency ablation.
- 83. A method of claim 78, wherein a subject in need thereof is administered one or more compositions in combination with any vaccine.
- 84. A method of claim 83, wherein said vaccine comprises a tumor antigen, tumor-associated antigen, tumor epitope, tumor antigen-containing fusion protein, tumor cell, or dendritic cell.
- 85. A method of claim 83, wherein said vaccine comprises a pathogen antigen, pathogen-associated antigen, pathogen epitope, or pathogen antigen-containing fusion protein.
- 86. A method of treatment of immune cells wherein said cells are contacted *ex vivo* or *in vitro* with a composition of claims 1-40.
- 87. A method of treatment of a neoplastic disease comprising administration to a subject in need thereof a composition of immune cells contacted with a composition of claims 1-40.
- 88. A method of inducing or promoting immune tolerance comprising administration to a subject in need thereof one or more compositions of claims 41-76.
- 89. A method of preventing or treating an autoimmune or inflammatory disease comprising administration to a subject in need thereof one or more compositions of claims 41-76.
- 90. A method of claim 89, wherein said subject is administered one or more compositions in combination with any anti-inflammatory or immunosuppressive therapy.
- 91. A method of treatment of immune cells wherein said cells are contacted *ex vivo* or *in vitro* with a composition of claims 41-76.
- 92. A method of treatment comprising administration to a subject in need thereof a composition of immune cells contacted with a composition of claims 41-76.

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Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
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Rajani	Ravi	Ruxtan, Maryland
<i>Additional inventors are being named on the _____ separately numbered sheets attached hereto</i>		
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☐ A check or money order is enclosed to cover the filing fee and application size fee (if applicable).		
☐ Payment by credit card. Form PTO-2038 is attached TOTAL FEE AMOUNT (\$)		
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SIGNATURE _____

Lisa A. Haile

Date January 24, 2011

TYPED or PRINTED NAME Lisa A. Haile, J.D., Ph.D.

REGISTRATION NO. 38,347
(if appropriate)

TELEPHONE 858.677.1456

Docket Number: JHU3260-3

TUMOR TARGETING COMPOSITIONS

BACKGROUND

[0001] 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

[0002] In one embodiment, a composition disclosed herein comprises an immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells. In one embodiment, the targeting moiety binds a component of a tumor cell, tumor cell surface molecule, tumor-associated cell, tumor microenvironment, tumor-associated immune cell, tumor antigen, tumor-associated antigen, tumor-antigen-containing fusion protein, or tumor vaccine. In another embodiment, the targeting moiety or moiety that inhibits the production or function of regulatory T cells binds a surface molecule on regulatory T cells. In one embodiment, the surface molecule is CD4, CD25, CTLA-4 or LAG-3. In another embodiment, the moiety that inhibits the production or function of regulatory T cells is a molecule that inhibits expression or function of STAT3. In yet another embodiment, the molecule that inhibits expression or function of STAT3 is a small interfering RNA. In one embodiment, the moiety that inhibits the production or function of regulatory T cells is a molecule that binds transforming growth factor β (TGF- β) or antagonizes the function of TGF- β . In one embodiment, the moiety that inhibits the production or function of regulatory T cells is a molecule that inhibits expression or function of Foxp3. In yet another embodiment, the molecule that inhibits expression or function of FOXP3 is a small interfering RNA. In one embodiment, the moiety that inhibits the production or function of regulatory T cells is a molecule that binds CTLA-4.

[0003] Also provided herein is a composition comprising a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β). In one embodiment, the tumor targeting moiety is conjugated or fused to said molecule that binds transforming growth factor beta (TGF- β). In another embodiment, the tumor targeting moiety is an antibody, antibody fragment, peptide, aptamer, ligand, or a combination thereof. In one embodiment, the tumor targeting moiety is an antibody or scFv. In yet another 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 or tumor antigen-containing fusion protein. In one embodiment, the tumor targeting moiety binds a growth factor receptor, growth factor, cytokine receptor, cytokine, or cell surface molecule. In another embodiment, the tumor targeting moiety binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), Insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, Vascular endothelial growth factor, CD20, CD30, CD33, CD40, or a combination thereof. In another embodiment, the tumor targeting moiety binds a regulatory T cell or a suppressor T cell. In yet another embodiment, the tumor targeting moiety binds CD4, CD25, CTLA-4, or a combination thereof. In one embodiment, the tumor targeting moiety is fused to the molecule that binds transforming growth factor beta (TGF- β), wherein said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence derived from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII). In one embodiment, the polypeptide sequence comprises SEQ ID NO. 1, 2 or 3.

[0004] In one embodiment, the tumor targeting moiety is an antibody or scFv that binds EGFR1. In yet another embodiment, the tumor targeting moiety is an antibody or scFv that binds CD20. In one embodiment, the tumor targeting moiety is an antibody or scFv that binds CD4. In another embodiment, the tumor targeting moiety is an antibody or scFv that binds CD25. In another embodiment, the tumor targeting moiety is an antibody or scFv that binds CTLA-4.

[0005] Also provided herein, is a composition as described herein, further comprising a tumor cell, tumor antigen, or tumor-associated antigen, immune cell, dendritic cell, or any combination thereof. In one embodiment, the composition further comprises a PAMP or DAMP. In another embodiment, the composition further comprises a tetanus antigen or a pDOM, or both. In yet another embodiment, the composition comprises a pDOM fused to a cationic polypeptide. In yet another embodiment, the composition further comprises a pDOM fused to arginine or histidine enriched polypeptide sequence. In one embodiment, the composition comprises a pDOM fused to polyarginine or polyhistidine polypeptide. In one embodiment, the pDOM fused to 1 to 20 arginines. In one embodiment, the pDOM is fused to 9 arginines. Also provided herein, is a composition as described herein, further comprising a chemotherapeutic agent. In one embodiment, the chemotherapeutic agent is doxorubicin.

[0006] In another embodiment, in a composition comprising a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β), the tumor targeting moiety is an antibody that binds to EGFR1. In another embodiment the tumor targeting moiety is cetuximab. In another embodiment, the tumor targeting moiety is antibody that binds to HER2/Neu. In one embodiment, the tumor targeting moiety is trastuzumab. In yet another embodiment, the tumor targeting moiety is antibody that binds to CD20. In one embodiment, the tumor targeting moiety is rituximab.

[0007] Also provided herein is a method of preventing or treating a neoplastic condition or disorder in a subject in need thereof comprising administering a composition as described herein. In another embodiment, the method of preventing or treating a neoplastic disorder in a subject in need thereof comprises administering an immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells. In yet another embodiment, a method of preventing or treating a neoplastic disorder in a subject in need thereof comprises administering a composition comprising a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β).

[0008] In one embodiment, a method of preventing or treating a neoplastic disorder in a subject in need thereof comprises administering a combination comprising: a cytotoxic anticancer agent and a moiety that reduces the number or function of a CD4⁺ regulatory T cell, wherein the cytotoxic anticancer agent is a chemotherapeutic agent, a kinase inhibitor, a tumor targeted antibody, or any combination thereof. In one embodiment, the neoplastic disorder is not a CD4-expressing T-cell malignancy. In another embodiment, the moiety that reduces the number or function of CD4⁺ regulatory T cells is an antibody or other moiety that binds CD4 or CD25.

[0009] In one embodiment, the method of preventing or treating a neoplastic disorder in a subject in need thereof further comprises administering to the subject an anticancer agent. In one embodiment, the anticancer agent is a chemotherapeutic agent, antibody, tyrosine kinase inhibitor, cytokine, targeted therapeutic agent, immunotherapeutic agent, hormonal therapeutic agent, or antiangiogenic agent. In yet another embodiment, the method of preventing or treating a neoplastic disorder in a subject in need thereof further comprises administering to the subject a vaccine, wherein the vaccine comprises a tumor cell, tumor antigen, tumor-associated antigen, dendritic cell, or any combination thereof. In yet another embodiment, the method of preventing or treating a neoplastic disorder in a subject in need thereof further comprises administering to the subject a PAMP or DAMP.

[0010] In one embodiment, the method of preventing or treating a neoplastic disorder in a subject in need thereof further comprises administering to the subject a tetanus antigen. In another embodiment, the method of preventing or treating a neoplastic disorder in a subject in need thereof further comprises administering to the subject pDOM. In one embodiment, the pDOM is fused to a cationic polypeptide. In yet another embodiment, the pDOM is fused to arginine or histidine enriched polypeptide sequence. In yet another embodiment, the pDOM is fused to polyarginine or polyhistidine polypeptide. pDOM fused to 1 to 20 arginines. In yet another embodiment, the pDOM fused to 9 arginines.

INCORPORATION BY REFERENCE

[0011] 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0013] **FIG. 1** illustrates immune cells play an instrumental role in the antitumor efficacy of cytotoxic anticancer agents.

[0014] **FIG. 2** illustrates regulatory T cells inhibit the antitumor efficacy of cytotoxic anticancer agents.

[0015] **FIG. 3** illustrates depletion of CD4⁺ T cells improves the efficacy of cytotoxic anticancer agents.

[0016] **FIG. 4** illustrates depletion of CD4⁺ T cells improves the efficacy of chemo-immunotherapy.

DETAILED DESCRIPTION

[0001] Before the present composition, methods, and methodologies are described, it is to be understood that the disclosure is not limited to particular compositions, methods, and experimental conditions described, as such compositions, methods, and conditions may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting.

[0002] 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 “a nucleic acid” includes one or more nucleic acids, and/or compositions of the type described herein which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0003] As used herein “immune effector cells” include T cells, NK cells, B cells, monocytes, macrophages, and dendritic cells (DC).

[0004] 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.

[0005] As used herein, “neoplasm,” 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, melanoma, sarcoma, liver, kidney, lung, testicular, breast, ovarian, pancreatic, brain, head and neck, melanoma, leukemia, lymphoma cancer, and the like.

[0006] 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.

[0007] As used herein “conjugation,” including grammatical variations thereof, means directly or indirectly linking, coupling, binding and the like of the foreign DNA or RNA with target-specific antibodies and/or peptides and/or tumor targeting moieties, either chemically, electrostatically, non-covalently, or by other techniques. For example, an isolated antibody-nucleic acid conjugate or peptide-nucleic acid conjugate as presently disclosed

would fall under this definition.

[0008] An “immunostimulatory nucleic acid sequence” (INAS) refers to a nucleic acid molecule that is a pathogen-associated molecular pattern (PAMP) or other motif that can activate immune cells, including, but not limited to, double stranded DNA (ds DNA), single stranded DNA (ss DNA), CpG DNA (CpG), herpes simplex virus (HSV) DNA, double stranded RNA (dsRNA), and single stranded RNA (ssRNA). In a related aspect, the INAS may be a coding or non-coding sequence. As illustrative examples, an INAS may be DNA or RNA.

[0009] 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.

[0010] 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.

[0011] 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. 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.

[0012] 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.

Targeting Moiety

[0013] Provided herein is a composition comprising a targeting moiety. Also provided herein is an immunoconjugate or fusion protein comprising a targeting moiety. In one embodiment, an immunoconjugate or

fusion protein comprises a targeting moiety and a moiety that inhibits the production or function of regulatory T cells. In another embodiment, a composition comprises a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β).

[0014] A targeting moiety refers to a molecule that has the ability to localize and bind to a molecule present on a cell or tissue, such as a normal cell or tissue, or a diseased cell or tissue, such as a cancer cell or tumor. In one embodiment, a targeting moiety can bind to a ligand (directly or indirectly), which is present on a cell. The targeting moiety can be an antibody, antibody fragment, polypeptide, peptide, aptamer, ligand, nucleic acid, or any combination thereof, that can bind a component of a target cell or molecule.

[0015] In one embodiment, the targeting moiety facilitates delivery of a biologically active agent to a target cell (e.g. via receptor-mediated endocytosis of antibodies binding target cell receptors). For example, a targeting moiety can facilitate delivery of the biologically active agent(s), immunogenic apoptotic material from antibody-bound tumor targets, or both, to an immune cell via interactions between their Fc and Fc receptors (on immune cells); this promotes internalization of nucleic acid via endocytosis and activation of endosomal pattern recognition receptors (e.g. Toll-like receptors).

[0016] In one embodiment, a targeting moiety is selected which is specific for a non-cancerous cell or tissue. For example, a targeting moiety can be specific for a molecule present normally on a particular cell or tissue. Furthermore, in some embodiments, the same molecule can be present on normal and cancer cells. Various cellular components and molecules are known. For example, if a targeting moiety is specific for EGFR, the resulting conjugate of the invention can target cancer cells expressing EGFR as well as normal skin epidermal cells expressing EGFR. Therefore, in some embodiments, a conjugate of the invention can operate by two separate mechanisms (targeting cancer and non-cancer cells), as further discussed herein.

[0017] In one embodiment, the targeting moiety binds a surface molecule on a regulatory T cell. In one embodiment, the surface molecule is CD4, CD25, CTLA-4 or LAG-3.

[0018] In another embodiment, the targeting moiety is a tumor targeting moiety. The tumor targeting moiety can bind 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.

[0019] The tumor targeting moiety can bind to a receptor or ligand on the surface of the cancer cell or may bind to an intracellular target of cancer cell provided that the target is accessible to the molecule. Accessibility to one or more intracellular cancer cell targets may arise in cancer cells that have a compromised plasma membrane such as cells which are undergoing apoptosis, necrosis, and the like. In one embodiment, a cancer targeting molecule can bind intracellular a portion of a cell that does not have a compromised plasma membrane.

[0020] In yet another embodiment, the targeting moiety, such as a tumor targeting moiety, binds a growth factor

receptor, growth factor, cytokine receptor, cytokine, or cell surface molecule. For example, the targeting moiety can bind epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), Insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, Vascular endothelial growth factor, CD20, CD30, CD33, CD40, or a combination thereof.

[0021] In another embodiment, the tumor targeting moiety binds a regulatory T cell or a suppressor T cell, such as CD4, CD25, CTLA-4, or a combination thereof.

[0022] For example, in various embodiments, a targeting moiety is specific for or binds to a component, which includes but is not limited to, epidermal growth factor receptor (EGFR, EGFR1, ErbB-1, HER1), ErbB-2 (HER2/neu), ErbB-3/HER3, ErbB-4/HER4, EGFR ligand family; insulin-like growth factor receptor (IGFR) family, IGF-binding proteins (IGFBPs), IGFR ligand family (IGF-1R); platelet derived growth factor receptor (PDGFR) family, PDGFR ligand family; fibroblast growth factor receptor (FGFR) family, FGFR ligand family, vascular endothelial growth factor receptor (VEGFR) family, VEGF family; HGF receptor family; TRK receptor family; ephrin (EPH) receptor family; AXL receptor family; leukocyte tyrosine kinase (LTK) receptor family; TIE receptor family, angiopoietin 1,2; receptor tyrosine kinase-like orphan receptor (ROR) receptor family; discoidin domain receptor (DDR) family; RET receptor family; KLG receptor family; RYK receptor family; MuSK receptor family; Transforming growth factor alpha (TGF-alpha) receptors, TGF-beta; Cytokine receptors, Class I (hematopoietin family) and Class II (interferon/IL-10 family) receptors, tumor necrosis factor (TNF) receptor superfamily (TNFRSF), death receptor family; 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-L-fucosyltransferase (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), HLA-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 I, NFYC, OGT, OS-9, pml-RARalpha fusion protein, PRDX5, PTPRK, K-ras (KRAS2), N-ras (NRAS), HRAS, RBAF600, SIRT2, SNRPD1, SYT-SSX1 or -SSX2 fusion protein, Triosephosphate Isomerase, BAGE, BAGE-1, BAGE-2,3,4,5, GAGE-1,2,3,4,5,6,7,8, GnT-V (aberrant N-acetyl glucosaminyl transferase V, MGAT5), HERV-K-MEL, KK-LC, KM-HN-1, LAGE, LAGE-1, CTL-recognized antigen on melanoma (CAMEL), MAGE-A1 (MAGE-1), MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, 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), EphA3, fibroblast growth factor-5 (FGF-5), glycoprotein 250 (gp250), EGFR (ERBB1), HER-2/neu (ERBB2), interleukin 13 receptor alpha2 chain (IL13Ralpha2), IL-6 receptor, intestinal carboxyl esterase (iCE), alpha-feto protein (AFP), M-CSF, mdm-2, MUC1, p53 (TP53), PBF, PRAME, 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, HCA661, LDHC, MORC, SGY-1, SPO11, TPX1, NY-SAR-35, FTHL17, NXF2, TDRD1, TEX15, FATE, TPTE, immunoglobulin idiotypes, Bence-Jones protein, estrogen receptors (ER), androgen receptors (AR), CD40, CD30, CD20, CD19, CD33, 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), carcinoembryogenic antigen peptide-1 (CAP-1), calcium-activated chloride channel-2 (CLCA2), cyclophilin B (Cyp-B), human signet ring tumor-2 (HST-2), Human papilloma virus (HPV) proteins (HPV-E6, HPV-E7, major or minor capsid antigens, others), Epstein-Barr virus (EBV) proteins (EBV latent membrane proteins—LMP1, LMP2; others), Hepatitis B or C virus proteins, and HIV proteins. A conjugate can further comprise the foregoing as a peptide/polypeptide and/or encoding the same.

Antibodies

[0023] In one embodiment, the targeting moiety 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 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.

[0024] 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 comprising either a VL or VH domain. In one embodiment, the targeting moiety is an antibody or scFv.

[0025] An antigen-binding antibody fragment, including single-chain antibody, 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. An antigen-binding fragment can also comprise any combination of variable region(s) with a

hinge region, CH1, CH2, and CH3 domains. Also included is an 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.

[0026] 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 comprising variable domain antigen-binding sequences derived from a non-human primate (e.g., Old World Monkey, Ape etc) and human constant region sequences.

[0027] 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.

[0028] In one embodiment, a hybridoma can produce a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β). For example, a hybridoma can produce an antibody, such as an anti-EGFR or anti-HER2 antibody linked to a molecule that binds TGF- β , such as a polypeptide comprising SEQ ID NO. 1, 2, or 3.

[0029] An antibody fragment can comprise a portion of an intact antibody, e.g. comprising 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).

[0030] An intact antibody is one which comprises 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 or any other modified Fc (e.g. glycosylation or other engineered Fc).

[0031] The intact antibody may have one or more “effector functions” which refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region or any other modified Fc region) of an antibody. Examples of antibody effector functions include Clq binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc.

[0032] Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different “classes.” There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into “subclasses” (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called alpha, Δ , ϵ , γ , and μ respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0033] Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as cetuximab (chimeric monoclonal antibody to epidermal growth factor receptor EGFR), panitumumab (anti-EGFR), nimotuzumab (anti-EGFR), B8, Rituximab (chimeric murine/human anti-CD20 MAb); Herceptin, trastuzumab (anti-Her2 hMAb); Panorex™ (17-1A) (murine monoclonal antibody); Panorex @ (17-1A) (chimeric murine monoclonal antibody); IDEC-Y2B8 (murine, anti-CD20 MAb); 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); MDX-210 (humanized anti-HER-2 bispecific antibody); 3622W94 MAb that binds to EGP40 (17-1A) pancarcinoma antigen on adenocarcinomas; Anti-VEGF, RhuMAb (Avastin; inhibits angiogenesis); Zenapax (SMART Anti-Tac (IL-2 receptor); SMART M195 Ab, humanized Ab, humanized); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-447 (humanized anti-EGF receptor bispecific antibody); 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 LL2 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.

[0034] In one aspect, an antibody or fragment thereof (e.g. scFv) is coupled, conjugated, linked or fused, either directly or via a linker, to a molecule that binds TGF- β , such as a polypeptide sequence derived from a TGF- β receptor. On one embodiment, the antibody or scFv fused or coupled to the molecule that binds TGF- β can be an antibody or scFv that binds HER2/neu, CD20, CD4, CD25, or CTLA-4. The linker can be an amino acid linker. In one embodiment, a linker is GGGGSGGGGSGGGGS (SEQ ID NO: 31). In another embodiment, a linker is GGGGS (SEQ ID NO: 32). In one embodiment, the TGF- β receptor is TGF- β receptor type II (TGF- β RII), or a

variant thereof. In another embodiment, the TGF- β receptor is TGF- β receptor type III (TGF- β RIII), or a variant thereof. The polypeptide derived from a TGF- β receptor can be any portion of the receptor. In one embodiment, the polypeptide is derived from the extracellular domain of a TGF- β receptor, such as the extracellular domain of TGF- β RII or TGF- β RIII, or a variant thereof. In one embodiment, the polypeptide sequence comprises SEQ. ID. NO: 1. In another embodiment, the polypeptide sequence comprises SEQ. ID. NO: 2. In another embodiment, the polypeptide sequence comprises SEQ. ID. 3.

[0035] In one embodiment, an antibody or fragment thereof is linked or fused to a polypeptide that binds TGF- β , with a linker or without a linker. In one embodiment, the conjugate comprises Cetuximab heavy chain linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Cetuximab heavy chain linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Cetuximab light chain linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Cetuximab light chain linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Cetuximab scFv linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Cetuximab scFv linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Trastuzumab heavy chain linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Trastuzumab heavy chain linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Trastuzumab light chain linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Trastuzumab light chain linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Trastuzumab scFv linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Trastuzumab scFv linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Rituximab heavy chain linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Rituximab heavy chain linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Rituximab light chain linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Rituximab light chain linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Rituximab scFv linked or fused to TGF- β -RII ECD. In another embodiment, the conjugate comprises Rituximab scFv linked or fused to TGF- β -RII ECD.

[0036] In another embodiment, the conjugate comprises Cetuximab heavy chain linked or fused to TGF- β -RIII ECD. In another embodiment, the conjugate comprises Cetuximab heavy chain linked or fused to TGF- β -RIII ECD. In another embodiment, the conjugate comprises Cetuximab light chain linked or fused to TGF- β -RIII ECD. In another embodiment, the conjugate comprises Cetuximab light chain linked or fused to TGF- β -RIII ECD. In another embodiment, the conjugate comprises Cetuximab scFv linked or fused to TGF- β -RIII ECD. In another embodiment, the conjugate comprises Cetuximab scFv linked or fused to TGF- β -RIII ECD. In another embodiment, the conjugate comprises Trastuzumab heavy chain linked or fused to TGF- β -RIII ECD. In another embodiment, the conjugate comprises Trastuzumab heavy chain linked or fused to TGF- β -RIII ECD. In another

embodiment, the conjugate comprises Trastuzumab light chain linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Trastuzumab light chain linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Trastuzumab scFv linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Trastuzumab scFv linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Rituximab heavy chain linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Rituximab heavy chain linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Rituximab light chain linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Rituximab light chain linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Rituximab scFv linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Rituximab scFv linked or fused to TGF β -RIII ECD.

Aptamers

[0037] In one aspect of the invention, the targeting moiety is an aptamer. For example, in some embodiments, the aptamer is comprised of a nucleic acid that functions as a targeting moiety, which is coupled to a molecule that binds transforming growth factor beta (TGF- β), such as a polypeptide sequence derived from a TGF- β receptor. In one embodiment, the TGF- β receptor is TGF- β receptor type II (TGF- β RII), or a variant thereof. In another embodiment, the TGF- β receptor is TGF- β receptor type III (TGF- β RIII), or a variant thereof. The polypeptide derived from a TGF- β receptor can be any portion of the receptor. In one embodiment, the polypeptide is derived from the extracellular domain of a TGF- β receptor, such as the extracellular domain of TGF- β RII or TGF- β RIII, or a variant thereof. In one embodiment, the polypeptide sequence comprises SEQ. ID. NO: 1. In another embodiment, the polypeptide sequence comprises SEQ. ID. NO: 2. In another embodiment, the polypeptide sequence comprises SEQ. ID. 3.

[0038] 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 or 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.

[0039] 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-methylg and g (Haller and Sarnow, *Proc. Natl. Acad. Sci. USA* 94:8521-8526 (1997))

or between D and L-tryptophan (supra, Gold et al.).

[0040] 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.

Peptides

[0041] In some aspects of the invention the targeting moiety is a peptide or polypeptide. For example, in some embodiments, the targeting polypeptide is coupled to a molecule that binds transforming growth factor beta (TGF- β), such as a polypeptide sequence derived from a TGF- β receptor. In one embodiment, the TGF- β receptor is TGF- β receptor type II (TGF- β RII), or a variant thereof. In another embodiment, the TGF- β receptor is TGF- β receptor type III (TGF- β RIII), or a variant thereof. The polypeptide derived from a TGF- β receptor can be any portion of the receptor. In one embodiment, the polypeptide is derived from the extracellular domain of a TGF- β receptor, such as the extracellular domain of TGF- β RII or TGF- β RIII, or a variant thereof. In one embodiment, the polypeptide sequence comprises SEQ. ID. NO: 1. In another embodiment, the polypeptide sequence comprises SEQ. ID. NO: 2. In another embodiment, the polypeptide sequence comprises SEQ. ID. 3.

[0042] 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, an alphavbeta3 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 an alphavbeta3 peptide in one or more of the assays.

[0043] 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 alphavbeta3 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.

[0044] 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.

[0045] 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.

[0046] 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.

Inhibition of Regulatory T Cell Function or Production

[0047] Also provided herein is a moiety that reduces the number or function of a CD4⁺ regulatory T cell. The moiety that reduces the number or function of a CD4⁺ regulatory T cell can be in a composition with a cytotoxic anticancer agent. The cytotoxic anticancer agent can be a chemotherapeutic agent, a kinase inhibitor, a tumor targeted antibody, or any combination thereof.

[0048] Without being bound by theory, immune cells can play a role in the antitumor efficacy of cytotoxic anticancer agents (chemotherapeutic agents, tumor targeted antibodies). Activated NK cells and antigen-specific CD4⁺ helper T cells and CD8⁺ cytotoxic T cells can induce or promote death of tumor cells via cytolytic products: Death ligands (TRAIL, FasL) which engage death receptors (TRAIL-R1, TRAIL-R2, Fas/CD95), granule proteases (Granzyme A, Granzyme B), Perforin, and Interferon- γ , which are believed to be repressed by TGF- β . Immune cells can cooperate with cytotoxic anticancer agents or tumor cell-targeted antibodies to induce tumor cell death: (i) Immune cell-mediated 'extrinsic' death signals cooperate with chemotherapeutic agent-induced 'intrinsic' death signals to induce tumor cell death; (ii) Immune cells promote antibody dependent cellular cytotoxicity. Depletion of CD8⁺ T cells can impair the *in vivo* response of tumors to cytotoxic anticancer agents (chemotherapeutic agents). Depletion of NK cells can also impair ADCC of tumor cells by tumor targeted antibodies.

[0049] Without being bound by theory, regulatory T cells can inhibit the antitumor efficacy of cytotoxic anticancer agents (chemotherapeutic agents, tumor targeted antibodies). Naïve CD4⁺T cells may differentiate into helper T cells (T_H1, T_H2) or regulatory T cells (T_{reg}). CD4⁺ T helper cells have a role in the induction and maintenance of immune responses and memory. CD4⁺ T cell help is typically used for priming and secondary expansion of CD8⁺ T cells. However, tumor cells can actively suppress immune responses by producing immunosuppressive cytokines (such as TGF- β) and increasing the number or function of regulatory T cells (CD4⁺ CD25⁺ Foxp3⁺). TGF- β can promote the number and function of regulatory T cells, and inhibit the cytotoxic ability of CD8⁺ T cells and NK cells. Regulatory T cells can express TGF- β and inhibit the generation or activation of CD4⁺ T helper cells or CD8⁺ cytotoxic T cells.

[0050] In one embodiment, the moiety that reduces the number or function of a CD4⁺ regulatory T cell is fused

or conjugated to a cytotoxic anticancer agent. In another embodiment, the moiety that reduces the number or function of a CD4+ regulatory T cell is fused to a targeting moiety.

[0051] In one embodiment, the moiety that reduces the number or function of a CD4+ regulatory T cell binds a surface molecule on a regulatory T cell. In one embodiment, the surface molecule is CD4, CD25, CTLA-4 or LAG-3. In one embodiment, the moiety that reduces the number or function of CD4+ regulatory T cells is an antibody or other moiety that binds CD4 or CD25. In another embodiment, the moiety that inhibits the production or function of regulatory T cells is a molecule that inhibits or antagonizes the expression or function of STAT3, Foxp3, CTLA-4, or TGF- β . In one embodiment, the molecule that inhibits or antagonizes the expression or function of STAT3, Foxp3, CTLA-4, or TGF- β is a small interfering RNA.

Compositions

[0052] Also provided herein is a composition comprising a cytotoxic anticancer agent and a moiety that reduces the number or function of a CD4+ regulatory T cell, wherein said cytotoxic anticancer agent is a chemotherapeutic agent, a kinase inhibitor, a tumor targeted antibody, or any combination thereof. In another embodiment, a composition comprises an immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells.

[0053] In yet another embodiment, a composition comprises a tumor targeting moiety and a molecule that binds TGF- β . In one embodiment, the targeting moiety is a tumor targeting moiety and the molecule that binds TGF- β is a polypeptide sequence derived from a TGF- β receptor. In one embodiment, the TGF- β receptor is TGF- β receptor type II (TGF- β RII), or a variant thereof. In another embodiment, the TGF- β receptor is TGF- β receptor type III (TGF- β RIII), or a variant thereof. The polypeptide derived from a TGF- β receptor can be any portion of the receptor. In one embodiment, the polypeptide is derived from the extracellular domain of a TGF- β receptor, such as the extracellular domain of TGF- β RII or TGF- β RIII, or a variant thereof. In one embodiment, the polypeptide sequence comprises SEQ. ID. NO: 1. In another embodiment, the polypeptide sequence comprises SEQ. ID. NO: 2. In another embodiment, the polypeptide sequence comprises SEQ. ID. 3.

[0054] The one or more compositions disclosed herein can further comprise a cytotoxic anticancer agent. In one embodiment, a cytotoxic anticancer agent can induce 'immunogenic' death of tumor cells. An anticancer agent can induce cellular stress or damage of tumor cells. Damaged tumor cells release tumor cell antigens and endogenous "danger signals" termed a "Damage-associated molecular patterns" (DAMPs/Alarmins). DAMPs/Alarmins alert the immune system by the following mechanisms: (i) Promoting uptake and cross-presentation of antigens from tumor cells by antigen presenting cells (APC) or dendritic cells (DCs); (ii) Inducing activation or maturation of DCs, leading to upregulation of co-stimulatory molecules and cytokines which promote activation of innate and adaptive immune responses; (i) Activation of Natural Killer (NK) Cells; and (ii) Activation of antigen-specific CD4+ T cells and CD8+ T cells.

[0055] In another embodiment, the one or more compositions disclosed herein can further comprise a

chemotherapeutic agent. The chemotherapeutic agent can be doxorubicin. In another embodiment, the chemotherapeutic agent is a topoisomerase-interacting agent, anthracycline, mitoxantrone, camptothecin, camptothecin analogue, epipodophyllotoxin, etoposide, alkylating agent, cyclophosphamide, cisplatin, cisplatin analogue, antimetabolite, fluoropyrimidine analogue, 5-fluorouracil, gemcitabine, azacytidine, antimicrotubule agent, taxane, paclitaxel, or docetaxel.

[0056] In one embodiment, the anticancer cytotoxic agent can be fused or conjugated to a moiety that reduces the number or function of a CD4⁺ regulatory T cell, an immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells, or a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β).

[0057] In another embodiment, the one or more compositions disclosed herein can further comprise a tumor cell, tumor antigen, or tumor-associated antigen, immune cell, dendritic cell, or any combination thereof. In yet another embodiment, the one or more compositions disclosed herein can further comprise a tetanus antigen. In yet another embodiment, the one or more compositions disclosed herein further comprises a pDOM. In one embodiment, the pDOM is fused to a cationic polypeptide. In another embodiment, the pDOM is fused to an arginine or histidine enriched polypeptide sequence. In yet another embodiment, the pDOM is fused to polyarginine or polyhistidine polypeptide. In one embodiment, the pDOM is fused to 1 to 20 arginines, such as to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 arginines.

[0058] Also provided herein is a method of preventing or treating a neoplastic disorder in a subject in need thereof comprising administering a compositions disclosed herein. In one embodiment, a cytotoxic anticancer agent and a moiety that reduces the number or function of a CD4⁺ regulatory T cell, wherein said cytotoxic anticancer agent is a chemotherapeutic agent, a kinase inhibitor, a tumor targeted antibody, or any combination thereof, is administered to the subject. In another embodiment, a composition comprising an immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells is administered to the subject. In yet another embodiment, a composition comprises a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β) is administered to the subject. In one embodiment, the neoplastic disorder is not a CD4-expressing T-cell malignancy.

[0059] In one embodiment, the method comprises administering a composition comprising a targeting moiety, which is coupled to a molecule that binds transforming growth factor beta (TGF- β), such as a polypeptide sequence derived from a TGF- β receptor. In one embodiment, the TGF- β receptor is TGF- β receptor type II (TGF- β RII), or a variant thereof. In another embodiment, the TGF- β receptor is TGF- β receptor type III (TGF- β RIII), or a variant thereof. The polypeptide derived from a TGF- β receptor can be any portion of the receptor. In one embodiment, the polypeptide is derived from the extracellular domain of a TGF- β receptor, such as the extracellular domain of TGF- β RII or TGF- β RIII, or a variant thereof. In one embodiment, the polypeptide sequence comprises SEQ. ID. NO: 1. In another embodiment, the polypeptide sequence comprises SEQ. ID. NO:

2. In another embodiment, the polypeptide sequence comprises SEQ. ID. 3.

[0060] In one embodiment, the method further comprises administering to the subject an anticancer agent, wherein said anticancer agent is a chemotherapeutic agent, antibody, tyrosine kinase inhibitor, cytokine, targeted therapeutic agent, immunotherapeutic agent, hormonal therapeutic agent, or antiangiogenic agent. The chemotherapeutic agent can be doxorubicin. In another embodiment, the chemotherapeutic agent is a topoisomerase-interacting agent, anthracycline, mitoxantrone, camptothecin, camptothecin analogue, epipodophyllotoxin, etoposide, alkylating agent, cyclophosphamide, cisplatin, cisplatin analogue, antimetabolite, fluoropyrimidine analogue, 5-fluorouracil, gemcitabine, azacytidine, antimicrotubule agent, taxane, paclitaxel, or docetaxel.

[0061] In another embodiment, the method further comprises administering to the subject a vaccine, wherein the vaccine comprises a tumor cell, tumor antigen, tumor-associated antigen, dendritic cell, or any combination thereof. In another embodiment, the method further comprises administering to the subject a PAMP or DAMP.

[0062] In another embodiment, the method further comprises administering to the subject a tetanus antigen. In yet another embodiment, In another embodiment, the method further comprises administering to the subject a pDOM. In one embodiment, the pDOM is fused to a cationic polypeptide. In another embodiment, the pDOM is fused to an arginine or histidine enriched polypeptide sequence. In yet another embodiment, the pDOM fused to polyarginine or polyhistidine polypeptide. In one embodiment, the pDOM is fused to 1 to 20 arginines, such as to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 arginines.

[0063] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

EXAMPLES

[0064] Example 1: Depletion of CD8⁺ T cells impairs the *in vivo* response of tumors to cytotoxic anticancer agents.

[0065] To determine whether CD4⁺ T cells help or inhibit the antitumor efficacy of cytotoxic anticancer agents *in vivo*, immunocompetent mice bearing syngeneic tumors were treated with cytotoxic anticancer agents with or without administration of an anti-CD4 antibody that depletes CD4⁺ T cells *in vivo*. Our studies indicate that the depletion of CD4⁺ regulatory T cells with an antibody targeting CD4 augments the response of tumors to cytotoxic chemotherapy or tumor-targeted antibodies. Improvement of the antitumor response to chemotherapy by depletion of CD4⁺ T cells is mediated via CD8⁺ T cells since combined depletion of CD8⁺ T cells and CD4⁺ T

cells results in loss of the antitumor effect of chemotherapy.

[0066] BALB/c mice were injected subcutaneously with syngeneic CT-26 mouse colon cancer cells (2×10^5). CT26 tumor-bearing BALB/c mice were treated with monoclonal antibodies to deplete specific lymphocyte sub-populations *in vivo* [anti-CD4 antibody (GK1.5), anti-CD8 antibody (GK2.43), and/or anti-NK cell antibody (asialo-GM)], and then treated with irinotecan (i.p. 50 mg/kg weekly x 3 weeks) or left untreated (FIG. 1). Analysis of tumor volume indicated that the antitumor effect of irinotecan was significantly impaired in animals depleted of NK cells and CD8⁺ T cells; in mice depleted of CD8⁺ T cells, tumors failed to respond to a dose of irinotecan that inhibited tumor growth in immunocompetent animals. These data indicate that the cellular immune system (CD8⁺ T cells) is instrumental in promoting the antitumor effects of chemotherapy *in vivo*.

[0067] Example 2: Depletion of CD4⁺ T cells improves the response of colorectal cancers to cytotoxic anticancer agents.

[0068] BALB/c mice bearing syngeneic CT-26 mouse colon cancer cells (2×10^5) were treated with irinotecan (i.p. 50 mg/kg weekly x 3 weeks) with or without administration of antibodies to deplete specific lymphocyte sub-populations *in vivo* [anti-CD4 antibody (GK1.5) and/or anti-CD8 antibody (GK2.43)]. Depletion of CD4⁺CD25⁺Foxp3⁺ regulatory T cells with the anti-CD4 antibody (confirmed by flow cytometry-FIG. 2C) improved the response of tumors to cytotoxic chemotherapy (FIG. 2 A, B). Improvement of the antitumor response to chemotherapy by depletion of CD4⁺ T cells was mediated via CD8⁺ T cells since combined depletion of CD8⁺ T cells and CD4⁺ T cells resulted in loss of the antitumor effect of chemotherapy (A, B).

[0069] Example 3: Depletion of CD4⁺ T cells improves the response of urothelial cancers to cytotoxic anticancer agents.

[0070] BALB/c mice bearing syngeneic MB49 mouse bladder cancer cells (5×10^5) were treated with cisplatin (i.p. 5 mg/kg weekly x 4) with or without administration of antibodies to deplete specific lymphocyte sub-populations *in vivo* [anti-CD4 antibody (GK1.5) and/or anti-CD8 antibody (GK2.43)]. Whereas depletion of CD8⁺ T cells resulted in loss of the efficacy of chemotherapy (FIG. 3 A, B), depletion of CD4⁺CD25⁺Foxp3⁺ regulatory T cells with the anti-CD4 antibody (confirmed by flow cytometry - FIG. 3 C) improved the antitumor efficacy of chemotherapy.

[0071] Example 4: Depletion of CD4⁺ regulatory T cells improves the response of urothelial cancers to chemo-immunotherapy.

[0072] BALB/c mice bearing syngeneic MB49 mouse bladder cancer cells (5×10^5) were treated with Doxorubicin (intratumoral 7.5 mg/kg weekly x 3), a cell permeable pathogen antigen-containing fusion protein (Arg9-pDOM)(intratumoral 10 μ g/dose weekly x3), or a combination of doxorubicin and Arg9-pDOM with or without administration of antibodies to deplete specific lymphocyte sub-populations *in vivo* [anti-CD4 antibody (GK1.5) and/or anti-CD8 antibody (GK2.43)]. Whereas depletion of CD8⁺ T cells resulted in loss of the antitumor efficacy of chemo-immunotherapy (FIG. 4 A, B), depletion of CD4⁺CD25⁺Foxp3⁺ regulatory T cells with the anti-CD4 antibody (confirmed by flow cytometry - FIG. 4 D) increased the percentage of CD8⁺ T cells

expressing interferon- γ (FIG. 4 C) and improved the response of tumors to chemoimmunotherapy. These data indicate that strategies to decrease the number or function of CD4⁺ regulatory T cells 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 chemo-immunotherapy (combination of chemotherapeutic agent with immunotherapeutic agents).

[0073] Example 5: A fusion protein comprising a tumor targeting antibody and a sequence from the extracellular domain of TGFBR2

[0074] A targeting moiety, such as an antibody, is coupled to a polypeptide derived from the extracellular domain of TGFBR2. The polypeptide sequence is selected from **Table 1**. The antibody, or a portion of antibody utilized for coupling with the polypeptide derived from TGFBR2 comprises sequences selected from **Table 2**. Examples of a fusion protein comprising a tumor targeting antibody and a sequence from the extracellular domain of TGFBR2 are shown in **Table 3**.

[0075] Table 1: Polypeptide Sequences Derived from the Extracellular Domain of TGF- β

SEQ. ID.	Sequence
1	MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDL LLVIFQ
2	TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD
3	PQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD

[0076] Table 2: Antibody Sequences for Coupling with A Polypeptide Derived from TGF- β RII

SEQ. ID.	Sequence
4	QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGNTD YNTPTFSTRLSINKDNSKSQVFFKMNSLQSDNTAIYYCARALTYDYEFAYWGQTLVT VSAASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVBPKSCDKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
5	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRITNGSPRLLIKYASESISGIPSRFS

	<p> GSGSGTDFTLINSVESEDIADYYCQNNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC </p>
6	<p> DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRNCGSPRLLIKYASESISGIPSRFS GSGSGTDFTLINSVESEDIADYYCQNNNNWPTTFGAGTKLELKGGGGSGGGGSGGGG SQVQLKQSGPGLVQPSQSLSTICTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNT DYNTPTTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGTLV TVSAGGGGS </p>
7	<p> EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYT RYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSF LYSKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPGK </p>
8	<p> DIQMTQSPSSLSASVGDRVITITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVP SRFSGSRSGTDFTLTISSLPEDFATYYCQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPS DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTL TLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC </p>
9	<p> DIQMTQSPSSLSASVGDRVITITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVP SRFSGSRSGTDFTLTISSLPEDFATYYCQHYTTPPTFGQGTKVEIKGGGGSGGGGSGG GGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTN GYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWG QGTLVTVSSGGGGGS </p>
10	<p> QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGD TSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAG TTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGSF FLYSKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPGK </p>
11	<p> QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWVQQKPGSSPKPWYATSNLASGVPVR </p>

	FSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
12	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWYATSNLASGVPVR FSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKGGGSGGGGSGGG GSQVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGN GDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWG AGTTVTVSAGGGGS

[0077] **Table 3. Examples of a Fusion Protein.**

SEQ. ID.	Sequence
13	QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWISGGNT DYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGT VTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCP APPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSTIPPHVQKSV NNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAVWR KNDENITLETVCHDPKLPYHDFILEDASPKCIMKEKKKPGETFFMCSSSDECNDNIIF SEEYNTSNPD
14	QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWISGGNT DYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGT VTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCP APPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSPQLCKFCDFR FSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAS PKCIMKEKKKPGETFFMCSSSDECNDNIIFSEEYNTSNPD
15	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQRTNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLEIKRTVAAPSVFIFPPSD

	EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTL TLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSTIPPHVQKSVNNDMIVT DNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITL ETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSN PD
16	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRNNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLSINSVESEDIADYYCQNNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTL TLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSPQLCKFCDVRFSTCDNQ KSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIM KEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
17	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRNNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLSINSVESEDIADYYCQNNNNWPTTFGAGTKLELKGGGGSGGGSGGG GSQVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWISGG NTDYNTPTTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQG TLVTVSAGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSC MSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEK KKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
18	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRNNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLSINSVESEDIADYYCQNNNNWPTTFGAGTKLELKGGGGSGGGSGGG GSQVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWISGG NTDYNTPTTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQG TLVTVSAGGGGSPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDEN ITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEY NTSNPD
19	EVQLVESGGGLVQPGGSLRLSAAAGFNKDTYIHWRQAPGKGLEWVARIYPTNGY TRYADSVKGRFTISADTSKNTAYLQMNSLRRAEDTAVYYCSRWGGDGFYAMDYWGQ GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNIHKPSNTKVDKKVEPKSCDKTHC PPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGKGGGGSTIPPH VQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAV

	AVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
20	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTILVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLQTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKITSKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGKGGGGSPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
21	DIQMTQSPSSLSASVGDRVITTCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPSPDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
22	DIQMTQSPSSLSASVGDRVITTCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPSPDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
23	DIQMTQSPSSLSASVGDRVITTCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTILVTVSSGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
24	DIQMTQSPSSLSASVGDRVITTCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGGGSGGGGS

	GGGGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIY PTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMD YWGQGTLLTVSSGGGGSPQLCKFCDFRFSTCDNQKSCMSNCSITSICEKPQEVCAV WRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECND NIIFSEYNTSNPD
25	QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNG DTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWG AGTTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSTIPP HVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRFSTCDNQKSCMSNCSITSICEKPQEV CAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDE CNDNIIFSEYNTSNPD
26	QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNG DTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWG AGTTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSPQL CKFCDFRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPY HDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPD
27	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFFQQKPGSSPKPWIYATSNLASGVPVR FSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPS DEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLTKADYEKKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSTIPPHVQKSVNNDMIVT DNNGAVKFPQLCKFCDFRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITL ETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSN PD
28	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFFQQKPGSSPKPWIYATSNLASGVPVR

	FSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPS DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSLSS LTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSPQLCKFCDVRFSTCDN QKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCI MKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
29	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVR FSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKGGGSGGGGSGG GGSQVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWWVKQTPGRGLEWIGAIYP GNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFN VWGAGTTVTVSAGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCD NQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKC IMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
30	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVR FSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKGGGSGGGGSGG GGSQVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWWVKQTPGRGLEWIGAIYP GNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFN VWGAGTTVTVSAGGGGSPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAV WRKNDENITLETVCHDPKLPYHDFILEDAAAPKCI MKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

[0017] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of preventing or treating a neoplastic disorder in a subject in need thereof comprising administering a combination comprising:
 - a. a cytotoxic anticancer agent and
 - b. a moiety that reduces the number or function of a CD4⁺ regulatory T cell, wherein said cytotoxic anticancer agent is a chemotherapeutic agent, a kinase inhibitor, a tumor targeted antibody, or any combination thereof.
2. The method of claim 1, wherein said neoplastic disorder is not a CD4-expressing T-cell malignancy.
3. The method of claim 1, wherein said moiety that reduces the number or function of CD4⁺ regulatory T cells is an antibody or other moiety that binds CD4 or CD25.
4. A method of preventing or treating a neoplastic disorder in a subject in need thereof comprising administering an immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells.
5. A method of preventing or treating a neoplastic disorder in a subject in need thereof comprising administering a composition comprising a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β).
6. The method of claims 1, 4 or 5, further comprising administering to said subject an anticancer agent, wherein said anticancer agent is a chemotherapeutic agent, antibody, tyrosine kinase inhibitor, cytokine, targeted therapeutic agent, immunotherapeutic agent, hormonal therapeutic agent, or antiangiogenic agent.
7. A method of claims 1 or 6, further comprising administering to a subject a vaccine, wherein said vaccine comprises a tumor cell, tumor antigen, tumor-associated antigen, dendritic cell, or any combination thereof.
8. The method of claims 1, 4 or 5, further comprising administering to said subject a PAMP or a DAMP.
9. The method of claims 1, 4 or 5, further comprising administering to said subject a tetanus antigen.
10. The method of claims 1, 4 or 5, further comprising administering to said subject pDOM.

11. The method of claims 1, 4 or 5, further comprising administering to said subject pDOM fused to a cationic polypeptide..
12. The method of claims 1, 4 or 5, further comprising administering to said subject pDOM fused to arginine or histidine enriched polypeptide sequence.
13. The method of claims 1, 4 or 5, further comprising administering to said subject pDOM fused to polyarginine or polyhistidine polypeptide.
14. The method of claims 1, 4 or 5, further comprising administering to said subject pDOM fused to 1 to 20 arginines.
15. The method of claims 1, 4 or 5, further comprising administering to said subject pDOM fused to 9 arginines.
16. An immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells.
17. The immunoconjugate or fusion protein of claim 16, wherein said targeting moiety binds a component of a tumor cell, tumor cell surface molecule, tumor-associated cell, tumor microenvironment, tumor-associated immune cell, tumor antigen, tumor-associated antigen, tumor-antigen-containing fusion protein, or tumor vaccine.
18. The immunoconjugate or fusion protein of claim 16, wherein said targeting moiety or moiety that inhibits the production or function of regulatory T cells binds a surface molecule on regulatory T cells.
19. The immunoconjugate or fusion protein of claim 18, wherein said surface molecule is CD4, CD25, CTLA-4 or LAG-3.
20. The immunoconjugate or fusion protein of claim 16, wherein said moiety that inhibits the production or function of regulatory T cells is a molecule that inhibits expression or function of STAT3.
21. The immunoconjugate or fusion protein of claim 20, wherein said molecule that inhibits expression or function of STAT3 is a small interfering RNA.

22. The immunoconjugate or fusion protein of claim 16, wherein said moiety that inhibits the production or function of regulatory T cells is a molecule that binds Transforming growth factor β (TGF- β) or antagonizes the function of TGF- β .
23. The immunoconjugate or fusion protein of claim 16, wherein said moiety that inhibits the production or function of regulatory T cells is a molecule that inhibits expression or function of Foxp3.
24. The immunoconjugate or fusion protein of claim 23, wherein said molecule that inhibits expression or function of FOXP3 is a small interfering RNA.
25. The immunoconjugate or fusion protein of claim 16, wherein said moiety that inhibits the production or function of regulatory T cells is a molecule that binds CTLA-4.
26. A composition comprising a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β).
27. The composition of claim 26, wherein said tumor targeting moiety is conjugated or fused to said molecule that binds transforming growth factor beta (TGF- β).
28. The composition of claim 26, wherein said tumor targeting moiety is an antibody, antibody fragment, peptide, aptamer, ligand, or a combination thereof.
29. The composition of claim 26, wherein said tumor targeting moiety is an antibody or scFv.
30. The composition of claim 26, wherein said 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 or tumor antigen-containing fusion protein.
31. The composition of claim 26, wherein said tumor targeting moiety binds a growth factor receptor, growth factor, cytokine receptor, cytokine, or cell surface molecule.
32. The composition of claim 26, wherein said tumor targeting moiety binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), Insulin-like growth factor receptor (IGF-1R), TRAIL-receptor,

epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, Vascular endothelial growth factor, CD20, CD30, CD33, CD40, or a combination thereof.

33. The composition of claim 26, wherein said tumor targeting moiety binds a regulatory T cell or a suppressor T cell.
34. The composition of claim 33, wherein said tumor targeting moiety binds CD4, CD25, CTLA-4, or a combination thereof.
35. The composition of claim 27, wherein said tumor targeting moiety is fused to said molecule that binds transforming growth factor beta (TGF- β), wherein said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence derived from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII).
36. The composition of claim 35, wherein said polypeptide sequence comprises SEQ ID NO: 1
37. The composition of claim 35, wherein said polypeptide sequence comprises: SEQ ID NO: 2.
38. The composition of claim 35, wherein said polypeptide sequence comprises: SEQ ID NO: 3
39. The composition of claim 26, wherein said tumor targeting moiety is an antibody or scFv that binds EGFR1 and said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII).
40. The composition of claim 26, wherein said tumor targeting moiety is an antibody or scFv that binds HER2/neu and said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII).
41. The composition of claim 26, wherein said tumor targeting moiety is an antibody or scFv that binds CD20

and said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII).

42. The composition of claim 26, wherein said tumor targeting moiety is an antibody or scFv that binds CD4 and said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII).

43. The composition of claim 26, wherein said tumor targeting moiety is an antibody or scFv that binds CD25 and said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII).

44. The composition of claim 26, wherein said tumor targeting moiety is an antibody or scFv that binds CTLA-4 and said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII).

45. A composition comprising the immunoconjugate of claim 16.

46. The composition of claim 26 or 45, further comprising a tumor cell, tumor antigen, or tumor-associated antigen, immune cell, dendritic cell, or any combination thereof.

47. The composition of claim 26 or 45, further comprising a PAMP or a DAMP.

48. The composition of claim 26 or 45, further comprising a tetanus antigen.

49. The composition of claim 26 or 45, further comprising a pDOM.

50. The composition of claim 26 or 45,, further comprising a pDOM fused to a cationic polypeptide.
51. The composition of claim 26 or 45,, further comprising a pDOM fused to arginine or histidine enriched polypeptide sequence.
52. The composition of claim 26 or 45,, further comprising a pDOM fused to polyarginine or polyhistidine polypeptide.
53. The composition of claim 26 or 45, further comprising a pDOM fused to 1 to 20 arginines.
54. The composition of claim 26 or 45, further comprising a pDOM fused to 9 arginines.
55. The composition of claim 26 or 45, further comprising a chemotherapeutic agent.
56. The composition of claim 26 or 45, further comprising doxorubicin.
57. The composition of claim 26 or 45, wherein said tumor targeting moiety is antibody that binds to EGFR1.
58. The composition of claim 26 or 45, wherein said tumor targeting moiety is cetuximab.
59. The composition of claim 26 or 45, wherein said tumor targeting moiety is antibody that binds to HER2/Neu.
60. The composition of claim 26 or 45, wherein said tumor targeting moiety is trastuzumab.
61. The composition of claim 26 or 45, wherein said tumor targeting moiety is antibody that binds to CD20.
62. The composition of claim 26 or 45, wherein said tumor targeting moiety is rituximab.

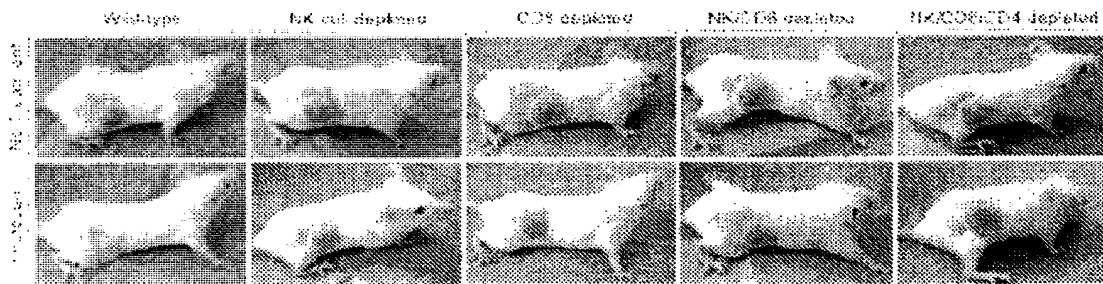
TUMOR TARGETING COMPOSITIONS

ABSTRACT

Disclosed herein are methods and compositions for treating or preventing neoplastic diseases or other disorders. In one embodiment, a composition comprising an immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells is provided. In another embodiment, a composition comprising a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β) is provided. Also provided herein are methods of producing and using said compositions.

FIGURE 1

A



B

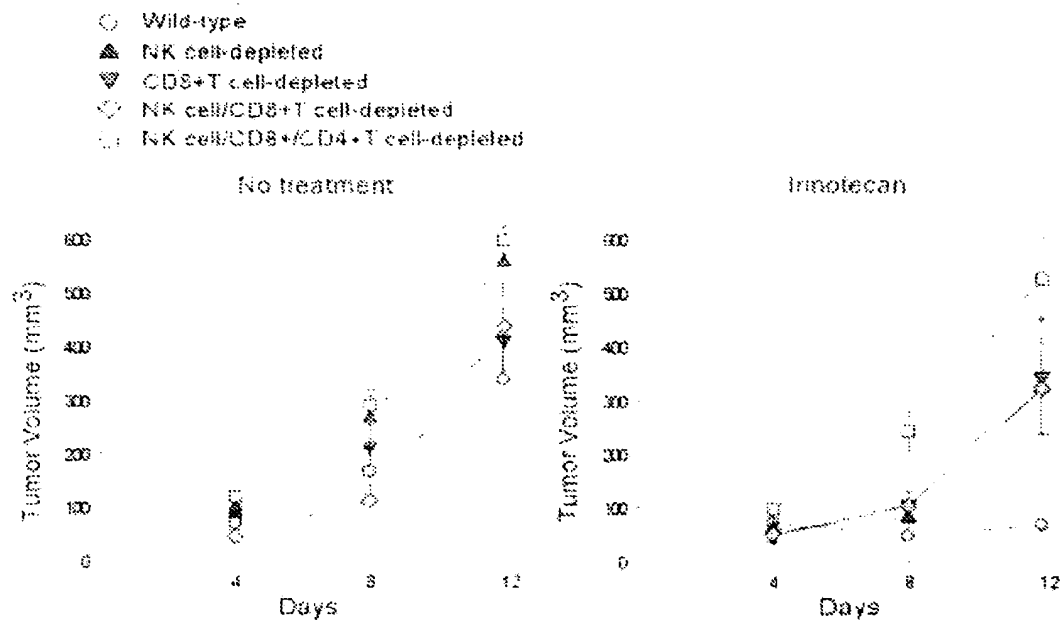
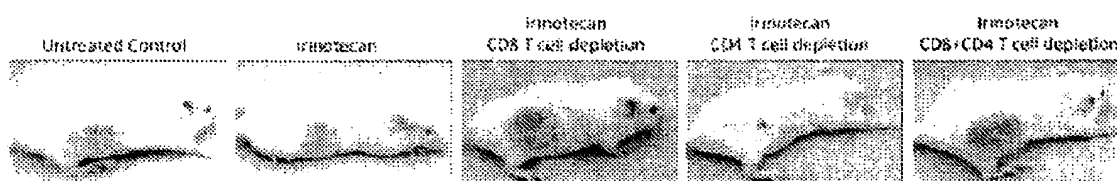
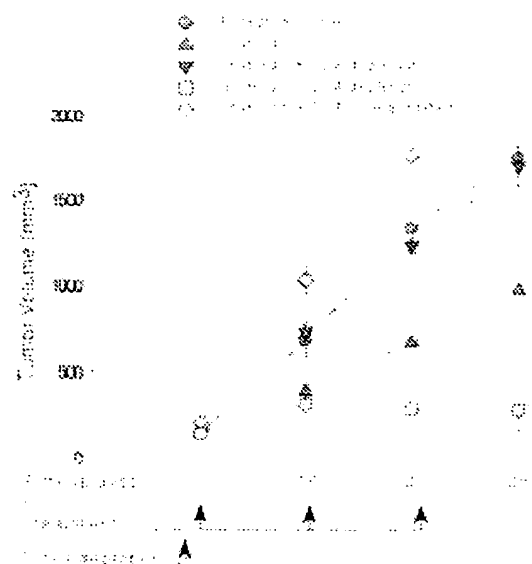


FIGURE 2

A



B



C.

FIGURE 2

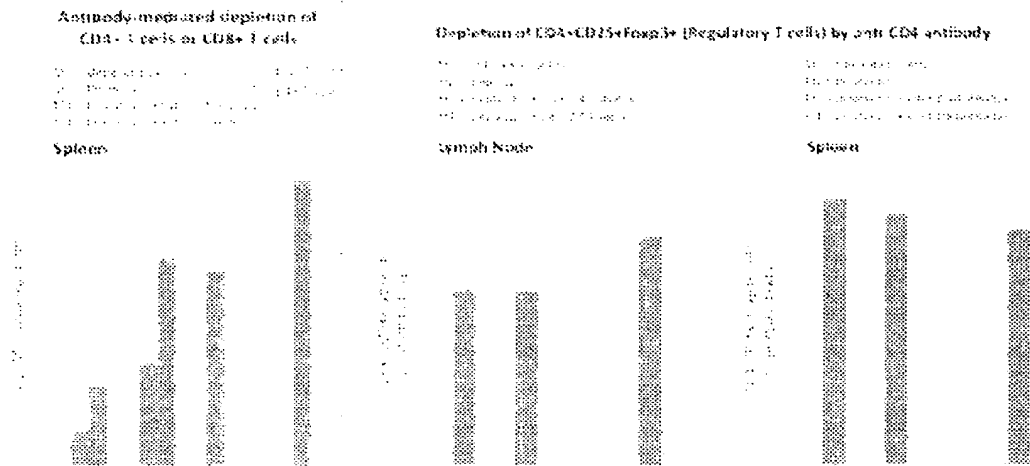


FIGURE 3

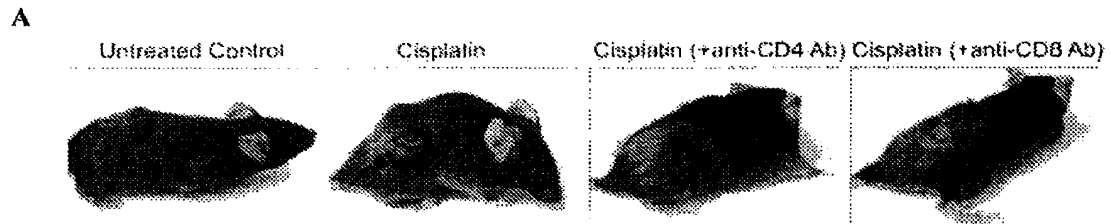
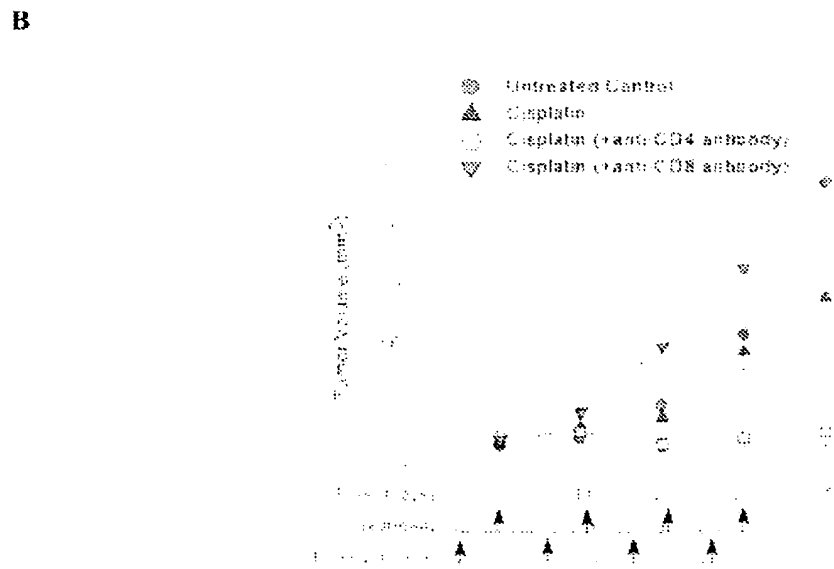


FIGURE 3

C

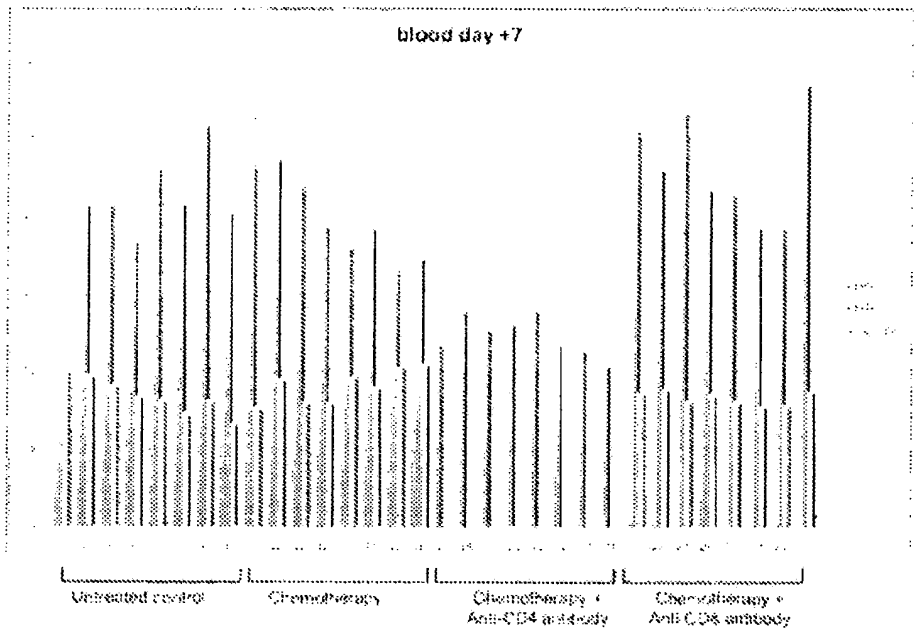
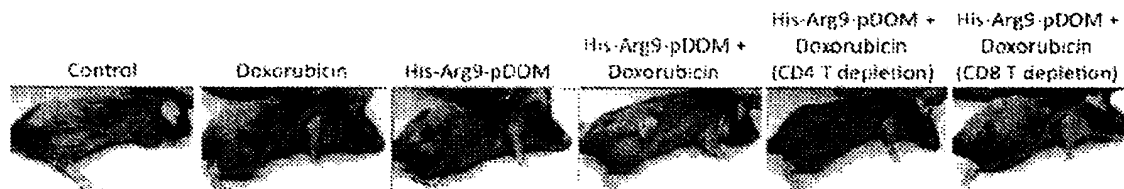
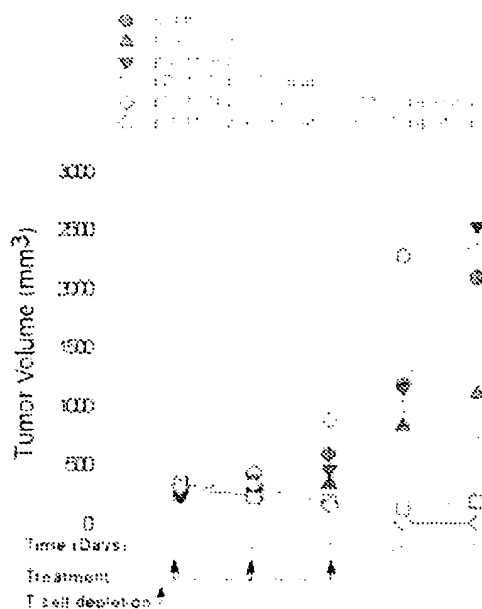


FIGURE 4

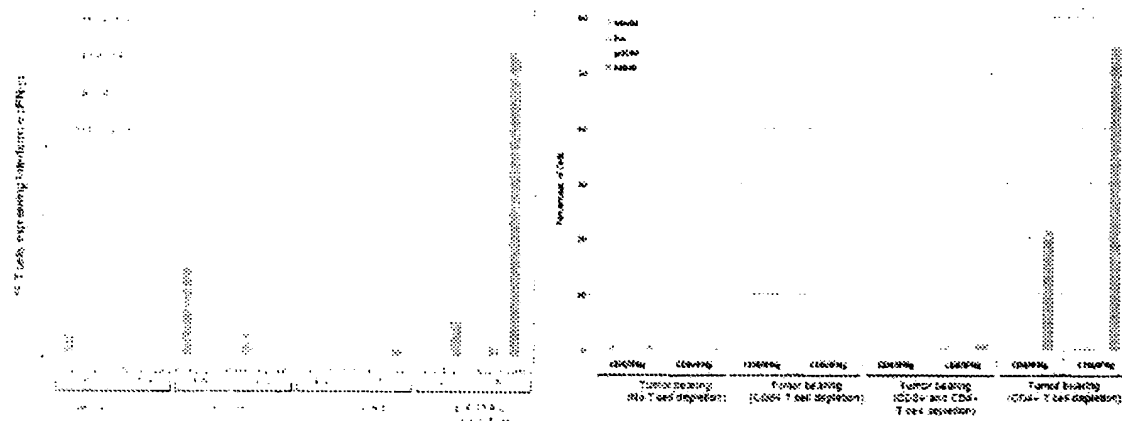
A



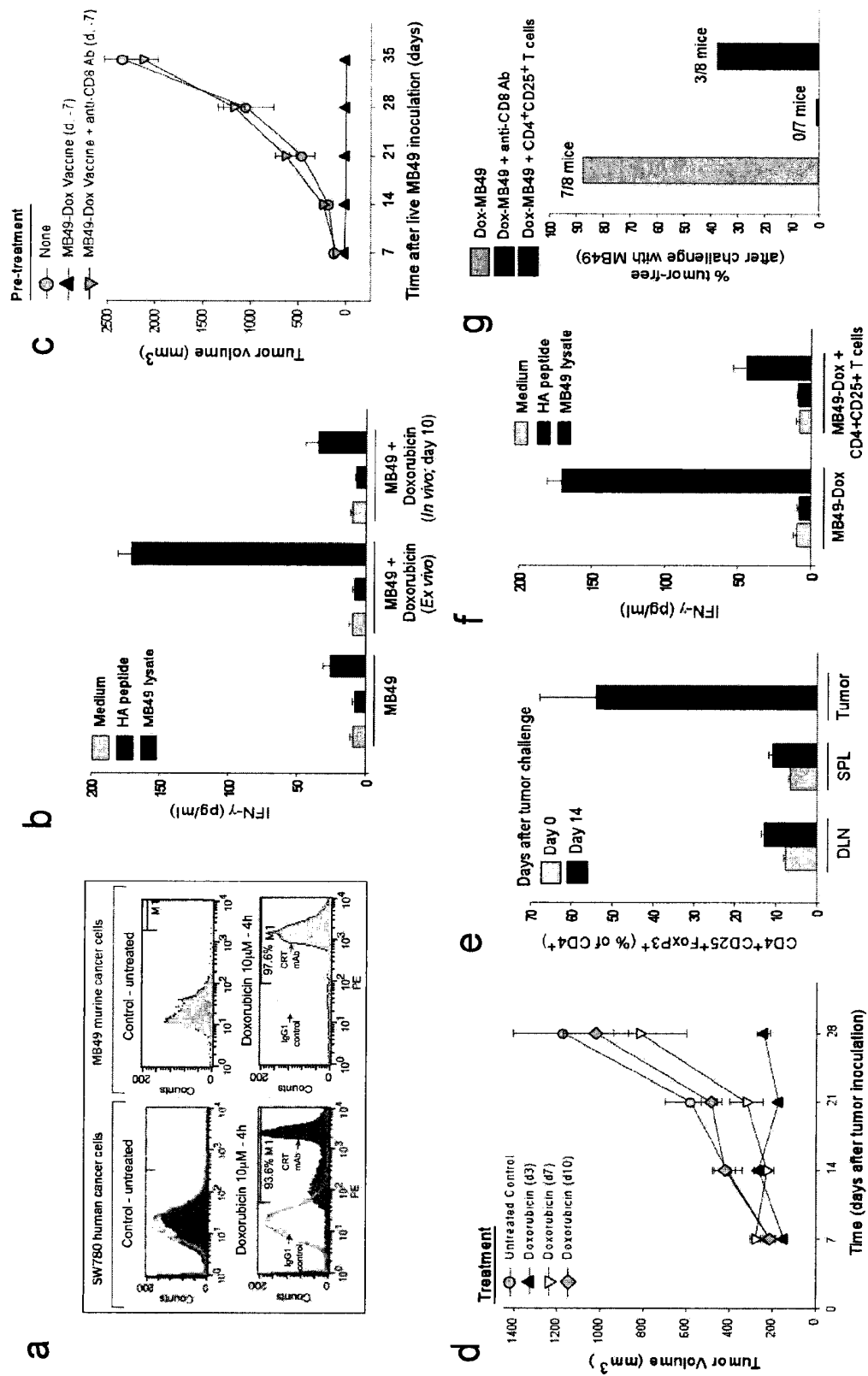
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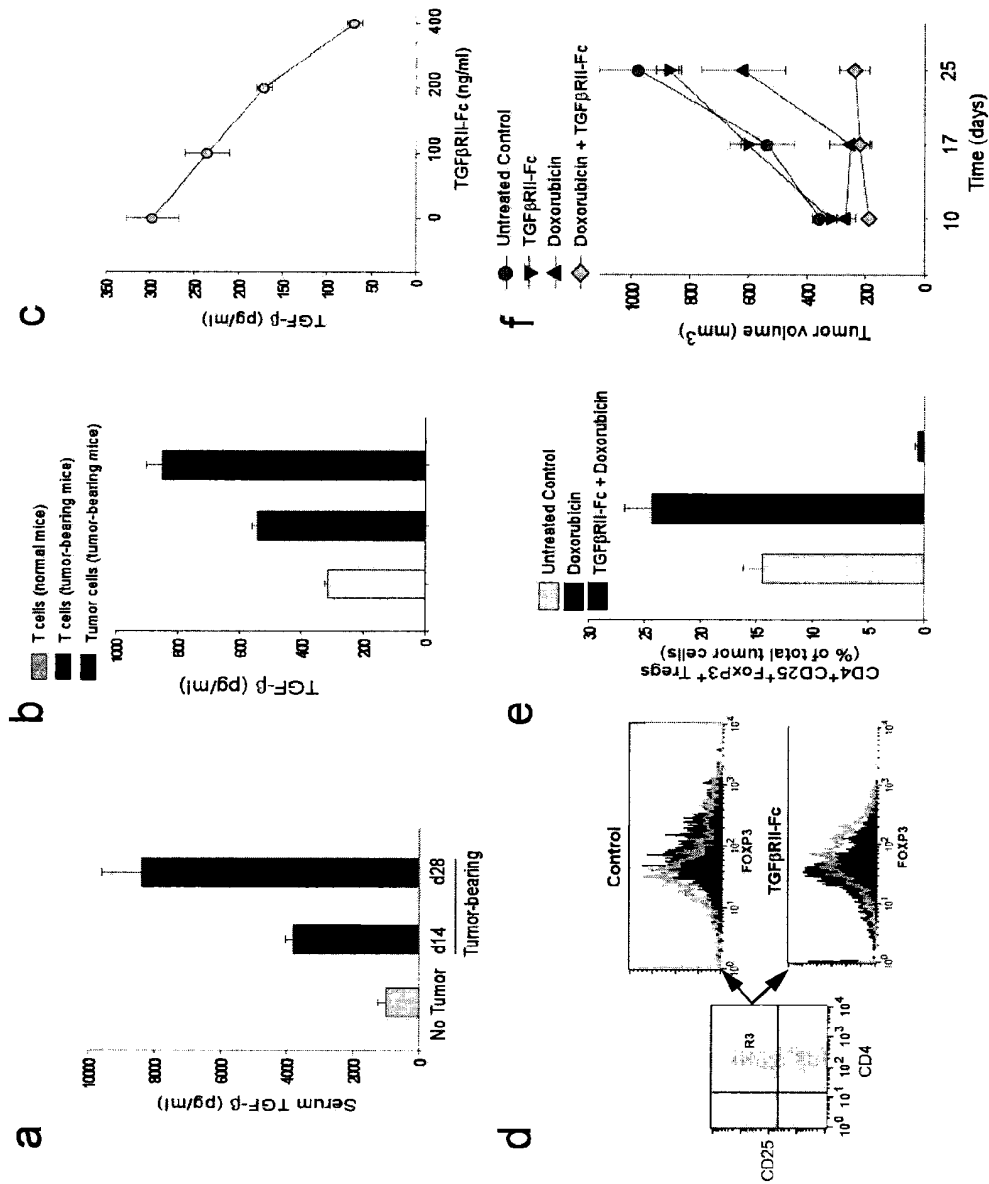


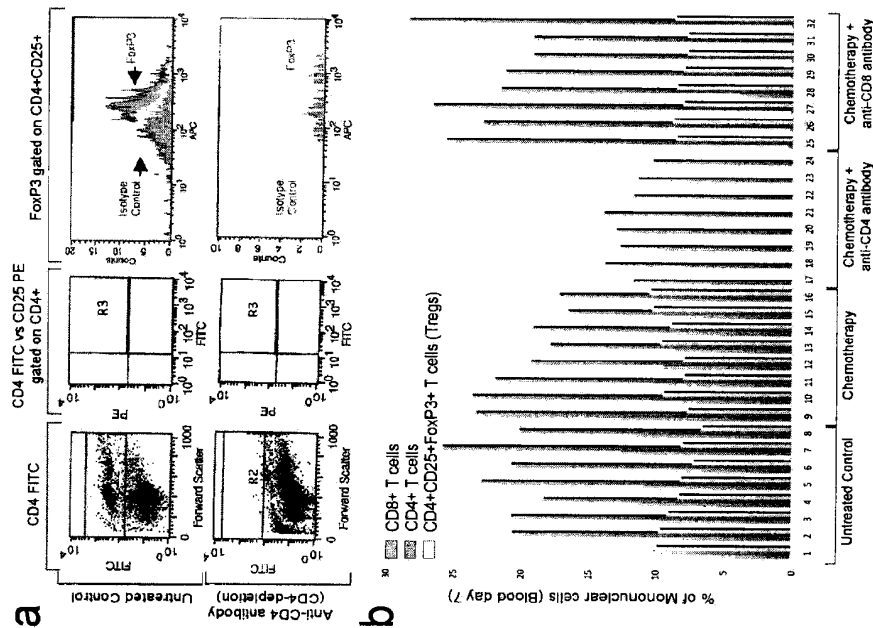
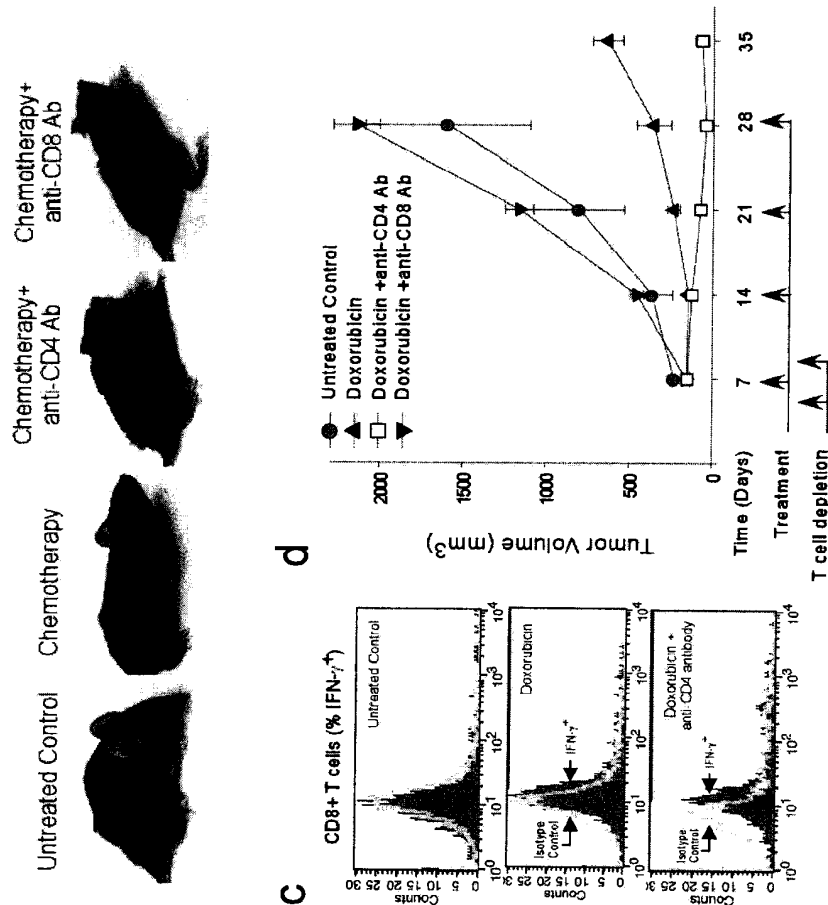
C

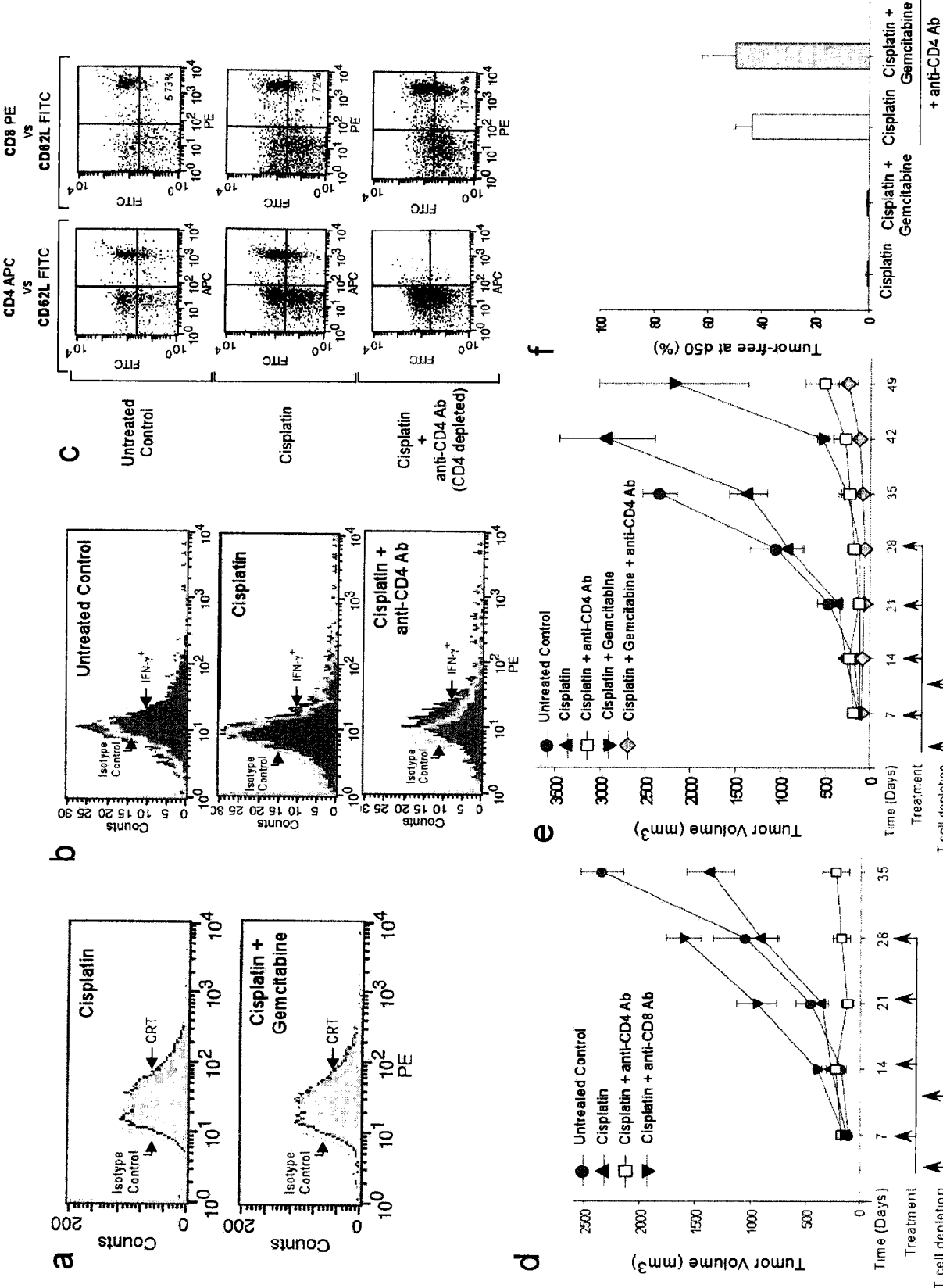


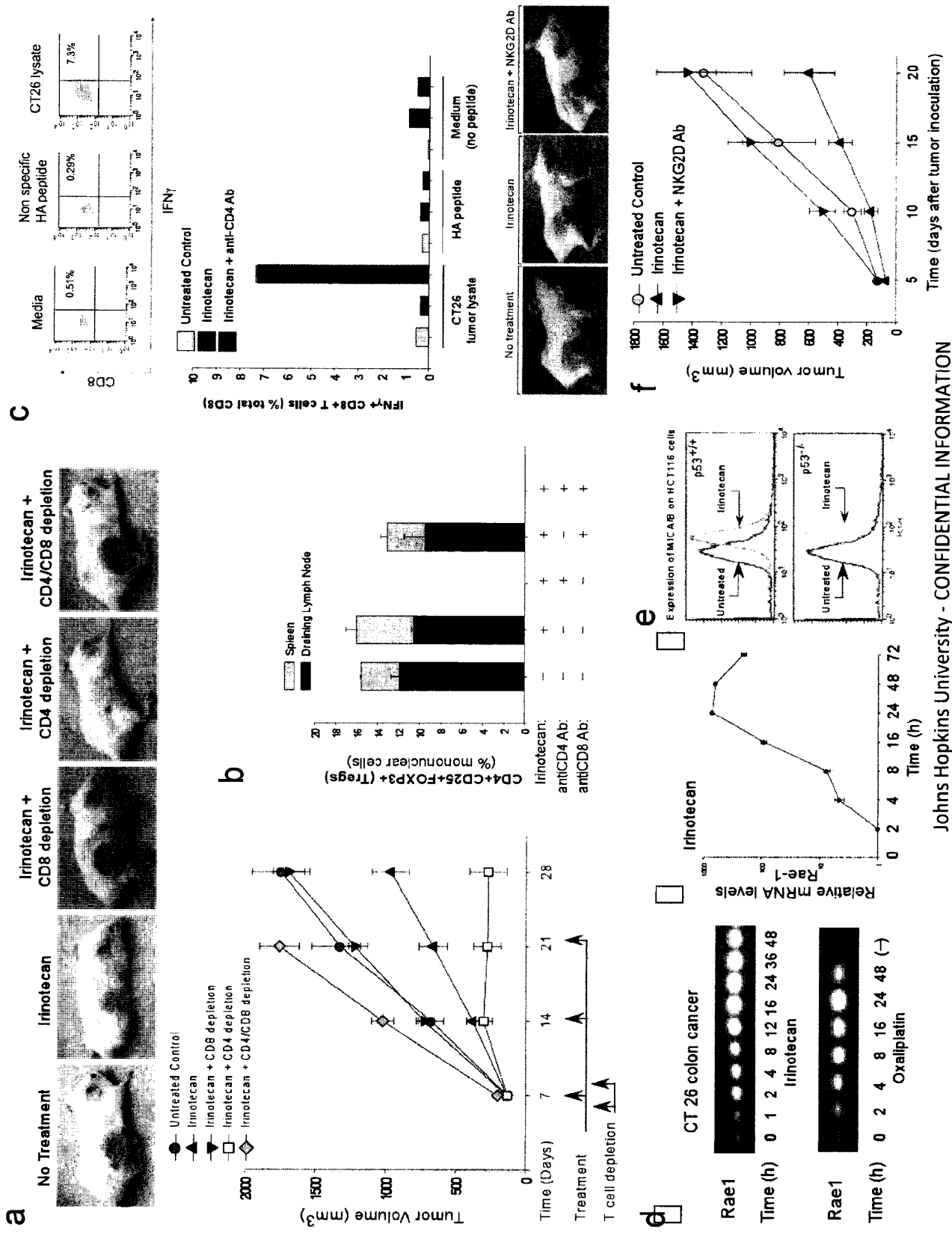
- Chemotherapy is a cornerstone of systemic treatment of patients with advanced cancers.
- The vast majority of human cancers harbor genetic alterations and signaling mechanisms that impair the direct death signaling pathways entrained by chemotherapeutic agents.
- Chemotherapeutic agents and tumor-targeted antibodies (mAbs) have immuno-adjvant effects which activate innate and adaptive antitumor immune responses that are crucial for their antitumor efficacy *in vivo*.
- Although chemotherapeutic agents and tumor-targeted mAbs can induce 'immunogenic' tumor cell death, 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.
- Cancer cells escape elimination by chemotherapeutic agents or tumor-targeted mAbs via specific immunosuppressive mechanisms in the tumor microenvironment:
 - Immunosuppressive cytokines - Transforming growth factor- β (TGF- β)
 - Regulatory T cells (T_{regs}) and Immunosuppressive myeloid DCs
- Strategies to counteract tumor-induced immune tolerance enhance the antitumor efficacy of chemotherapy by activating and leveraging T cell-mediated adaptive antitumor immunity against resistant or disseminated cancer cells.

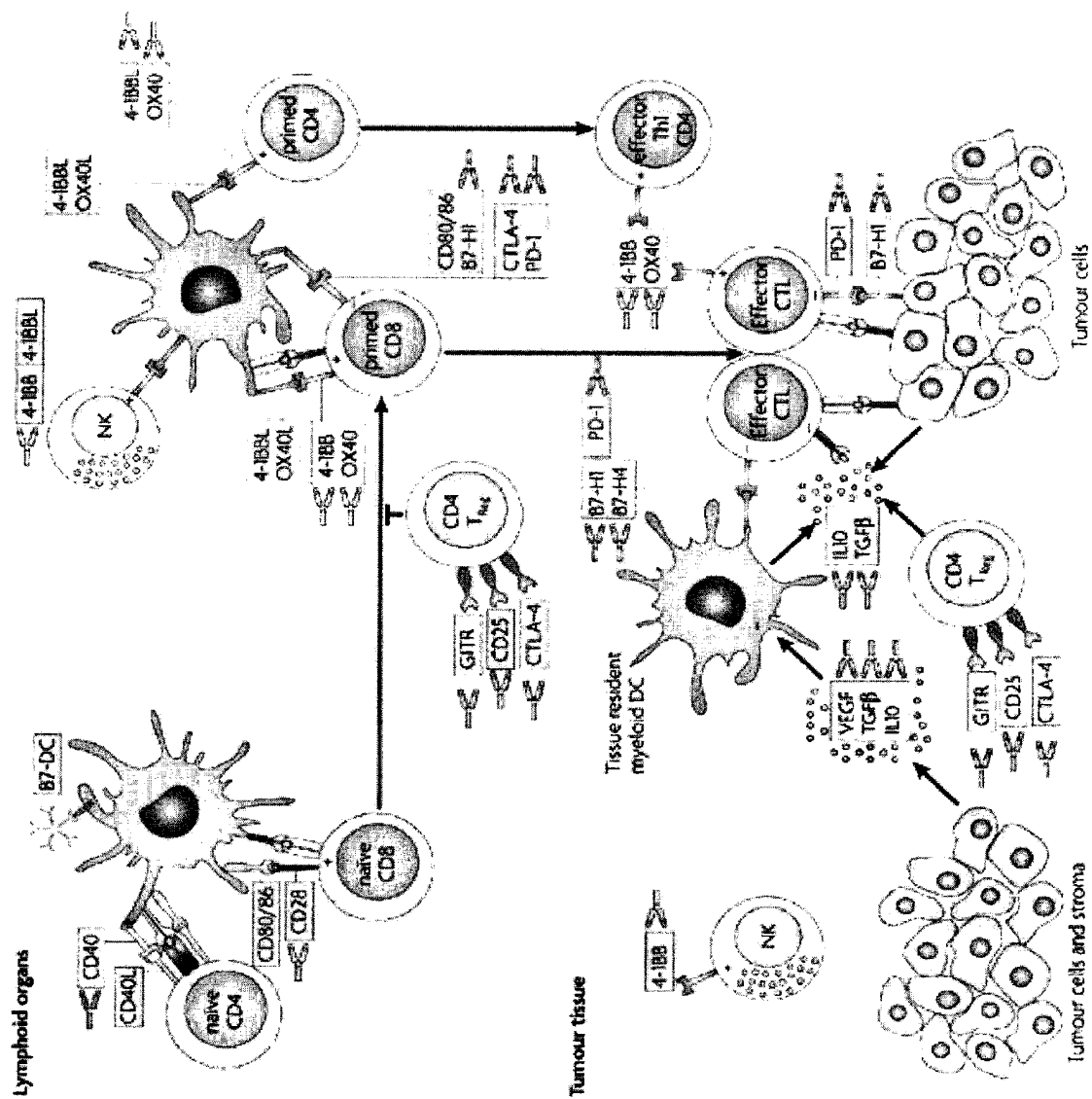






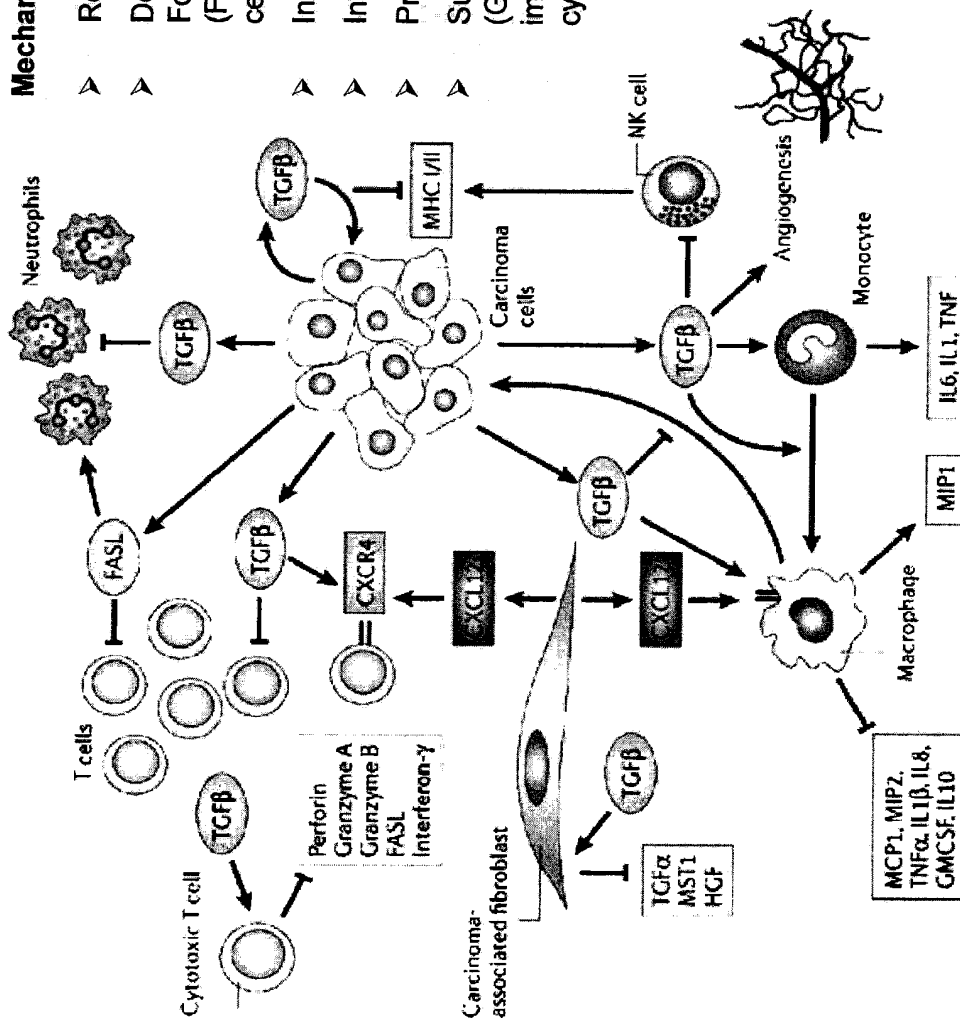






Mechanisms of TGF- β -induced immune tolerance:

- Restricts the maturation and function of DCs;
- Downregulates activating Fc receptors (Fc γ RI and Fc γ RIII) and upregulates the inhibitory Fc receptor (Fc γ RIIB), thereby limiting antibody-dependent cell mediated cytotoxicity (ADCC);
- Inhibits the proliferation of naïve T cells;
- Inhibits the differentiation of CD4⁺ helper T cells;
- Promotes number/function of regulatory Tregs;
- Suppresses expression of cytotoxic molecules (Granzyme A/B, FasL, Apo2L/TRAIL, IFN- γ) in immune effector cells, thereby attenuating the cytotoxic function of CD8⁺ T cells and NK cells.



Tumor-targeted antibodies that interfere with key immunosuppressive molecules responsible for immune evasion in the tumor microenvironment

The novelty of tumor-targeted immunostimulatory mAbs is their ability to generate and boost antitumor immunity to cross-presented tumor antigens obtained from the tumor during the course of therapy (as an *in situ* tumor vaccine), while simultaneously leveraging the antitumor immune response to enhance the direct cytotoxic efficacy of chemotherapy.

This approach is distinguished from and superior to conventional tumor antigen-, allogeneic tumor cell- or DC-based vaccines in 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-adjutant effects of chemotherapy and tumor-targeted immunostimulatory antibody occurs in tandem with the sensitization of tumor cells to immune effector-mediated cytotoxicity, thereby establishing a positive feedback loop that augments tumor cytorreduction and reinforces adaptive antitumor immunity.
- (iv) They have broad clinical relevance for advancing the treatment of many types of human cancers.

- I (A).** A molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:
- (a) said targeting moiety binds a specific target molecule, and
 - (b) said immunomodulatory moiety binds Transforming growth factor-beta (TGF- β)
- Claims: 1-24
SEQ. ID.: 1-8
1. Fusion protein comprising Anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).
 2. Fusion protein comprising Anti-EGFR1 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).
 3. Fusion protein comprising Anti-CD20 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).
 4. Fusion protein comprising anti-VEGF antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).
 5. Fusion protein comprising anti-human CTLA-4 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).
 - * Fusion protein comprising anti-CD4 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD)
 6. Fusion protein comprising IL-2, Fc, and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).
 7. Fusion protein comprising anti-CD25 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).
 - * Fusion protein comprising anti-PD1 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain.
 8. Fusion protein 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)

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I (B). A molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:

- (a) said targeting moiety binds a specific target molecule, and
- (b) said immunomodulatory moiety is a molecule that binds Programmed Death-1 ligand [PD-L1 (B7-H1) or PD-L2 (B7-DC)].

Claims: 24-40
SEQ. ID.: 9-16

9. Fusion protein comprising Anti-HER2/neu antibody and PD-1 Ectodomain.
10. Fusion protein comprising Anti-EGFR1 antibody and PD-1 Ectodomain.
11. Fusion protein comprising Anti-CD20 antibody and PD-1 Ectodomain.
12. Fusion protein comprising Anti-VEGF antibody and PD-1 Ectodomain.
13. Fusion protein comprising anti-human CTLA-4 antibody and PD-1 Ectodomain.
- * Fusion protein comprising anti-Transforming growth factor-beta (TGFβ) antibody and PD-1 Ectodomain
14. Fusion protein comprising Transforming growth factor-beta receptor II (TGFβ-RII) ectodomain, Fc, and PD-1 Ectodomain:
(TGFβ-RII ectodomain – Fc - PD-1 ectodomain)
- * Fusion protein comprising anti-CD4 antibody and PD-1 Ectodomain.
15. Fusion protein comprising anti-CD25 antibody and PD-1 Ectodomain.
16. Fusion protein comprising IL-2, Fc, and PD-1 ectodomain.

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- II (A) A molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:
- (a) said targeting moiety binds a specific target molecule, and
 - (b) said immunomodulatory moiety is a molecule that binds Programmed death-1 (PD-1)

Claims: 41-59

SEQ. ID.: 17-26

- 17. Fusion protein comprising anti-tumor necrosis factor (TNF α) antibody and PD-1 ligand.
- 18. Fusion protein comprising TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand: (TNFR2 ECD - IgG C γ 1 - PD-1L1).
- 19. Fusion protein comprising PD-1 ligand, Fc, and TNFR2 Extracellular ligand binding domain: (PD1-L1 - IgG C γ 1 - TNFR2 ECD)
- 20. Fusion protein comprising anti-CD20 antibody and PD-1 ligand 1 (PD-L1).
- * Fusion protein comprising anti-CD4 antibody and PD-1 ligand 1 (PD-L1).
- 21. Fusion protein comprising anti-CD25 antibody and PD-1 ligand 1 (PD-L1).
- 22. Fusion protein comprising IL-2, Fc, and PD-1 ligand 1 (PD-L1).
- 23. Fusion protein 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-1L1)
- 24. Fusion protein 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-1L1 - IgG C γ 1 - CTLA-4 ECD)
- 25. Fusion protein comprising transforming growth factor- β (TGF- β), Immunoglobulin Fc (IgG C γ 1), and a sequence from PD-1 ligand (PD-L1): (TGF β -1 - Fc - PD-1L1)
- 26. Fusion protein comprising a sequence from PD-1 ligand (PD-L1), Immunoglobulin Fc (IgG C γ 1), and Transforming growth factor beta (TGF- β): (PD-1L1 - Fc - TGF β -1)

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- II (B).** A molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:
- (a) said targeting moiety binds a specific target molecule, and
 - (b) said immunomodulatory moiety is a molecule that binds Transforming growth factor-beta receptor (TGF-βR).
- Claims: 60-75
SEQ. ID.: 27-34
- 27. Fusion protein comprising an antibody that binds TNF-α, and a sequence from transforming growth factor-β (TGF-β).
 - 28. Fusion protein comprising TNFR2 Extracellular ligand binding domain, Fc, and a sequence from transforming growth factor-β (TGF-β): (TNFR2 ECD- IgG Cγ1- TGF-β)
 - 29. Fusion protein comprising a sequence from transforming growth factor-β (TGF-β), Fc, and TNFR2 Extracellular ligand binding domain: (TGF-β - IgG Cγ1 - TNFR2 ECD)
 - 30. Fusion protein comprising anti-CD20 antibody and a sequence from transforming growth factor-β (TGF-β).
 - * Fusion protein comprising anti-CD4 antibody and a sequence from transforming growth factor-β (TGF-β).
 - 31. Fusion protein comprising anti-CD25 antibody and a sequence from transforming growth factor-β (TGF-β).
 - 32. Fusion protein comprising IL-2, Fc, and a sequence from transforming growth factor-β (TGF-β).
 - 33. Fusion protein comprising the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from a sequence from transforming growth factor-β (TGF-β): (Oncostatin M signal peptide - CTLA-4 ECD - IgG Cγ1 - TGF-β1)
 - 34. Fusion protein comprising a sequence from transforming growth factor-β (TGF-β), Immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4: (TGF-β1 - IgG Cγ1 - CTLA-4 ECD)

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CLASSES OF MOLECULES – SEQUENCES (Examples – SEQ. ID):

I. Targeted Immunostimulatory Antibodies and Fusion Proteins:

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 5. Fusion protein comprising anti-human CTLA-4 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD).
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 13. Fusion protein comprising anti-human CTLA-4 antibody and PD-1 Ectodomain.

- * Fusion protein comprising anti-Transforming growth factor-beta (TGF β) antibody and PD-1 Ectodomain
- 14. Fusion protein comprising Transforming growth factor-beta receptor II (TGF β -RII) ectodomain, Fc, and PD-1 Ectodomain:
(TGF β -RII ectodomain – Fc – PD-1 ectodomain)
- * Fusion protein comprising anti-CD4 antibody and PD-1 Ectodomain.
- 15. Fusion protein comprising anti-CD25 antibody and PD-1 Ectodomain.
- 16. Fusion protein comprising IL-2, Fc, and PD-1 ectodomain.

II. Targeted Immunosuppressive Antibodies and Fusion Proteins:

- II (A)** A molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:
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Claims: 41-59
SEQ. ID.: 17-26

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 - * Fusion protein comprising anti-CD4 antibody and PD-1 ligand 1 (PD-L1).
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 - 22. Fusion protein comprising IL-2, Fc, and PD-1 ligand 1 (PD-L1).
 - 23. Fusion protein comprising the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C γ 1), and a sequence from PD-1 ligand (PD-L1):
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 - 24. Fusion protein 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-1L1 – IgG C γ 1 – CTLA-4 ECD)
 - 25. Fusion protein comprising transforming growth factor- β (TGF- β), Immunoglobulin Fc (IgG C γ 1), and a sequence from PD-1 ligand (PD-L1):
(TGF β -1 – Fc – PD-1L1)
 - 26. Fusion protein comprising a sequence from PD-1 ligand (PD-L1), Immunoglobulin Fc (IgG C γ 1), and Transforming growth factor beta (TGF- β):
(PD-1L1 – Fc – TGF β -1)
- II (B).** A molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:
- (a) said targeting moiety binds a specific target molecule, and
 - (b) said immunomodulatory moiety is a molecule that binds Transforming growth factor-beta receptor (TGF- β R).

Claims: 60-75
SEQ. ID.: 27-34

27. Fusion protein comprising an antibody that binds TNF- α , and a sequence from transforming growth factor- β (TGF- β).
28. Fusion protein 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)
29. Fusion protein comprising a sequence from transforming growth factor- β (TGF- β), Fc, and TNFR2 Extracellular ligand binding domain: (TGF- β - IgG C γ 1 -TNFR2 ECD)
30. Fusion protein comprising anti-CD20 antibody and a sequence from transforming growth factor- β (TGF- β).
- * Fusion protein comprising anti-CD4 antibody and a sequence from transforming growth factor- β (TGF- β).
31. Fusion protein comprising anti-CD25 antibody and a sequence from transforming growth factor- β (TGF- β).
32. Fusion protein comprising IL-2, Fc, and a sequence from transforming growth factor- β (TGF- β).
33. Fusion protein 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)
34. Fusion protein 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)

CLASSES OF MOLECULES – SEQUENCES (Examples – SEQ. ID):

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- I (A). A molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:
- (a) said targeting moiety binds a specific target molecule, and
 - (b) said immunomodulatory moiety binds Transforming growth factor-beta (TGF-β)

Claims: 1-24

SEQ. ID.: 1-8

Said immunomodulatory moiety binds Transforming growth factor-beta (TGF-β) may be the following:

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)

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPDL LLVIFQVTGI SLLPPLGVAI
SVIIIFYCYR VNRQOKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELKGQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQGVG
TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC WDHDPEARLT AQCVAERFSE
LEHLDRLSGR SCSEEKIPED GSLNTTK

[Red – Extracellular domain of Transforming growth factor beta Receptor II (TGFβ RII)]
[Underlined = TGFβ RII Extracellular domain (ECD) region that binds TGF-β]

- (ii) Transforming growth factor beta receptor type IIB (TGF-β-RIIB)

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND
MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS ICEKPQEVCV AVWRKNDENI
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEYNT
SNPDLLLVIQVTGISLLPP LGVAISVIII FYCYRVNRQQ KLSSTWETGK TRKLMEFSEH
CAIILEDERS DISSTCANNI NHNTLLPIE LDTLVGKGRF AEVYKAKLKQ NTSEQFETVA
VKI FP YEEYASWKTE KDIFSDINLK HENILQFLTA EERKTELKGQ YWLITAFHAK
GNLQEYLTRH VISWEDLRKL GSSLARGIAH LHSDHTPCGR PKMPIVHRDL KSSNILVKND
LTCCLCDFGL SLRLDPTLSV DDLANSQGVG TARYMAPEVL ESRMNLENVE SFKQTDVYSM
ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI PSFWLNHQGI
QMVCETLTEC WDHDPEARLT AQCVAERFSE LEHLDRLSGR SCSEEKIPED GSLNTTK

[Red – Extracellular domain of Transforming growth factor beta Receptor IIB (TGFβ RIIB)]
[Underlined = TGFβ RIIB Extracellular domain (ECD) region that binds TGF-β]

2. Truncated mutants of Transforming growth factor beta Receptor II (TGF-β-RII) or TGF-β-

RIIB comprising the Extracellular domain (ECD) region that binds TGF- β

(i) **TGF- β R-II (Δ C terminus):** TGF β RII lacking the last 38 amino acids from the C-terminus

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

TGF- β R-IIB (Δ C terminus): TGF β RIIB lacking the last 38 aa from the C-terminus

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SDVEMEAQKD EIICPSCNRT
AHPLRHINND MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS ICEKPQEVCV
AVWRKNDENI TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND
NIIFSEEYNT SNPDL LLVIF QVTGISLLPP LGVAISVIII FYCYRVNRQQ KLSSTWETGK
TRKLMEFSEH CAIILEDERS DISSTCANNI NHNTPELLPIE LDTLVGKGRF AEVYKAKLKQ
NTSEQFETVA VKI FP YEEYASWKTE KDIFSDINLK HENILQFLTA EERKTELGKQ
YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH LHSDHTPCGR PKMPIVHRDL
KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQVVG TARYMAPEVL ESRMNLENVE
SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI
PSFWLNHQGI QMVCETLTEC WDHDPEARL

(ii) **TGF- β R-II (Δ cyt):** TGF β RII lacking the kinase domain and the juxtamembrane region.

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

TGF- β R-IIB (Δ cyt): TGF β RIIB lacking the kinase domain and the juxtamembrane region.

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND
MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS ICEKPQEVCV AVWRKNDENI
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT
SNPDL LLVIF QVTGISLLPP LGVAISVIII FYCYRVNRQQ KLSS

(iii) **TGF- β R-II containing the N-terminus region including the extracellular domain.**

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

TGF- β R-IIB containing the N-terminus region including the extracellular domain.

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND
MIVTDNNGAV KFPQLCKFCD VRFSTCDNQK SCMSNCSITS ICEKPQEVCV AVWRKNDENI
TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT
SNPDL LLVIF Q

(iv) **TGF- β R-II containing the extracellular domain that binds TGF- β**

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

TGF- β R-II containing the extracellular domain that binds TGF- β

TIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND MIVTDNNGAV KFPQLCKFCD
VRFSTCDNQK SCMSNCSITS ICEKPQEVCAVVRKNDENI TLETVCHDPK LPYHDFILED
AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEYNT SNPD

(v) TGF- β R-II containing the region of the extracellular domain that binds TGF- β

PQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCAVVRK NDENITLETV
CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

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

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

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCAVVRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPDL LLVIFQVTGI SLLPPLGVAI
SVIIIFYCYR VNRQQLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SRLDPTLSV DDLANSQVVG
TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
HPCVESMKDA SGIQMV CETL 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.

Preferred embodiment: Sequence used in examples (SEQ. ID: 1-8):

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

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

Protein Sequence:

Anti-HER2/neu heavy chain+ TGFβ-RII ECD fusion amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVSSASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSST
LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
EVTCCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFVMSHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EBYNTSNPD

Anti-HER2/neu light chain amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFRSGS
RSGTDFTLTISLQPEDFATYYCQHQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC

Blue: Anti-HER2 antibody variable region

Black: Anti-HER2 antibody constant region

Green: Linker

Red: TGFβRII ectodomain

cDNA Sequence:

Heavy chain sequence:

XhoI
SacI *PvuII* *BamHI*
GAGCTCGAGGTGCAGCTGGTGGAAATCCGGCGGAGGACTGGTGCAGCCTGGCGGATCCCTG
1 -----+-----+-----+-----+-----+-----+
CTCGAGCTCCACGTCGACCACCTTAGGCCGCTCCTGACCACGTCGGACCGCCTAGGGAC
E V Q L V E S G G G L V Q P G G S L
1 3 5 7 9 11 13 15 17

AGACTGTCTTGCGCCGCTCCGGCTTCAACATCAAGGACACCTACATCCACTGGGTGCGA
61 -----+-----+-----+-----+-----+-----+
TCTGACAGAACGCGCGGAGGCCGAAGTTGTAGTTCCTGTGGATGTAGGTGACCCACGCT
R L S C A A S G F N I R D T Y I H W V R
19 21 23 25 27 29 31 33 35 37

CAGGCCCTGGCAAGGACTGGAATGGGTGGCCCGGATCTACCCACCAACGGCTACACC
121 -----+-----+-----+-----+-----+-----+
GTCCGGGGACCGTTCCTGACCTTACCCACCGGCCTAGATGGGGTGGTTGCCGATGTGG

Q A P G K G L E W V A R I Y P T N G Y T
 39 41 43 45 47 49 51 53 55 57

 AGATACGCCGACTCCGTGAAGGGCCGGTTACCATCTCCGCCGACACCTCCAAGAACACC
 181 -----+-----+-----+-----+-----+
 TCTATGCGGCTGAGGCACTTCCCGCCAAGTGGTAGAGGCGGCTGTGGAGGTTCTTGTGG
 R Y A D S V K G R F T I S A D T S K N T
 59 61 63 65 67 69 71 73 75 77

PstI
BspMI
 GCCTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCGTGTACTACTGCTCCAGA
 241 -----+-----+-----+-----+-----+
 CGGATGGACGTCTACTTGAGGGACGCGCGGCTCCTGTGGCGGCACATGATGACGAGGTCT
 A Y L Q M N S L R A E D T A V Y Y C S R
 79 81 83 85 87 89 91 93 95 97

NcoI
 TGGGGAGGCGACGGCTTCTACGCCATGGACTACTGGGGCCAGGGCACCCCTGGTGACAGTG
 301 -----+-----+-----+-----+-----+
 ACCCCTCCGCTGCCGAAGATGCGGTACCTGATGACCCCGGTCCCGTGGGACCACTGTCAC
 W G G D G F Y A M D Y W G Q G T L V T V
 99 101 103 105 107 109 111 113 115 117

ApaI
 TCCTCTGCCTCCACCAAGGGCCCCCTCTGTGTTCCTCTGGCCCCCTTCCAGCAAGTCCACA
 361 -----+-----+-----+-----+-----+
 AGGAGACGGAGGTGGTTCCTCGGGGAGACACAAGGGAGACCGGGGAAGGTCGTTAGGTGT
 S S A S T K G P S V F P L A P S S K S T
 119 121 123 125 127 129 131 133 135 137

 TCTGGCGGCACCGCGCTCTGGGCTGCCTGGTGAAAGACTACTTCCCCGAGCCCGTGACC
 421 -----+-----+-----+-----+-----+
 AGACCGCCGTGGCGGCGAGACCCGACGGACCATTCTGATGAAGGGGCTCGGGCACTGG
 S G G T A A L G C L V K D Y F P E P V T
 139 141 143 145 147 149 151 153 155 157

NarI
KasI *PstI*
 GTGTCCTGGAACCTCTGGCGCCCTGACCTCCGGCGTGACACACCTTTCCAGCCGTGCTGCAG
 481 -----+-----+-----+-----+-----+
 CACAGGACCTTGAGACCGCGGGACTGGAGGCCGACGTGTGGAAAGGTCGGCACGACGTC
 V S W N S G A L T S G V H T F P A V L Q
 159 161 163 165 167 169 171 173 175 177

BstEII
 TCCTCCGGCCTGTACTCCCTGTCTCCGTGGTGACCGTGCCCTCCAGCTCTCTGGGCACC
 541 -----+-----+-----+-----+-----+
 AGGAGGCCGACATGAGGGACAGGAGGCACCACTGGCACGGGAGGTCGAGAGACCCGTGG
 S S G L Y S L S S V V T V P S S S L G T
 179 181 183 185 187 189 191 193 195 197

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E E M T K N Q V S L T C L V K G F Y P S
359 361 363 365 367 369 371 373 375 377

EcoRV

GATATCGCCGTGGAATGGGAGTCCAACGGCCAGCCCGAGAACAACACTACAAGACCACCCCC
1141 -----+-----+-----+-----+-----+
CTATAGCGGCACCTTACCCTCAGGTTGCCGGTCGGGCTCTTGTGATGTTCTGGTGGGGG
D I A V E W E S N G Q P E N N Y K T T P
379 381 383 385 387 389 391 393 395 397

CCTGTGCTGGACTCCGACGGCTCATTCTTCTGTACTCCAAGCTGACCGTGGACAAGTCC
1201 -----+-----+-----+-----+-----+
GGACACGACCTGAGGCTGCCGAGTAAGAAGGACATGAGGTTGACTGGCACCTGTTTCAGG
P V L D S D G S F F L Y S K L T V D K S
399 401 403 405 407 409 411 413 415 417

CGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCAC
1261 -----+-----+-----+-----+-----+
GCCACCGTCGTCCCGTTGCACAAGAGGACGAGGCACTACGTGCTCCGGGACGTGTTGGTG
R W Q Q G N V F S C S V M H E A L H N H
419 421 423 425 427 429 431 433 435 437

TACACCCAGAAGTCCCTGTCCCTGAGCCCTGGAAAGGGCGGAGGCGGCTCTGGTGGTGGGA
1321 -----+-----+-----+-----+-----+
ATGTGGGTCTTCAGGGACAGGGACTCGGGACCTTTCCCGCCTCCGCGGAGACCACCACCT
Y T Q K S L S L S P G K G G G G S G G G
439 441 443 445 447 449 451 453 455 457

GGCTCTGGAGGCGGAGGCTCTACCATCCCTCCACACGTGCAGAAATCCGTGAACAACGAC
1381 -----+-----+-----+-----+-----+
CCGAGACCTCCGCCTCCGAGATGGTAGGGAGGTGTGCACGTCTTTAGGCACTTGTGTGCTG
G S G G G G S T I P P H V Q K S V N N D
459 461 463 465 467 469 471 473 475 477

NarI

KasI

PvuII

ATGATCGTGACCGACAACAACGGCGCCGTGAAGTTCCCCCAGCTGTGCAAGTTCTGCGAC
1441 -----+-----+-----+-----+-----+
TACTAGCACTGGCTGTTGTTGCCGCGGCACTTCAAGGGGGTCGACACGTTCAAGACGCTG
M I V T D N N G A V K F P Q L C K F C D
479 481 483 485 487 489 491 493 495 497

BspMI

GTGCGGTTCTCTACCTGCGACAACCAGAAATCCTGCATGTCCAAGTCTCCATCACCTCC
1501 -----+-----+-----+-----+-----+
CACGCCAAGAGATGGACGCTGTTGGTCTTTAGGACGTACAGGTTGACGAGGTAGTGGAGG
V R F S T C D N Q K S C M S N C S I T S
499 501 503 505 507 509 511 513 515 517

ATCTGCGAGAAGCCCCAGGAAGTGTGCGTGGCCGTGTGGCGGAAGAACGACGAGAATCATC
1561 -----+-----+-----+-----+-----+
TAGACGCTCTTCGGGGTCCTTCACACGCACCGGCACACCGCCTTCTTGCTGCTCTTGTAG

181 -----+-----+-----+-----+-----+-----+
 GGTAGGGCCAAGAGGCCGAGATCTAGGCCGTGGCTGAAGTGGGACTGGTAGAGGTCGGAC
 P S R F S G S R S G T D F T L T I S S L
 59 61 63 65 67 69 71 73 75 77

CAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGCACTACACCACCCCCCTACCTTC
 241 -----+-----+-----+-----+-----+-----+
 GTCGGGCTCCTGAAGCGGTGGATGATGACGGTCGTCGTGATGTGGTGGGGGGATGGAAG
 Q P E D F A T Y Y C Q Q H Y T T P P T F
 79 81 83 85 87 89 91 93 95 97

GGCCAGGGCACCAAGGTGGAATCAAGCGGACCGTGGCCGCTCCCTCCGTGTTCATCTTC
 301 -----+-----+-----+-----+-----+-----+
 CCGGTCCCGTGGTTCACCTTTAGTTCGCTGGCACCGGCGAGGGAGGCACAAGTAGAAG
 G Q G T K V E I K R T V A A P S V F I F
 99 101 103 105 107 109 111 113 115 117

PvuII

CCACCCCTCCGACGAGCAGCTGAAGTCTGGCACCGCCTCCGTGCTGCTGAACAAC
 361 -----+-----+-----+-----+-----+-----+
 GGTGGGAGGCTGCTCGTCGACTTCAGACCGTGGCGGAGGCAGCACACGGACGACTTGTG
 P P S D E Q L K S G T A S V V C L L N N
 119 121 123 125 127 129 131 133 135 137

PstI

TTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGGCAAC
 421 -----+-----+-----+-----+-----+-----+
 AAGATGGGGCGCTCCGGTTCACGTACCTTCCACCTGTTGCGGGACGTCAGGCCGTTG
 F Y P R E A K V Q W K V D N A L Q S G N
 139 141 143 145 147 149 151 153 155 157

TCCCAGGAATCCGTGACCGAGCAGGACTCCAAGGACAGCACCTACTCCCTGTCTCCACC
 481 -----+-----+-----+-----+-----+-----+
 AGGGTCCTTAGGCACTGGCTCGTCCTGAGGTTCTGTGCTGGATGAGGGACAGGAGGTGG
 S Q E S V T E Q D S K D S T Y S L S S T
 159 161 163 165 167 169 171 173 175 177

CTGACCCTGTCCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAAGTGACCCAC
 541 -----+-----+-----+-----+-----+-----+
 GACTGGGACAGGTTCCGGCTGATGCTCTTCGTGTTCCACATGCGGACGCTTCACTGGGTG
 L T L S K A D Y E K H K V Y A C E V T H
 179 181 183 185 187 189 191 193 195 197

KpnI

CAGGGCCTGTCCAGCCCCGTGACCAAGTCCTTCAACCGGGGCGAGTGCTGAGGTACC
 601 -----+-----+-----+-----+-----+-----+
 GTCCCCGACAGGTCGGGGCACTGGTTCAAGGAAGTTGGCCCCGCTCACGACTCCATGG
 Q G L S S P V T K S F N R G E C *
 199 201 203 205 207 209 211 213 215

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

Protein sequence:

Anti-EGFR1 heavy chain + TGFβ-RII ECD fusion amino acid sequence:

QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPFTS
RLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTVTVSAASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
GTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVDVDSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGKGGGGSG
GGSGGGGS TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN
CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASP K CIMKEKKKPG
ETFFMCSCSS DECNDNIIFS EYNTSNPD

Anti-EGFR1 light chain amino acid sequence:

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQRTNGSPRLLIKYASESISGIPSRFSGS
GSGTDFTLINSVESEDIADYYCQQNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC

Blue: Anti-EGFR antibody variable region
Black: Anti-EGFR antibody constant region
Green: Linker
Red: TGFβRII ectodomain

cDNA Sequence:

Heavy chain sequence:

SacI *PvuII*
GAGCTCCAGGTGCAGCTGAAGCAGTCCGGCCCAGGACTGGTGCAGCCTTCCCAGTCCCTG
1 -----+-----+-----+-----+-----+-----+-----+
CTCGAGGTCCACGTCGACTTCGTCAGGCCGGGTCCTGACCACGTCGGAAGGGTCAGGGAC
 Q V Q L K Q S G P G L V Q P S Q S L
 1 3 5 7 9 11 13 15 17

TCCATCACCTGTACCGTGTCCGGCTTCTCCCTGACCAACTACGGCGTGCAGTGGGTCCGA
61 -----+-----+-----+-----+-----+-----+-----+
AGGTAGTGGACATGGCACAGGCCGAAGAGGGACTGGTTGATGCCGCACGTGACCCAGGCT
S I T C T V S G F S L T N Y G V H W V R
19 21 23 25 27 29 31 33 35 37

CAGTCCCCAGGCAAGGGCCTGGAATGGCTGGGAGTGATTGGAGCGGCGGCAACACCGAC
121 -----+-----+-----+-----+-----+-----+-----+
GTCAGGGGTCCGTTCCTCGGACCTTACCGACCTCACTAAACCTCGCCGCCGTTGTGGCTG
Q S P G K G L E W L G V I W S G G N T D

39 41 43 45 47 49 51 53 55 57
 TACAACACCCCCTTCACCTCCCGGCTGTCCATCAACAAGGACAACCTCCAAGTCCCAGGTG
 181 -----+-----+-----+-----+-----+-----+-----+
 ATGTTGTGGGGGAAGTGGAGGGCCGACAGGTAGTTGTTCCCTGTTGAGGTTTCAGGGTCCAC
 Y_N_T_P_F_T_S_R_L_S_I_N_K_D_N_S_K_S_Q_V
 59 61 63 65 67 69 71 73 75 77

PstI
 TTCTTCAAGATGAACTCCCTGCAGTCCAACGACACCGCCATCTACTACTGCGCCAGAGCC
 241 -----+-----+-----+-----+-----+-----+-----+
 AAGAAGTTCTACTTGAGGGACGTCAAGTTGCTGTGGCGGTAGATGATGACGCGGTCTCGG
 F_F_K_M_N_S_L_Q_S_N_D_T_A_I_Y_Y_C_A_R_A
 79 81 83 85 87 89 91 93 95 97

BstEII
 CTGACCTACTATGACTACGAGTTTCGCCTACTGGGGACAGGGCACCCCTGGTCACCGTGTCT
 301 -----+-----+-----+-----+-----+-----+-----+
 GACTGGATGATACTGATGCTCAAGCGGATGACCCCTGTCCCGTGGGACCAGTGGCACAGA
 L_T_Y_Y_D_Y_E_F_A_Y_W_G_Q_G_T_L_V_T_V_S
 99 101 103 105 107 109 111 113 115 117

ApaI
 GCCGCCTCTACCAAGGGCCCCCTCCGTGTTTCCCCTGGCCCCCTCCAGCAAGTCCACATCT
 361 -----+-----+-----+-----+-----+-----+-----+
 CGGCGGAGATGGTTCCCGGGGAGGCACAAAGGGGACCGGGGAGGTCTGTTTCAGGTGTAGA
 A_A_S_T_K_G_P_S_V_F_P_L_A_P_S_S_K_S_T_S
 119 121 123 125 127 129 131 133 135 137

 GGCGGCACCGCCGCTCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAGCCCGTGACCGTG
 421 -----+-----+-----+-----+-----+-----+-----+
 CCGCCGTGGCGGCGAGACCCGACGGACAGTTCCTGATGAAGGGGCTCGGGCACTGGCAC
 G_G_T_A_A_L_G_C_L_V_K_D_Y_F_P_E_P_V_T_V
 139 141 143 145 147 149 151 153 155 157

NarI
KasI *PstI*
 TCCTGGAACCTCTGGCGCCCTGACCTCCGGCGTGACACCTTTCCAGCCGTGCTGCAGTCC
 481 -----+-----+-----+-----+-----+-----+-----+
 AGGACCTTGAGACCGCGGACTGGAGGCCGCACGTGTGGAAGGTCTGGCACGACGTCAGG
 S_W_N_S_G_A_L_T_S_G_V_H_T_F_P_A_V_L_Q_S
 159 161 163 165 167 169 171 173 175 177

 TCCGGCCTGTACTCCCTGTCCTCCGTCGTGACCGTGCCCTCCAGCTCTCTGGGCACCCAG
 541 -----+-----+-----+-----+-----+-----+-----+
 AGGCCGGACATGAGGGACAGGAGGCAGCACTGGCACGGGAGGTCTGAGAGACCCGTGGGTC
 S_G_L_Y_S_L_S_S_V_T_V_P_S_S_S_L_G_T_Q
 179 181 183 185 187 189 191 193 195 197

 ACCTACATCTGCAACGTGAACCACAAGCCCTCCAACACCAAGGTGGACAAGCGGGTGGAA
 601 -----+-----+-----+-----+-----+-----+-----+
 TGGATGTAGACGTTGCACTTGGTGTTCGGGAGGTTGTGGTTCCACCTGTTTCGCCACCTT

T_Y_I_C_N_V_N_H_K_P_S_N_T_K_V_D_K_R_V_E
 199 201 203 205 207 209 211 213 215 217

661 CCCAAGTCCTGCGACAAGACCCACACCTGTCCCCCTGCCCTGCCCCTGAACTGCTGGGA
 -----+-----+-----+-----+-----+
 GGGTTCAGGACGCTGTTCTGGGTGTGGACAGGGGGACGGGACGGGACTTGACGACCCCT
 P_K_S_C_D_K_T_H_T_C_P_P_C_P_A_P_E_L_L_G
 219 221 223 225 227 229 231 233 235 237

721 GGCCCTTCCGTGTTCTCTGTTCACCAAGCCCAAGGACACCCCTGATGATCTCCCGGACC
 -----+-----+-----+-----+-----+
 CCGGGAAGGCACAAGGACAAGGGGGGTTTCGGGTTCCTGTGGGACTACTAGAGGGCCTGG
 G_P_S_V_F_L_F_P_P_K_P_K_D_T_L_M_I_S_R_T
 239 241 243 245 247 249 251 253 255 257

BspMI

841 CCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCACGAGGACCCCTGAAGTGAAGTTCAAT
 -----+-----+-----+-----+-----+
 GGGCTTCACTGGACGCACCACCACCTGCACAGGGTGCTCCTGGGACTTCACTTCAAGTTA
 P_E_V_T_C_V_V_V_D_V_S_H_E_D_P_E_V_K_F_N
 259 261 263 265 267 269 271 273 275 277

841 TGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCCAGAGAGGAACAGTAC
 -----+-----+-----+-----+-----+
 ACCATGCACCTGCCGCACCTTCACGTGTTGCGGTTCTGTTTCGGGTCTCTCCTTGTTCATG
 W_Y_V_D_G_V_E_V_H_N_A_K_T_K_P_R_E_E_Q_Y
 279 281 283 285 287 289 291 293 295 297

901 AACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGC
 -----+-----+-----+-----+-----+
 TTGAGGTGGATGGCCCACCACAGGCACGACTGGCACGACGTGGTCTGACCGACTTGCCG
 N_S_T_Y_R_V_V_S_V_L_T_V_L_H_Q_D_W_L_N_G
 299 301 303 305 307 309 311 313 315 317

BsaI

961 AAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAAAAGACCATC
 -----+-----+-----+-----+-----+
 TTTCTCATGTTACGTTCCAGAGGTTGTTCCGGGACGGACGGGGGTAGCTTTTCTGGTAG
 K_E_Y_K_C_K_V_S_N_K_A_L_P_A_P_I_E_K_T_I
 319 321 323 325 327 329 331 333 335 337

1021 TCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACTCTGCCTCCCAGCCGGGAC
 -----+-----+-----+-----+-----+
 AGGTTCCGGTTCCCGGTCGGGGCGCTCGGAGTCCACATGTGAGACGGAGGGTCGGCCCTG
 S_K_A_K_G_Q_P_R_E_P_Q_V_Y_T_L_P_P_S_R_D
 339 341 343 345 347 349 351 353 355 357

EcoRV

1081 GAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTCAAGGGCTTCTACCCCTCCGAT
 -----+-----+-----+-----+-----+
 CTCGACTGGTCTTGGTCCACAGGGACTGGACAGACCAGTTCCCGAAGATGGGGAGGCTA

E L T K N Q V S L T C L V K G F Y P S D
 359 361 363 365 367 369 371 373 375 377

 1141 ATCGCCGTGGAATGGGAGTCCAACGGCCAGCCCGAGAACAACACTACAAGACCACCCCCCT
 -----+-----+-----+-----+-----+-----+
 TAGCGGCACCTTACCCTCAGGTTGCCGGTCGGGCTCTTGTTGATGTTCTGGTGGGGGGGA
I A V E W E S N G Q P E N N Y K T T P P
 379 381 383 385 387 389 391 393 395 397

 1201 GTGCTGGACTCCGACGGCTCATTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGG
 -----+-----+-----+-----+-----+-----+
 CACGACCTGAGGCTGCCGAGTAAGAAGGACATGAGGTTGCGACTGGCACCTGTTTCAGGGCC
V L D S D G S F F L Y S K L T V D K S R
 399 401 403 405 407 409 411 413 415 417

 1261 TGGCAGCAGGGCAACGTGTTCTCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTAC
 -----+-----+-----+-----+-----+-----+
 ACCGTCGTCCCGTTGCACAAGAGGACGAGGCACCTACGTGCTCCGGGACGTGTTGGTGATG
W Q Q G N V F S C S V M H E A L H N H Y
 419 421 423 425 427 429 431 433 435 437

 1321 ACCCAGAAAGTCCCTGTCTCTGAGCCCCGGCAAGGGCGGAGGCGGATCTGGTGGTGGTGGC
 -----+-----+-----+-----+-----+-----+
 TGGGTCTTCAGGGACAGAGACTCGGGGCCGTTCCCGCCTCCGCCTAGACCACCACCACCG
T Q K S L S L S P G K G G G S G G G G
 439 441 443 445 447 449 451 453 455 457

 1381 TCTGGTGGCGGAGGCTCTACCATCCCTCCACACGTGCAGAAATCCGTGAACAACGACATG
 -----+-----+-----+-----+-----+-----+
 AGACCACCGCCTCCGAGATGGTAGGGAGGTGTGCACGTCTTTAGGCACCTGTTGCTGTAC
S G G G G S T I P P H V Q K S V N N D M
 459 461 463 465 467 469 471 473 475 477

NarI
KasI *PvuII*
 1441 ATCGTGACCGACAACAACGGCGCCGTGAAGTTCCCCCAGCTGTGCAAGTTCTGCGACGTG
 -----+-----+-----+-----+-----+-----+
 TAGCACTGGCTGTTGTTGCGCGGCACCTCAAGGGGGTCGACACGTTCAAGACGCTGCAC
I V T D N N G A V K F P Q L C K F C D V
 479 481 483 485 487 489 491 493 495 497

BspMI
 1501 CGGTTCTCTACCTGCGACAACCAGAAATCCTGCATGTCCAACCTGCTCCATCACCTCCATC
 -----+-----+-----+-----+-----+-----+
 GCCAAGAGATGGACGCTGTTGGTCTTTAGGACGTACAGGTTGACGAGGTAGTGGAGGTAG
R F S T C D N Q K S C M S N C S I T S I
 499 501 503 505 507 509 511 513 515 517

 1561 TGCGAGAAGCCCCAGGAAGTGTGCGTCGCCGTCTGGCGGAAGAACGACGAGAACATCACC
 -----+-----+-----+-----+-----+-----+
 ACGCTCTTCGGGGTCCTTCACACGCAGCGGCAGACCGCCTTCTTGCTGCTCTTGTAGTGG
C E K P Q E V C V A V W R K N D E N I T
 519 521 523 525 527 529 531 533 535 537

CTGGAAACCGTGTGCCACGACCCCAAGCTGCCCTACCACGACTTCATCCTGGAAGATGCC
1621 -----+-----+-----+-----+-----+-----+-----+
GACCTTTGGCACACGGTGCTGGGGTTCGACGGGATGGTGCTGAAGTAGGACCTTCTACGG
L E T V C H D P K L P Y H D F I L E D A
539 541 543 545 547 549 551 553 555 557

GCCTCCCCCAAGTGCATCATGAAGGAAAAGAAGAAGCCCGGCGAGACTTTCTTCATGTGC
1681 -----+-----+-----+-----+-----+-----+-----+
CGGAGGGGGTTCACGTAGTACTTCTTTTCTTCTCGGGCCGCTCTGAAAGAAGTACACG
A S P K C I M K E K K K P G E T F F M C
559 561 563 565 567 569 571 573 575 577

AGCTGCTCCTCCGACGAGTGCAACGACAACATCATCTTCTCCGAAGAGTACAACACCTCC
1741 -----+-----+-----+-----+-----+-----+-----+
TCGACGAGGAGGCTGCTCAGTTGCTGTTGTAGTAGAAGAGGCTTCTCATGTTGTGGAGG
S C S S D E C N D N I I F S E E Y N T S
579 581 583 585 587 589 591 593 595 597

KpnI
AACCCCGACTGAGGTACC
1801 -----+-----
TTGGGGCTGACTCCATGG
N P D *
599 601

Light chain sequence:

SacI EcoRV
GAGCTCGATATCCTGCTGACCCAGTCCCCCGTGATCCTGTCCGTGTCTCCTGGCGAGCGG
1 -----+-----+-----+-----+-----+-----+-----+
CTCGAGCTATAGGACGACTGGGTCAGGGGGCACTAGGACAGGCACAGAGGACCGTCCGCC
D I L L T Q S P V I L S V S P G E R
1 3 5 7 9 11 13 15 17

GTGTCCTTCTCCTGCCGGGCCTCCAGTCCATCGGCACCAACATCCACTGGTATCAGCAG
61 -----+-----+-----+-----+-----+-----+-----+
CACAGGAAGAGGACGGCCCGAGGGTCAGGTAGCCGTGGTTGTAGGTGACCATAGTCGTC
V S F S C R A S Q S I G T N I H W Y Q Q
19 21 23 25 27 29 31 33 35 37

CGGACCAACGGCTCCCCTCGGCTGCTGATTAAGTACGCCTCCGAGTCTATCTCCGGCATC
121 -----+-----+-----+-----+-----+-----+-----+
GCCTGGTTGCCGAGGGGAGCCGACGACTAATTCATGCGGAGGCTCAGATAGAGGCCGTAG
R T N G S P R L L I K Y A S E S I S G I
39 41 43 45 47 49 51 53 55 57

CCCTCCCGGTTCTCCGGCTCTGGCTCCGGCACCGACTTCACCCTGTCCATCAACTCCGTG
181 -----+-----+-----+-----+-----+-----+-----+
GGGAGGGCCAAGAGGCCGAGACCGAGGCCGTGGCTGAAGTGGGACAGGTAGTTGAGGCAC

P S R F S G S G S G T D F T L S I N S V
59 61 63 65 67 69 71 73 75 77

EcoRV

GAATCCGAGGATATCGCCGACTACTACTGCCAGCAGAACAACAACTGGCCCACCACCTTC
241 -----+-----+-----+-----+-----+
CTTAGGCTCCTATAGCGGCTGATGATGACGGTCGTCTTGTTGACCGGGTGGTGAAG
E S E D I A D Y Y C Q Q N N N W P T T F
79 81 83 85 87 89 91 93 95 97

GGCGCTGGCACCAAGCTGGAAGTGAAGCGGACCGTGGCCGCTCCCTCCGTGTTTCATCTTC
301 -----+-----+-----+-----+-----+
CCGCGACCGTGGTTCGACCTTGACTTCGCCTGGCACCGGCGAGGGAGGCACAAGTAGAAG
G A G T K L E L K R T V A A P S V F I F
99 101 103 105 107 109 111 113 115 117

PvuII

CCACCCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTCGTGTCCTGCTGAACAAC
361 -----+-----+-----+-----+-----+
GGTGGGAGGCTGCTCGTCGACTTCAGGCCGTGGCGGAGGCAGCACACGGACGACTTGTG
P P S D E Q L K S G T A S V V C L L N N
119 121 123 125 127 129 131 133 135 137

PstI

TTCTACCCCCGCGAGGCCAAGGTGCAGTGAAGGTGGACAACGCCCTGCAGTCCGGCAAC
421 -----+-----+-----+-----+-----+
AAGATGGGGGCGCTCCGGTTCCACGTCACCTTCCACCTGTTGCGGGACGTCAGGCCGTTG
F Y P R E A K V Q W K V D N A L Q S G N
139 141 143 145 147 149 151 153 155 157

TCCCAGGAATCCGTCACCGAGCAGGACTCCAAGGACAGCACCTACTCCCTGTCCTCCACC
481 -----+-----+-----+-----+-----+
AGGGTCCTTAGGCAGTGGCTCGTCCTGAGGTTCTGTCGTGGATGAGGGACAGGAGGTGG
S Q E S V T E Q D S K D S T Y S L S S T
159 161 163 165 167 169 171 173 175 177

CTGACCCTGTCCAAGGCCGACTACGAGAAGCACAAAGGTGTACGCCTGCGAAGTGACCCAC
541 -----+-----+-----+-----+-----+
GACTGGGACAGGTTCCGGCTGATGCTCTTCGTGTTCCACATGCGGACGCTTCACTGGGTG
L T L S K A D Y E K H K V Y A C E V T H
179 181 183 185 187 189 191 193 195 197

KpnI

CAGGGCCTGTCCAGCCCCGTGACCAAGTCCTTCAACCGGGGCGAGTGTGAGGTACC
601 -----+-----+-----+-----+-----+
GTCCCGACAGGTCGGGGCACTGGTTTCAGGAAGTTGGCCCCGCTCACGACTCCATGG
Q G L S S P V T K S F N R G E C *
199 201 203 205 207 209 211 213 215

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

Protein Sequence:

Anti-CD20 heavy chain+ TGFβ-RII ECD fusion amino acid sequence:

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFK
GKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
SLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAPIEKTIISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

Anti-CD20 light chain amino acid sequence:

QIVLSQSPAILASAPGEKVTMTCRASSSVSYIHWFAQKPGSSPKPWIYATSNLASGVPVRFSGSG
SGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

Blue: Anti-CD20 antibody variable region

Black: Anti-CD20 antibody constant region

Green: Linker

Red: TGFβRII ectodomain

cDNA Sequence:

Heavy chain sequence:

```

                PvuII           NarI
                PstI           KsiI
SacI
GAGCTCCAGGTGCAGCTGCAGCAGCCTGGCGCCGAGCTGGTCAAGCCTGGCGCTTCCGTG
1  -----+-----+-----+-----+-----+-----+-----+
CTCGAGGTCCACGTCGACGTCGTCGGACCGCGGCTCGACCAGTTTCGGACCGCGAAGGCAC
      Q  V  Q  L  Q  Q  P  G  A  E  L  V  K  P  G  A  S  V
      1    3    5    7    9   11  13  15  17

                StuI
AAGATGTCCTGCAAGGCCTCCGGCTACACCTTACCAGCTACAACATGCACTGGGTCAAG
61 -----+-----+-----+-----+-----+-----+
TTCTACAGGACGTTCCGGAGGCCGATGTGGAAGTGGTCGATGTTGTACGTGACCCAGTTC
K  M  S  C  K  A  S  G  Y  T  F  T  S  Y  N  M  H  W  V  K
19   21  23   25   27   29   31  33   35  37
```


NarI
KasI

StuI

121 CAGACCCCCGGCAGAGGCCTGGAATGGATCGGCGCCATCTACCCCGGCAACGGCGACACC
 -----+-----+-----+-----+-----+-----+-----+
 GTCTGGGGGGCCGTCTCCGACCTTACCTAGCCGCGGTAGATGGGGCCGTTGCCGCTGTGG
 Q T P G R G L E W I G A I Y P G N G D T
 39 41 43 45 47 49 51 53 55 57

181 TCCTACAACCAGAAGTTCAAGGGAAAGGCCACCCTGACCGCCGACAAGTCCTCCTCCACC
 -----+-----+-----+-----+-----+-----+-----+
 AGGATGTTGGTCTTCAAGTTCCTTTCCGGTGGGACTGGCGGCTGTTCAGGAGGAGGTGG
 S Y N Q K F K G K A T L T A D K S S S T
 59 61 63 65 67 69 71 73 75 77

PvuII

241 GCCTACATGCAGCTGTCTCCCTGACCTCCGAGGACTCCGCCGTGTACTACTGCGCCCGG
 -----+-----+-----+-----+-----+-----+-----+
 CGGATGTACGTCGACAGGAGGGACTGGAGGCTCCTGAGGCGGCACATGATGACGCGGGCC
 A Y M Q L S S L T S E D S A V Y Y C A R
 79 81 83 85 87 89 91 93 95 97

301 TCCACCTACTACGGCGGCGACTGGTACTTCAACGTGTGGGGCGCTGGCACCACCGTGACC
 -----+-----+-----+-----+-----+-----+-----+
 AGGTGGATGATGCCGCCGCTGACCATGAAGTTGCACACCCCGCGACCGTGGTGGCACTGG
 S T Y Y G G D W Y F N V W G A G T T V T
 99 101 103 105 107 109 111 113 115 117

ApaI

361 GTGTCTGCCGCTCTACCAAGGGCCCCTCCGTGTTTCCCCTGGCCCCCTCCAGCAAGTCC
 -----+-----+-----+-----+-----+-----+-----+
 CACAGACGGCGGAGATGGTTCCCGGGGAGGCACAAAGGGGACCGGGGGAGGTGCTTCAGG
 V S A A S T K G P S V F P L A P S S K S
 119 121 123 125 127 129 131 133 135 137

421 ACATCTGGCGGCACCGCCGCTCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAGCCCGTG
 -----+-----+-----+-----+-----+-----+-----+
 TGTAGACCGCCGTGGCGGCGAGACCCGACGGACCAGTTCCTGATGAAGGGGCTCGGGCAC
 T S G G T A A L G C L V K D Y F P E P V
 139 141 143 145 147 149 151 153 155 157

NarI
KasI

PstI

481 ACAGTGTCTGGAACCTCTGGCGCCCTGACCAGCGCGTGACACCTTTCCAGCCGTGCTG
 -----+-----+-----+-----+-----+-----+-----+
 TGTACAGGACCTTGAGACCGCGGACTGGTCGCCGACGTGTGGAAAGGTCGGCACGAC
 T V S W N S G A L T S G V H T F P A V L
 159 161 163 165 167 169 171 173 175 177

541 CAGTCCTCTGGCCTGTACTCCCTGTCCAGCGTCGTGACCGTGCCCTCCAGCTCTCTGGGC
 -----+-----+-----+-----+-----+-----+-----+
 GTCAGGAGACCGGACATGAGGGACAGGTCGCAGCACTGGCACGGGAGGTCGAGAGACCCG

T I S K A K G Q P R E P Q V Y T L P P S
 339 341 343 345 347 349 351 353 355 357

1081 CGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTCAAGGGCTTCTACCCC
 -----+-----+-----+-----+-----+
 GCCCTGCTCGACTGGTTCTTGGTCCACAGGGACTGGACAGACCAGTTCCCGAAGATGGGG
 R D E L T K N Q V S L T C L V K G F Y P
 359 361 363 365 367 369 371 373 375 377
EcoRV
 TCCGATATCGCCGTGGAATGGGAGTCCAACGGCCAGCCCGAGAACAACACTACAAGACCACC
 1141 -----+-----+-----+-----+-----+
 AGGCTATAGCGGCACCTTACCCTCAGGTTGCCGGTCGGGCTCTTGTTGATGTTCTGGTGG
 S D I A V E W E S N G Q P E N N Y K T T
 379 381 383 385 387 389 391 393 395 397

1201 CCCCCTGTGCTGGACTCCGACGGCTCATTCTTCCTGTACTCCAAGCTGACCGTGGACAAG
 -----+-----+-----+-----+-----+
 GGGGGACACGACCTGAGGCTGCCGAGTAAGAAGGACATGAGGTTGACTGGCACCTGTTTC
 P P V L D S D G S F F L Y S K L T V D K
 399 401 403 405 407 409 411 413 415 417

1261 TCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAAC
 -----+-----+-----+-----+-----+
 AGGGCCACCGTCGTCCCGTTGCACAAGAGGACGAGGCACTACGTGCTCCGGGACGTGTTG
 S R W Q Q G N V F S C S V M H E A L H N
 419 421 423 425 427 429 431 433 435 437

1321 CACTACACCCAGAAGTCCCTGTCCCTGAGCCCCGAAAGGGCGGAGGCGGATCTGGTGGT
 -----+-----+-----+-----+-----+
 GTGATGTGGGTCTTCAGGGACAGGGACTCGGGGCCCTTCCCGCCTCCGCCTAGACCACCA
 H Y T Q K S L S L S P G K G G G G S G G
 439 441 443 445 447 449 451 453 455 457

1381 GGAGGATCAGGCGGCGGAGGCTCTACCATCCCCCACACGTGCAGAAATCCGTGAACAAC
 -----+-----+-----+-----+-----+
 CCTCCTAGTCCGCCGCCTCCGAGATGGTAGGGGGTGTGCACGTCTTTAGGCACTTGTG
 G G S G G G S T I P P H V Q K S V N N
 459 461 463 465 467 469 471 473 475 477

NarI
KasI *PvuII*
 GACATGATCGTGACCGACAACAACGGCGCCGTGAAGTTCCCCCAGCTGTGCAAGTTCTGC
 1441 -----+-----+-----+-----+-----+
 CTGTACTAGCACTGGCTGTTGTTGCCGCGGCACTTCAAGGGGTGACACGTTCAGACG
 D M I V T D N N G A V K F P Q L C K F C
 479 481 483 485 487 489 491 493 495 497

BspMI
 GACGTGCGGTTCTCTACCTGCGACAACCAGAAATCCTGCATGTCCAACGTGCTCCATCACC
 1501 -----+-----+-----+-----+-----+
 CTGCACGCCAAGAGATGGACGCTGTTGGTCTTTAGGACGTACAGGTTGACGAGGTAGTGG
 D V R F S T C D N Q K S C M S N C S I T
 499 501 503 505 507 509 511 513 515 517

TCCATCTGCGAGAAGCCCCAGGAAGTGTGCGTCGCCGTCTGGCGGAAGAACGACGAGAAC
 1561 -----+-----+-----+-----+-----+-----+
 AGGTAGACGCTCTTCGGGGTCCTTCACACGCAGCGGCAGACCGCCTTCTTGCTGCTCTTG
S I C E K P Q E V C V A V W R K N D E N
 519 521 523 525 527 529 531 533 535 537

 ATCACCCTGGAAACCGTGTGCCACGACCCCAAGCTGCCCTACCACGACTTCATCTGGAA
 1621 -----+-----+-----+-----+-----+-----+
 TAGTGGGACCTTTGGCACACGGTGCTGGGGTTCGACGGGATGGTGCTGAAGTAGGACCTT
I T L E T V C H D P K L P Y H D F I L E
 539 541 543 545 547 549 551 553 555 557
 GATGCCGCCTCCCCAAGTGCATCATGAAGGAAAAGAAGAAGCCCGCGAGACTTTCCTTC
 1681 -----+-----+-----+-----+-----+-----+
 CTACGGCGGAGGGGGTTCACGTAGTACTTCCTTTCTTCTTCGGGCCGCTCTGAAAGAAG
D A A S P K C I M K E K K K P G E T F F
 559 561 563 565 567 569 571 573 575 577

 ATGTGCTCTTGCTCCTCCGACGAGTGCAACGACAACATCATCTTCTCCGAAGAGTACAAC
 1741 -----+-----+-----+-----+-----+-----+
 TACACGAGAACGAGGAGGCTGCTCACGTTGCTGTTGTAGTAGAAGAGGCTTCTCATGTTG
M C S C S S D E C N D N I I F S E E Y N
 579 581 583 585 587 589 591 593 595 597

KpnI

 ACCTCCAACCCCGACTGAGGTACC
 1801 -----+-----+-----+-----+-----+
 TGGAGGTTGGGGCTGACTCCATGG
T S N P D *
 599 601 603

Light chain sequence:

SacI *NheI*
 GAGCTCCAGATCGTGTGTCCCAGTCCCCCGCCATCCTGTCTGCTAGCCCTGGCGAGAAA
 1 -----+-----+-----+-----+-----+-----+
 CTCGAGGTCTAGCACGACAGGGTCAGGGGGCGGTAGGACAGACGATCGGGACCGCTCTTT
Q I V L S Q S P A I L S A S P G E K
 1 3 5 7 9 11 13 15 17

BspMI

 GTGACAATGACCTGCCGGGCCTCCTCCTCCGTGTCTTACATCCACTGGTTCCAGCAGAAG
 61 -----+-----+-----+-----+-----+-----+
 CACTGTACTGGACGGCCCGGAGGAGGAGGCACAGGATGTAGGTGACCAAGGTCGTCTTC
V T M T C R A S S S V S Y I H W F Q Q K
 19 21 23 25 27 29 31 33 35 37

PflMI

 CCCGGCTCCAGCCCCAAGCCCTGGATCTACGCCACCTCCAACCTGGCCTCCGGCGTGCCA
 121 -----+-----+-----+-----+-----+-----+
 GGGCCGAGGTCGGGGTTCGGGACCTAGATGCGGTGGAGGTTGGACCGGAGGCCGCACGGT

P G S S P K P W I Y A T S N L A S G V P
 39 41 43 45 47 49 51 53 55 57

SmaI

GTGCGGTTCTCTGGCTCCGGCTCCGGCACCTCCTACTCCCTGACCATCTCCCGGGTGGAA
 181 -----+-----+-----+-----+-----+-----+-----+
 CACGCCAAGAGACCGAGGCCGAGGCCGTGGAGGATGAGGGACTGGTAGAGGGCCACCTT
 V R F S G S G S G T S Y S L T I S R V E
 59 61 63 65 67 69 71 73 75 77

GCCGAGGACGCCGCCACCTACTACTGCCAGCAGTGGACCTCCAACCCCCCACCTTTGGC
 241 -----+-----+-----+-----+-----+-----+-----+
 CGGCTCCTGCGGCGGTGGATGATGACGGTCGTCACCTGGAGGTTGGGGGGGTGGAAACCG
 A E D A A T Y Y C Q Q W T S N P P T F G
 79 81 83 85 87 89 91 93 95 97

GGAGGCACCAAGCTGGAAATCAAGCGGACCGTGGCCGCTCCCTCCGTGTTTCATCTTCCCA
 301 -----+-----+-----+-----+-----+-----+-----+
 CCTCCGTGGTTTCGACCTTTAGTTTCGCCTGGCACCGGCGAGGGAGGCACAAGTAGAAGGGT
 G G T K L E I K R T V A A P S V F I F P
 99 101 103 105 107 109 111 113 115 117

PvuII

CCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTCGTGTGCCTGCTGAACAACTTC
 361 -----+-----+-----+-----+-----+-----+-----+
 GGGAGGCTGCTCGTCGACTTCAGGCCGTGGCGGAGGCAGCACACGGACGACTTGTTGAAG
 P S D E Q L K S G T A S V V C L L N N F
 119 121 123 125 127 129 131 133 135 137

PstI

TACCCCCGCGAGGCCAAGGTGCAGTGAAGGTGGACAACGCCCTGCAGTCCGGCAACTCC
 421 -----+-----+-----+-----+-----+-----+-----+
 ATGGGGGCGCTCCGGTTCCACGTACCTTCCACCTGTTGCGGGACGTGAGGCCGTTGAGG
 Y P R E A K V Q W K V D N A L Q S G N S
 139 141 143 145 147 149 151 153 155 157

CAGGAATCCGTCACCGAGCAGGACTCCAAGGACAGCACCTACAGCCTGTCTCCACCCTG
 481 -----+-----+-----+-----+-----+-----+-----+
 GTCCTTAGGCAGTGGCTCGTCTGAGGTTCTGTCGTGGATGTGCGACAGGAGGTGGGAC
 Q E S V T E Q D S K D S T Y S L S S T L
 159 161 163 165 167 169 171 173 175 177

ACCCTGTCCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAAGTGACCCACCAG
 541 -----+-----+-----+-----+-----+-----+-----+
 TGGGACAGGTTCCGGCTGATGCTCTTCGTGTTCCACATGCGGACGCTTCACTGGGTGGTC
 T L S K A D Y E K H K V Y A C E V T H Q
 179 181 183 185 187 189 191 193 195 197

KpnI

GGCCTGTCCAGCCCCGTGACCAAGTCCTTCAACCGGGGCGAGTGCTGAGGTACC
 601 -----+-----+-----+-----+-----+-----+-----+
 CCGGACAGGTCGGGGCACTGGTTTCAGGAAGTTGGCCCCGCTCACGACTCCATGG

G L S S P V T K S F N R G E C *
199 201 203 205 207 209 211 213

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

Protein Sequence:

Anti-VEGF heavy chain+ TGFβ-RII ECD fusion amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGE
PTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWFYFDVWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL
YSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP
QEVCAVVRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS
DECNDNIIFS EYNTSNPD

Anti-VEGF Light chain sequence:

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSR
FSGSGSGTDFTLTISLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Blue: Anti-VEGF antibody variable region

Black: Anti-VEGF antibody constant region

Green: Linker

Red: TGFβRII ectodomain

cDNA Sequence:

Heavy chain sequence:

XhoI
SacI *PvuII* *StuI* *BamHI*

1 GAGCTCGAGGTGCAGCTGGTGGAAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGATCCCTG
-----+-----+-----+-----+-----+-----+-----+
CTCGAGCTCCACGTCGACCACCTTAGGCCGCCTCCGACACAGGTCGGACCGCCTAGGGAC
 E V Q L V E S G G G L V Q P G G S L
 1 3 5 7 9 11 13 15 17

61 AGACTGTCTGTGCCGCCTCCGGCTACACCTTCACCAACTACGGCATGAACCTGGGTCCGA
-----+-----+-----+-----+-----+-----+-----+
TCTGACAGGACACGGCGGAGGCCGATGTGGAAGTGGTTGATGCCGTACTTGACCCAGGCT
R L S C A A S G Y T F T N Y G M N W V R
19 21 23 25 27 29 31 33 35 37

CAGGCCCCCTGGCAAGGGCCTGGAATGGGTCTGGATGGATCAACACCTACACCGGCGAGCCC
 121 -----+-----+-----+-----+-----+-----+-----+
 GTCCGGGGACCGTTCCCGGACCTTACCCAGCCTACCTAGTTGTGGATGTGGCCGCTCGGG
 Q A P G R G L E W V G W I N T Y T G E P
 39 41 43 45 47 49 51 53 55 57

 ACCTACGCCGCCGACTTCAAGCGGCGGTTCACCTTCTCCCTGGACACCTCCAAGTCCACC
 181 -----+-----+-----+-----+-----+-----+-----+
 TGGATGCGGCGGCTGAAGTTTCGCCGCCAAGTGAAGAGGGACCTGTGGAGGTTTCAGGTGG
 T Y A A D F K R R F T F S L D T S K S T
 59 61 63 65 67 69 71 73 75 77

PstI
BspMI
 GCCTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAG
 241 -----+-----+-----+-----+-----+-----+-----+
 CGGATGGACGTCTACTTTGAGGGACGCCCCGGCTCCTGTGGCGGCACATGATGACGCGGTTC
 A Y L Q M N S L R A E D T A V Y Y C A K
 79 81 83 85 87 89 91 93 95 97

BstEII
 TACCCCCACTACTACGGCTCCTCCCACTGGTACTTTCGACGTGTGGGGCCAGGGCACCCCTG
 301 -----+-----+-----+-----+-----+-----+-----+
 ATGGGGGTGATGATGCCGAGGAGGGTGACCATGAAGCTGCACACCCCGTCCCGTGGGAC
 Y P H Y Y G S S H W Y F D V W G Q G T L
 99 101 103 105 107 109 111 113 115 117

ApaI
 GTCACCGTGTCTCCGCCTCTACCAAGGGCCCCCTCCGTGTTCCCTCTGGCCCCCTCCAGC
 361 -----+-----+-----+-----+-----+-----+-----+
 CAGTGGCACAGGAGGCGGAGATGGTTCCCGGGGAGGCACAAGGGAGACCGGGGGAGGTCTG
 V T V S S A S T K G P S V F P L A P S S
 119 121 123 125 127 129 131 133 135 137

 AAGTCCACATCTGGCGGCACCGCCGCTCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAG
 421 -----+-----+-----+-----+-----+-----+-----+
 TTCAGGTGTAGACCGCCGTGGCGGCGAGACCCGACGGACCACTTCTGATGAAGGGGCTC
 K S T S G G T A A L G C L V K D Y F P E
 139 141 143 145 147 149 151 153 155 157

NarI
KasI
 CCCGTGACCGTGTCTGGAACCTCTGGCGCCCTGACCTCCGGCGTGCACACCTTTCCAGCC
 481 -----+-----+-----+-----+-----+-----+-----+
 GGGCACTGGCACAGGACCTTGAGACCGCGGGACTGGAGGCCGCACGTGTGGAAGGTCTCG
 P V T V S W N S G A L T S G V H T F P A
 159 161 163 165 167 169 171 173 175 177

PstI
 GTGCTGCAGTCTCCGGCCTGTACTCCCTGTCCTCCGTGCTGACCGTGCCCTCCAGCTCT
 541 -----+-----+-----+-----+-----+-----+-----+
 CACGACGTCAGGAGGCCGGACATGAGGGACAGGAGGCAGCACTGGCACGGGAGGTCTGAGA

V L Q S S G L Y S L S S V V T V P S S S
 179 181 183 185 187 189 191 193 195 197

601 CTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCTCCAACACCAAGGTGGAC
 -----+-----+-----+-----+-----+-----+
 GACCCGTGGGTCTGGATGTAGACGTTGCACTTGGTGTTCGGGAGGTTGTGGTTCCACCTG
 L G T Q T Y I C N V N H K P S N T K V D
 199 201 203 205 207 209 211 213 215 217

661 AAGAAGGTGGAACCCAAGTCCTGCGACAAGACCCACACCTGTCCCCCTGCCCTGCCCT
 -----+-----+-----+-----+-----+-----+
 TTCTTCCACCTTGGGTTCAGGACGCTGTTCTGGGTGTGGACAGGGGGGACGGGACGGGGA
 K K V E P K S C D K T H T C P P C P A P
 219 221 223 225 227 229 231 233 235 237

721 GAACTGCTGGGAGGCCCTAGCGTGTTCTGTTCCTGTTCCTCCCAAGCCCAAGGACACCTGATG
 -----+-----+-----+-----+-----+-----+
 CTTGACGACCTCCGGGATCGCACAAGGACAAGGGGGGTTTCGGGTTCCTGTGGGACTAC
 E L L G G P S V F L F P P K P K D T L M
 239 241 243 245 247 249 251 253 255 257

BspMI

781 ATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCACGAGGACCCTGAA
 -----+-----+-----+-----+-----+-----+
 TAGAGGGCCTGGGGGCTTCACTGGACGCACCACCTGCACAGGGTGCTCCTGGGACTT
 I S R T P E V T C V V V D V S H E D P E
 259 261 263 265 267 269 271 273 275 277

841 GTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGACAACGCCAAGACCAAGCCCAGA
 -----+-----+-----+-----+-----+-----+
 CACTTCAAGTTAACCATGCACCTGCCGCACCTTACGTGTTGCGGTTCTGGTTCGGGTCT
 V K F N W Y V D G V E V H N A K T K P R
 279 281 283 285 287 289 291 293 295 297

901 GAGGAACAGTACAACCTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
 -----+-----+-----+-----+-----+-----+
 CTCCTTGTCATGTTGAGGTGGATGGCCCACCACAGGCACGACTGGCACGACGTGGTCCTG
 E E Q Y N S T Y R V V S V L T V L H Q D
 299 301 303 305 307 309 311 313 315 317

961 TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCATC
 -----+-----+-----+-----+-----+-----+
 ACCGACTTGCCGTTTCTCATGTTTCACGTTCCACAGGTTGTTCCGGGACGGACGGGGTAG
 W L N G K E Y K C K V S N K A L P A P I
 319 321 323 325 327 329 331 333 335 337

1021 GAAAAGACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACTCTGCCC
 -----+-----+-----+-----+-----+-----+
 CTTTTCTGGTAGAGGTTCCGGTTCCCGGTCGGGGCGCTCGGAGTCCACATGTGAGACGGG
 E K T I S K A K G Q P R E P Q V Y T L P
 339 341 343 345 347 349 351 353 355 357

1081 CCTAGCCGGAAGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTCAAGGGCTTC
 -----+-----+-----+-----+-----+-----+
 GGATCGGCCCTTCTCTACTGGTTCTTGGTCCACAGGGACTGGACAGACCAGTTCCCCGAAG
 P S R E E M T K N Q V S L T C L V K G F
 359 361 363 365 367 369 371 373 375 377

EcoRV

1141 TACCCCTCCGATATCGCCGTGGAATGGGAGTCCAACGGCCAGCCCCGAGAACAACACTACAAG
 -----+-----+-----+-----+-----+-----+
 ATGGGGAGGCTATAGCGGCACCTTACCCTCAGGTTGCCGGTCGGGCTCTTGTGTGATGTTTC
 Y P S D I A V E W E S N G Q P E N N Y K
 379 381 383 385 387 389 391 393 395 397

1201 ACCACCCCCCTGTGCTGGACTCCGACGGCTCATTCCTCTGCTACTCCAAGCTGACCGTG
 -----+-----+-----+-----+-----+-----+
 TGGTGGGGGGGACACGACCTGAGGCTGCCGAGTAAGAAGGACATGAGGTTCCGACTGGCAC
 T T P P V L D S D G S F F L Y S K L T V
 399 401 403 405 407 409 411 413 415 417

1261 GACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCTGCTCCGTGATGCACGAGGCCCTG
 -----+-----+-----+-----+-----+-----+
 CTGTTTCAGGGCCACCGTCGTCCCGTTGCACAAGAGGACGAGGCACTACGTGCTCCGGGAC
 D K S R W Q Q G N V F S C S V M H E A L
 419 421 423 425 427 429 431 433 435 437

1321 CACAACCACTACACCCAGAAGTCCCTGTCCCTGAGCCCAGGCAAGGGCGGAGGCGGATCT
 -----+-----+-----+-----+-----+-----+
 GTGTTGGTGATGTGGGTCTTCAGGGACAGGGAAGTCCGGTCCGTTCCCGCCTCCGCCTAGA
 H N H Y T Q K S L S L S P G K G G G G S
 439 441 443 445 447 449 451 453 455 457

1381 GGTGGTGGAGGATCAGGCGGCGGAGGCTCTACCATCCCCCACACGTGCAGAAATCCGTG
 -----+-----+-----+-----+-----+-----+
 CCACCACCTCCTAGTCCGCCCTCCGAGATGGTAGGGGGGTGTGCACGTCTTTAGGCAC
 G G G G S G G G S T I P P H V Q K S V
 459 461 463 465 467 469 471 473 475 477

NarI

KasI

PvuII

1441 AACAACGACATGATCGTGACCGACAACAACGGCGCCGTGAAGTCCCCCAGCTGTGCAAG
 -----+-----+-----+-----+-----+-----+
 TTGTTGCTGTACTAGCACTGGCTGTTGTTGCCGCGGCACTTCAAGGGGGTGCACACGTTTC
 N N D M I V T D N N G A V K F P Q L C K
 479 481 483 485 487 489 491 493 495 497

BspMI

1501 TTCTGCGACGTGCGGTTCTCTACCTGCGACAACCAGAAATCCTGCATGTCCAACCTGCTCC
 -----+-----+-----+-----+-----+-----+
 AAGACGCTGCACGCCAAGAGATGGACGCTGTTGGTCTTTAGGACGTACAGGTTGACGAGG
 F C D V R F S T C D N Q K S C M S N C S
 499 501 503 505 507 509 511 513 515 517

ATCACCTCCATCTGCGAGAAGCCCCAGGAAGTGTGCGTCGCCGTCTGGCGGAAGAACGAC
 1561 -----+-----+-----+-----+-----+-----+-----+
 TAGTGGAGGTAGACGCTCTTCGGGGTCCTTCACACGCAGCGGCAGACCGCCTTCTTGCTG
 I T S I C E K P Q E V C V A V W R K N D
 519 521 523 525 527 529 531 533 535 537

GAGAACATCACCTGGAAACCGTGTGCCACGACCCCAAGCTGCCCTACCACGACTTCATC
 1621 -----+-----+-----+-----+-----+-----+-----+
 CTCTTGTAGTGGGACCTTTGGCACACGGTGTCTGGGGTTCGACGGGATGGTGCTGAAGTAG
 E N I T L E T V C H D P K L P Y H D F I
 539 541 543 545 547 549 551 553 555 557

CTGGAAGATGCCGCCTCCCCCAAGTGCATCATGAAGGAAAAGAAGCCCGGCGAGACT
 1681 -----+-----+-----+-----+-----+-----+-----+
 GACCTTCTACGGCGGAGGGGGTTCACGTAGTACTTCCTTTTCTTCTTCGGGCCGCTCTGA
 L E D A A S P K C I M K E K K K P G E T
 559 561 563 565 567 569 571 573 575 577

PvuII

TTCTTCATGTGCAGCTGCTCCTCCGACGAGTGCAACGACAACATCATCTTCTCCGAAGAG
 1741 -----+-----+-----+-----+-----+-----+-----+
 AAGAAGTACACGTGACGAGGAGGCTGCTACGTTGCTGTTGTAGTAGAAGAGGCTTCTC
 F F M C S C S S D E C N D N I I F S E E
 579 581 583 585 587 589 591 593 595 597

KpnI

TACAACACCTCCAACCCCGACTGAGGTACC
 1801 -----+-----+-----+-----+-----+-----+-----+
 ATGTTGTGGAGGTTGGGGCTGACTCCATGG
 Y N T S N P D *
 599 601 603 605

Light chain sequence:

SacI EcoRV

GAGCTCGATATCCAGATGACCCAGTCCCCCTCCAGCCTGTCCGCCTCTGTGGGCGACAGA
 1 -----+-----+-----+-----+-----+-----+-----+
 CTCGAGCTATAGGTCTACTGGGTCTAGGGGAGGTCGGACAGGCGGAGACACCCGCTGTCT
 D I Q M T Q S P S S L S A S V G D R
 1 3 5 7 9 11 13 15 17

GTGACCATCACCTGTTCCGCCAGCCAGGACATCTCCAACCTACCTGAACTGGTATCAGCAG
 61 -----+-----+-----+-----+-----+-----+-----+
 CACTGGTAGTGGACAAGGCGGTCTGGTCTGTAGAGGTTGATGGACTTGACCATAGTCGTC
 V T I T C S A S Q D I S N Y L N W Y Q Q
 19 21 23 25 27 29 31 33 35 37

AAGCCCGGCAAGGCCCTAAGGTGCTGATCTACTTCACCTCCTCCCTGCACTCCGGCGTG
 121 -----+-----+-----+-----+-----+-----+-----+
 TTCGGGCCGTTCCGGGGATTCCACGACTAGATGAAGTGGAGGAGGGACGTGAGGCCGCAC
 K P G K A P K V L I Y F T S S L H S G V
 39 41 43 45 47 49 51 53 55 57

PstI

181 CCCTCCAGATTCTCCGGCTCTGGCTCCGGCACCAGCTTTACCCTGACCATCTCCAGCCTG
 -----+-----+-----+-----+-----+-----+
 GGGAGGTCTAAGAGGCCGAGACCGAGGCCGTGGCTGAAATGGGACTGGTAGAGGTCGGAC
 P_S_R_F_S_G_S_G_S_G_T_D_F_T_L_T_I_S_S_L_
 59 61 63 65 67 69 71 73 75 77

ScaI

241 CAGCCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACTCCACCGTGCCCTGGACCTTC
 -----+-----+-----+-----+-----+-----+
 GTCGGGCTCCTGAAGCGGTGGATGATGACGGTCGTCATGAGGTGGCAGGGACCTGGAAG
 Q_P_E_D_F_A_T_Y_Y_C_Q_Q_Y_S_T_V_P_W_T_F_
 79 81 83 85 87 89 91 93 95 97

301 GGCCAGGGCACCAAGGTGGAATCAAGCGGACCGTGGCCGCTCCCTCCGTGTTTCATCTTC
 -----+-----+-----+-----+-----+-----+
 CCGGTCCCGTGGTTCCACCTTTAGTTCGCCTGGCACCGGCGAGGGAGGCACAAGTAGAAG
 G_Q_G_T_K_V_E_I_K_R_T_V_A_A_P_S_V_F_I_F_
 99 101 103 105 107 109 111 113 115 117

PvuII

361 CCACCCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTCGTGTGCCTGCTGAACAAC
 -----+-----+-----+-----+-----+-----+
 GGTGGGAGGCTGCTCGTCGACTTCAGGCCGTGGCGGAGGCAGCACACGACGACTTGTTC
 P_P_S_D_E_Q_L_K_S_G_T_A_S_V_V_C_L_L_N_N_
 119 121 123 125 127 129 131 133 135 137

PstI

421 TTCTACCCCCGCGAGGCCAAGGTGCAGTGAAGGTGGACAACGCCCTGCAGTCCGGCAAC
 -----+-----+-----+-----+-----+-----+
 AAGATGGGGGCGCTCCGGTTCACGTCACCTTCCACCTGTTGCGGGACGTCAGGCCGTTG
 F_Y_P_R_E_A_K_V_Q_W_K_V_D_N_A_L_Q_S_G_N_
 139 141 143 145 147 149 151 153 155 157

481 TCCCAGGAATCCGTCACCGAGCAGGACTCCAAGGACAGCACCTACTCCCTGTCCTCCACC
 -----+-----+-----+-----+-----+-----+
 AGGGTCCTTAGGCAGTGGCTCGTCCTGAGGTTCTGTCGTGGATGAGGGACAGGAGGTGG
 S_Q_E_S_V_T_E_Q_D_S_K_D_S_T_Y_S_L_S_S_T_
 159 161 163 165 167 169 171 173 175 177

541 CTGACCCTGTCCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAAGTGACCCAC
 -----+-----+-----+-----+-----+-----+
 GACTGGGACAGGTTCCGGCTGATGCTCTTCGTGTTCCACATGCGGACGCTTCACTGGGTG
 L_T_L_S_K_A_D_Y_E_K_H_K_V_Y_A_C_E_V_T_H_
 179 181 183 185 187 189 191 193 195 197

KpnI

601 CAGGGCCTGTCCAGCCCCGTGACCAAGTCCTTCAACCGGGGCGAGTGCTGAGGTACC
 -----+-----+-----+-----+-----+-----+
 GTCCCGGACAGGTCGGGGCACTGGTTTCAGGAAGTTGGCCCCGCTCACGACTCCATGG
 Q_G_L_S_S_P_V_T_K_S_F_N_R_G_E_C_*_
 201 203 205 207 209 211 213 215 217 219

199 201 203 205 207 209 211 213 215

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

Protein Sequence:

Sequence: Anti-CTLA-4 heavy chain + TGFβ-RII Extracellular domain fusion amino acid sequence:

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

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

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

Green: Linker

Red: TGFβRII ectodomain

Sequence: Anti-CTLA-4 light chain

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

Blue: Anti-CTLA-4 antibody light chain variable region

Black: Anti-CTLA-4 antibody light chain constant region

- * Fusion protein comprising anti-CD4 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD)
6. Fusion protein comprising IL-2, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD).

Protein Sequence:

(a)

TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP
 QEVCAVVRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS
 DECNDNIIFS EEEYNTSNPD GGGGSGGGGSGGGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTL
 MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
 KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
 ESNGQOPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSVHEALHNHYTQKSLSLSPG
 KGGGSGGGGSGGGGS

(b)

APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKP
 LEEVLNLAQSKNFHLRPRDLISNINIVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIIS
 TLTGGGGSGGGGSGGGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
 PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQOPENNYKTTTPVLD
 SDGSFFLYSKLTVDKSRWQQGNVFSVMSVHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
 TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP
 QEVCAVVRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS
 DECNDNIIFS EEEYNTSNPD

Red: TGFβRII ectodomain

Green: Linker (Optional; any linker sequence)

Blue: Fc

Black: IL2 fragment

(Can replace linker 1 with EPKSCDK)

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

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

Protein Sequence:

(a)

anti-CD25 and PD-1 ectodomain - Heavy chain

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY
NQKFKGKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGGT LVTVSSASTK
GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
LSSVTVFPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMEHA LHNHYTQKSL SLSPGK GGGSGGGSGGGSGTIPPHVQK SVNNDMIVTD
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCAVWRK NDENITLETV
CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

Black: Anti-CD25 antibody heavy chain

Green: Linker

Red: TGFβRII ectodomain

anti-CD25 - Light chain

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

(b)

anti-CD25 and PD-1 ectodomain - Heavy chain

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPG QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDIYGY YFDWFGQGT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVTVFPSS LGTQTYICNV NHKPSNTKVD KRVEPPKSCD KTHTCPPCPA PELLGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMEHA LHNHYTQKSL SLSPGK GGGSGGGSGGGSGTIPPHVQK SVNNDMIVTD
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCAVWRK NDENITLETV
CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

Black: Anti-CD25 antibody heavy chain

Green: Linker

Red: TGFβRII ectodomain

anti-CD25 – Light chain

QIVSTQSPAI MSASPGEKVT MTCSASSRS YMOWYQQKPG TSPKRWIYDT SKLASGVPAR
 FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGK LEIKRTVAAP SVFIFPPSDE
 QLKSGTASVV CLLNNFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSLTLSK
 ADYEKHKVYA CEVTHQGLSS PVTKSFNRRGE

* **Fusion protein comprising anti-PD1 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain.**

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

Protein Sequence:

(a) PD-1 ectodomain – Fc - TGFβRII ectodomain

PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWMSPSNQTDKL
 AAFPEDRSQPGQDCFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESL
 RAE LRVTERRAEVPTAHPSPPRPAGQFQTLVGGGGSGGGSGGGGSTHTCPPCPAPEL
 LGGPSVFLFPKPKDTLMISRTPVETCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
 EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLTP
 PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
 VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGGSTIPPHVQK
 SVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWR
 KNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNII
 FSEYNTSNPD

(b) PD-1 ectodomain – Fc - TGFβRII ectodomain

PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWMSPSNQTDKL
 AAFPEDRSQPGQDCFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESL
 RAE LRVTERRAEVPTAHPSPPRPAGQFQTLV (EPKSCDK) THTCPPCPAPELLGGPSV
 FLFPKPKDTLMISRTPVETCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
 YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLTPPSREEM
 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
 QQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGGSTIPPHVQKSVNNDM
 IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENI
 TLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNIIIFSEYNT
 SNPD

Green: PD-1 ectodomain

Red: Linker

Blue: IgG1 Fc

Black: TGFβRII ectodomain

(Note: optional sequence in parentheses)

- I (B).** A molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:
- (a) said targeting moiety binds a specific target molecule, and
 - (b) said immunomodulatory moiety is a molecule that binds Programmed Death-1 ligand [PD-L1 (B7-H1) or PD-L2 (B7-DC)].

Claims: 24-40
SEQ. ID.: 9-16

Said immunomodulatory moiety that binds Programmed Death-1 ligand [PD-L1 (B7-H1) or PD-L2 (B7-DC)] may be the following:

- (i) Full-length PD-1 or fragment thereof:

I MQIPQAPWPV VWAVLQLGWR PGWFLDSPDR PWNPTFSPA LLVVTEGDNA TFTCSFSNTS
61 ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT
121 YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPPSP RPAGQFQTLV VGVVGGLLGS
181 LVLLVWVLAV ICSRAARGTI GARRTGQPLK EDPSAVPVFS VDYGELDFQW REKTPEPPVP
241 CVPEQTEYAT IVFPSGMGTS SPARRGSADG PRSAQPLRPE DGHCSWPL

Green: PD-1 ectodomain (ligand-binding domain – underlined)

- (ii) PD-1 extracellular domain (ectodomain) or fragment thereof:

PGWFLDSPDR PWNPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA
AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA
ELRVTERRAE VPTAHPSPPSP RPAGQFQTLV

- (iii) PD-1 extracellular domain (ectodomain) ligand-binding region

DSPDR PWNPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA
AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA
ELRVTERRAE VPTAHPSPPSP 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)]

Preferred embodiment: Sequence used in examples (SEQ. ID: 9-16):

PGWFLDSPDR PWNPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA
AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA
ELRVTERRAE VPTAHPSPPSP RPAGQFQTLV

9. Fusion protein comprising Anti-HER2/neu antibody and PD-1 Ectodomain.

Protein Sequence:

Anti-HER2/neu heavy chain + PD-1 ectodomain fusion amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVSSASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
EVTCTVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKGGGGS
GGGGSGGGGS PGWFLDSPDR PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM
SPSNQTDKLA AFPEDRSQPG QDCRFRTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP
KAQIKESLRA ELRVTERRAE VPTAHPSPPSP RPAGQFQTLV

Anti-HER2/neu light chain amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFYLYSGVPSRFSGS
RSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC

Blue color: Anti-HER2/neu Variable region
Black color: Anti-HER2/neu Constant region
Pink Color: Linker region
Green: PD-1 ectodomain

10. Fusion protein comprising Anti-EGFR1 antibody and PD-1 Ectodomain.

Protein Sequence:

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

QVQLKQSGPGLVQPSSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSSGGNTDYNTPFTS
RLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTVTVSAASTKGPSVFPPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSL
GTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSG
GGGSGGGGS PGWFLDSPDR PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWMYRM
SPSNQTDKLA AFPEDRSQPG QDCRFRTVQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP
KAQIKESLRA ELRVTERRAE VPTAHPSPPSP RPAGQFQTLV

Anti-EGFR light chain amino acid sequence:

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGS
GSGTDFTLSINSVESEDIADYYCQNNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC

Blue color: Anti-EGFR Variable region
Black color: Anti-EGFR Constant region
Pink Color: Linker region
Green: PD-1 ectodomain

11. Fusion protein comprising Anti-CD20 antibody and PD-1 Ectodomain.

Protein Sequence:

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

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFK
GKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTTVTVSAASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
SLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMHEALHNHYTQKSLSLSPGKGGGG
SGGGGSGGGGSPGWFLDSPDR PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM
SPSNQTDKLA AFPEDRSQPG QDCRFRTVL PNGRDFHMSV VRARRNDSGT YLCGAISLAP
KAQIKESLRA ELRVTERRAE VPTAHPSPPSP RPAGQFQTLV

Anti-CD20 light chain amino acid sequence:

QIVLSQSPAILASAPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSG
SGTSYSLTISRVEAEDAATYYCQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEV
THQGLSPVTKSFNRGEC

Blue color: Anti-CD20 Variable region
Black color: Anti-CD20 Constant region
Pink Color: Linker region
Green: PD-1 ectodomain

12. Fusion protein comprising Anti-VEGF antibody and PD-1 Ectodomain.

Protein Sequence:

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

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGE
PTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA
AFPEDRSQPG QDCRFRTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA
ELRVTERRAE VPTAHPSPPSP RPAGQFQTLV

Anti-VEGF Light chain sequence:

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Blue color: Anti-VEGF Variable region
Black color: Anti-VEGF Constant region
Pink Color: Linker region
Green: PD-1 ectodomain

13. Fusion protein 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

Protein Sequence:

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

QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYTMHWVRQA PGKGLEWVTF ISYDGNNKYY
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGQ GTLVTVSSAS
TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL
YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPELLGGPS
VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ
GNVFCSSVMH EALHNHYTQKSLSLSPGKGGGSGGGGSGGGGSPGWFLDSPDR PWNPTTFSPA
LLVVTEDDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL
PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP
RPAGQFQTLV

Anti-CTLA-4 light chain

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

Blue color: Anti-CTLA-4 Variable region
Black color: Anti-CTLA-4 Constant region
Pink Color: Linker region
Green: PD-1 ectodomain

- * **Fusion protein comprising anti-Transforming growth factor-beta (TGF β) antibody and PD-1 Ectodomain**
- 14. **Fusion protein comprising Transforming growth factor-beta receptor II (TGF β -RII) ectodomain, Fc, and PD-1 Ectodomain:**

Protein Sequence:

(a) TGF β RII ectodomain – Fc - PD-1 ectodomain

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQ
 EVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKKPGETFFMCSCSS
 DECNDNIIFSEEYNTSNPDGGGGSGGGSGGGGSTHTCPPCPAPELLGGPSVFLFPPKP
 KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
 TCLVKGFPYSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
 CSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGSGGGSPGWFLDSPDRPWNPTTFSPAL
 LVVTEGDNATFTCSFSNTSESFVLNWMSPSNQTDKLAAPEDRSQPGQDCRFRVTQL
 PNGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPS
 PRPAGQFQTLV

Black: TGF β RII ectodomain

Red: Linker

Blue: IgG1 Fc

Green: PD-1 ectodomain

(b) TGF β RII ectodomain – Fc - PD-1 ectodomain

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQ
 EVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKKPGETFFMCSCSS
 DECNDNIIFSEEYNTSNPD (EPKSCDK) THTCPPCPAPELLGGPSVFLFPPKPKDTLMI
 SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
 WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
 FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
 CSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGSGGGSPGWFLDSPDRPWNPTTFSPALLVVTEG
 DNATFTCSFSNTSESFVLNWMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLPNGRDF
 HMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQ
 FQTLV

Black: TGF β RII ectodomain

Blue: IgG1 Fc

Red: Linker

Green: PD-1 ectodomain

(Note: Optional sequence in parentheses)

* **Fusion protein comprising anti-CD4 antibody and PD-1 Ectodomain.**

15. Fusion protein comprising anti-CD25 antibody and PD-1 Ectodomain.

Protein Sequence:

(a)

anti-CD25 (Daclizumab) and PD-1 ectodomain - Heavy chain

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY
NQKFKGKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGGGT LVTVSSASTK
GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSINKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK GGGGSGGGGSGGGGSPGWFLDSPDRPWNPPTFSPAL
LVVTEGDNATFTCSFNTSESFVLNWMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLPNGRDF
HMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSRPAGQFQTLV

Black: anti-CD25 heavy chain

Red: Linker

Green: PD-1 ectodomain

anti-CD25 (Daclizumab) - Light chain

DIQMTQSPST LSASVGDRVIT ITCSASSSIS YMHYQQKPG KAPKLLIYTT SNLASGVPAR
FSGSGSGTEF LTITSSLPD DFATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS
DEQLKSGTAS VVCLLNFPYF REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSTLTTL
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

(b)

anti-CD25 (Basiliximab) and PD-1 ectodomain - Heavy chain

QLQQSGTVLA RPGASVKMSC KASGYSTFT WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDIYGY YFDWGGGT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPPKSCD KTHTCPPCPA PELLGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSINKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK GGGGSGGGGSGGGGSPGWFLDSPDRPWNPPTFSPAL
LVVTEGDNATFTCSFNTSESFVLNWMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLPNGRDF
HMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSRPAGQFQTLV

Black: anti-CD25 heavy chain

Red: Linker

Green: PD-1 ectodomain

anti-CD25 (Basiliximab) – Light chain

QIVSTQSPAI MSASPGEKVT MTCSASSRS YMQWYQOKPG TSPKRWIYDT SKLASGVPAR
 FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGK LEIKRTVAAP SVFIFPPSDE
 QLKSGTASVV CLLNNFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK
 ADYEKHKVYA CEVTHQGLSS PVTKSFNRGE

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

Protein Sequence:

(a)

PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWMSPSNQTDKLAAPFED
 RSQPGQDCFRVLTQLPNGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRVTERRAE
 VPTAHPSPPRPAQGFQTLVGGGGSGGGSGGGSGTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
 ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
 EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE
 SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSVHEALHNHYTQKSLSLSPGK
 GGGSGGGSGGGSGGGSG

(b)

APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLCLEELKP
 LEEVLNLAQSKNFHLRPRDLISNINIVIVLELKGSETTFMCEYADETATVEFLNRWITFCQSIIS
 TLTGGGSGGGSGGGSGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
 PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
 TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLD
 SDGSFFLYSKLTVDKSRWQQGNVFSVMSVHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGSGPG
 WFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWMSPSNQTDKLAAPFEDRS
 QPGQDCFRVLTQLPNGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRVTERRAEVP
 TAHPSPPRPAQGFQTLV

Black: IL2 fragment

Red: Linker (Optional – any linker sequence)

Blue: Fc

Green: human PD-1 ectodomain

(Can replace linker 1 with EPKSCDK)

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

II. Targeted Immunosuppressive Antibodies and Fusion Proteins:

- II (A). A molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:
- (a) said targeting moiety binds a specific target molecule, and
 - (b) said immunomodulatory moiety is a molecule that binds Programmed death-1 (PD-1)

Claims: 41-59
SEQ. ID.: 17-26

Said immunomodulatory moiety that binds Programmed Death-1 (PD-1) may be a PD-1 ligand [PD-L1 (B7-H1) or PD-L2 (B7-DC)] or a fragment thereof:

- (i) Full-length human PD-1 ligand 1 (B7-H1; PDCD1L1; PDL1; CD274) protein or a fragment thereof:

MRIFAVFIFM TYWHLLNAFT VTPPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME
DKNIIQFVHG EEDLKVQHSS YRQARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG
ADYKRITVKV NAPYNKINQR ILVVDPTSE HELTCQAEY PKAEVIWTSS DHQVLSGKTT
TTNSKREEKL FNVSTLRLIN TTTNEIFYCT FRRLDPEENH TAEIPIPELP LAHPPNERTH
LVILGAILLC LGVALTFIFR LRKGRMDVK KCGIQDTNSK KQSDTHLEET

Extracellular Binding domain = underlined sequence

- (ii) PD-L1 extracellular binding domain (ectodomain) or fragment thereof:

AFT VTPPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG EEDLKVQHSS
YRQARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV NAPYNKINQR
ILVVDPTSE HELTCQAEY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL FNVSTLRLIN
TTTNEIFYCT FRRLDPEENH TAEIPI

Green: PD-L1 extracellular binding domain (ectodomain) – underlined.

- (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).

Preferred embodiment: Sequence used in examples (SEQ. ID: 17-26):

RIFAVFIFM TYWHLLNAFT VTPPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME
DKNIIQFVHG EEDLKVQHSS YRQARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG
ADYKRITVKV NAPYNKINQR ILVVDPTSE HELTCQAEY PKAEVIWTSS DHQVLSGKTT
TTNSKREEKL FNVSTLRLIN TTTNEIFYCT FRRLDPEENH TAEIPIPELP LAHPPNERTH
LVILGAILLC LGVALTFIFR LRKGRMDVK KCGIQDTNSK KQSDTHLEET

Green: PD-L1 extracellular binding domain (ectodomain) – underlined.

17. Fusion protein comprising anti-tumor necrosis factor (TNF α) antibody and PD-1 ligand.

Protein Sequence:

Heavy chain:

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSV
GRFTISRDNKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGTLLTVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
SLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKGGGG
SGGGGSGGGGSRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIV
YWEMEDKNI IQFVHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGG
ADYKRITVKVNAPYNKINQRILVVDVPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSK
REEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLC
LGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET

Black color: anti-TNF α heavy chain variable region

Blue color: IgG1 heavy chain constant region

Pink Color: Linker region

Green Color: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)

(Note: Options – replace underlined KKAEE with KRVE or KKVE)

Light Chain:

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLPGKAPKLLIYAASLTQSGVPSRFGSG
GSGTDFTLTISLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC

Black color: anti-TNF α light chain variable region

Blue color: IgG1 light chain constant region

Fusion protein comprising TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand:

- 18. Fusion protein comprising TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand: (TNFR2 ECD - IgG Cy1 – PD-1L1).**

TNFR2 ECD - IgG Cy1 – PD-1L1

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLW
 NWVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKEGECRLCAPLRKCRPGFGVARP
 GTETSDVVCKPCAPGTFSNTTSSTDI CRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLP
 QPVSTRSQHTQPTPEPSTAPSTSFL LPMGPSPPAEGSTGDEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
 LTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
 GFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
 YTQKSLSLSPGKGGGSGGGGSGGGGSRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIE
CKFPVEKQLDLAALIVWEMEDKNIIQFVHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDV
KLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRI LVVDPVTSEHELTCQAEGYPKAEVIWTS
SDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHP
 PNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET

Black color: TNFR2 ligand-binding domain

Blue Color: human IgG1 heavy chain constant region (IgG Cy1 domain = H, CH2, CH3)

Pink Color: Linker region

Green Color: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)

- 19. Fusion protein comprising PD-1 ligand, Fc, and TNFR2 Extracellular ligand binding domain: (PD1-L1 - IgG Cy1 -TNFR2 ECD)**

PD1-L1 - IgG Cy1 -TNFR2 ECD

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNII
QFVHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKV
 NAPYNKINQRI LVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVT
TLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFR
 LRKGRMMDVKKCGIQDTNSKKQSDTHLEETGGGGSGGGGSGGGGSEPKSCDKTHTCPPCPAPELL
 GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
 RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
 TCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
 ALHNHNTQKSLSLSPGKGGGSGGGGSGGGGSLPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCS
 KCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWNWVPECLSCGSRCSSDQVETQACTREQNRICTC
 RPGWYCALSKEGECRLCAPLRKCRPGFGVARPGTETSDVVCKPCAPGTFSNTTSSTDI CRPHQIC
 NVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFL LPMGPSPP
 AEGSTGD

Green Color: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)

Blue Color: human IgG1 heavy chain constant region (IgG Cy1 domain = H, CH2, CH3)

Pink Color: Linker region (optional); any linker

Black color: TNFR2 ligand-binding domain

20. Fusion protein comprising anti-CD20 antibody and PD-1 ligand 1 (PD-L1).

Protein Sequence:

Heavy chain sequence:

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFK
GKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
SLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAIEKTIKAKGQPREPQVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGSRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLA
ALIVYWEMEDKNI IQFVHGEEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMI
SYGGADYKRITVKVNAPYNKINQRILVVDVPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTT
TNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELV IPELPLAHPPNERTHLVILGA
ILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET

Blue color: anti-CD20 Heavy chain Variable region

Black color: anti-CD20 Heavy chain Constant region

Pink Color: Linker region

Green Color: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)

Light chain sequence:

QIVLSQSPAILASAPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSG
SGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSKADYEKHKVYACEV
THQGLSPVTKSFNRGEC

Blue color: anti-CD20 Light chain Variable region

Black color: anti-CD20 Light chain Constant region

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

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

(i)

anti-CD25 and PD-1L1 - Heavy chain

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY
NQKFKGKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LTVSSASTK
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 GGGGSGGGGSGGGGSRIFAVFIFMTYWHLNNAFTVT
VPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQRARL
LKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDVPTSEHE
LTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQ
SDTHLEET

Black: anti-CD25 heavy chain

Red: Linker

Green Color: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)

anti-CD25 - Light chain

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

(ii)

anti-CD25 and PD-1 ectodomain - Heavy chain

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDIYGY YFDFWGQGT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPPKSCD KTHTCPPCPA PELLGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK GGGGSGGGGSGGGGSRIFAVFIFMTYWHLNNAFTVT
VPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQRARL
LKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDVPTSEHE
LTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQ
SDTHLEET

Black: anti-CD25 heavy chain

Red: Linker

Green Color: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)

anti-CD25 - Light chain

QIVSTQSPAI MSASPGEKVT MTCSASSRS YMOWYQQKPG TSPKRWIYDT SKLASGVPAR
 FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGK LEIKRTVAAP SVFIFPPSDE
 QLKSGTASVV CLLNNFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK
 ADYEKHKVYA CEVTHQGLSS PVTKSFNERGE

22. Fusion protein comprising IL-2, Fc, and PD-1 ligand 1 (PD-L1).

(i) Fusion protein: hPD-1 ligand 1, Fc, and IL-2

MRIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQDLAALIVWEMEDKNII
QFVHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKV
 NAPYNKINQRILVVDVPTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVT
TLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFR
 LRKGRMDVKKCGIQDTNSKKQSDTHLEETGGGGSGGGSGGGSGGSGTHTCPPCPAPPELLGGPSVFL
 FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
 VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF
 YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYT
 QKSLSLSPGKGGGGSGGGSGGGGS

(ii) Fusion protein: IL-2, Fc, and PD-1 ligand 1

APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLCLEELKP
 LEEVLNLAQSKNFHLRPDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIIS
 TLTGGGGSGGGSGGGSGGSGTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
 EDPVEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 SDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGKGGGGSGGGSGGGGSRI
 FAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQDLAALIVWEMEDKNIIQFV
HGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
PNKINQRILVVDVPTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLR
 GRMDVKKCGIQDTNSKKQSDTHLEET

Black: IL2 fragment

Red: Linker (optional: any sequence)

Blue: Fc

Green Color: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)

(Can replace linker 1 with EPKSCDK)

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

Fusion proteins comprising CTLA-4-Ig and PD-1 ligand

23. Fusion protein 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-1L1

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQ
ADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYL
GIGNGTQIYVIDPEPCPDSD(Q)EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSVMHEALHNHYTQKSLSLSPGKGGGGS
GGGSGGGGSRIFAVFIFMTYWHLLNAFTVTPPKDLYVVEYGSNMTIECKFPVEKQDLAALIVY
WEMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGG
ADYKRITVKVNAPYNKINQRILVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKR
EEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCL
GVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET

Black color: Oncostatin M Signal Peptide (-25 to -1)

Blue color: CTLA-4 extracellular domain (1-125)

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

Pink Color: Optional Linker region (any linker)

Green Color: human PD-1 ligand 1 (PD-1L) or extracellular binding domain of PD-1L (underlined)

24. Fusion protein 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-1L1 - IgG C γ 1 - CTLA-4 ECD

MRIFAVFIFMTYWHLLNAFTVTPPKDLYVVEYGSNMTIECKFPVEKQDLAALIVYWEMEDKNI
IQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKV
NAPYNKINQRILVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVT
TLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFR
LRKGRMMDVKKCGIQDTNSKKQSDTHLEETGGGSGGGSGGGSGGGSEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSVMH
EALHNHYTQKSLSLSPGKGGGSGGGSGGGGSAMHVAQPAVVLASSRGIASFVCEYASPGKATEV
RVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELM
YPPPYLGIGNGTQIYVIDPEPCPDSD

Green Color: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)

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

Pink Color: Optional Linker region (any linker)

Blue color: CTLA-4 extracellular domain (1-125)

Fusion proteins comprising Transforming growth factor- β and PD-1 ligand:

- 25. Fusion protein comprising a sequence of transforming growth factor- β (TGF- β), Immunoglobulin Fc (IgG C γ 1), and a sequence of PD-1 ligand (PD-L1):**

TGF β -1 – Fc – PD-1L1:

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALY
NQHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCSGGGGSGGGSGGGGSTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGGSRIFAVFIFMTYWHLLNAFTVTV
KDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQARLLK
DQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYKNINQIRILVVDVPTSEH
ELTCQAEQYPAEVIWTSDDHQLVSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEEN
HTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGMMMDVKKCGIQDTNSKKQSD
THLEET

Black: TGF β -1

Red: Linker (optional: any sequence)

Blue: Fc

Green Color: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)
(Can replace linker 1 with EPKSCDK)

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

- 26. 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-1L1 – Fc - TGF β -1:

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNII
QFVHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKV
NAPYKNINQIRILVVDVPTSEHELTCQAEQYPAEVIWTSDDHQLVSGKTTTTNSKREEKLFNVT
STLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFR
LRKGRMMMDVKKCGIQDTNSKKQSDTHLEETGGGGSGGGSGGGGSTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYT
QKSLSLSPGKGGGGSGGGSGGGGSALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYH
ANFCLGPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMI
VRSCKCS

Black: TGF β -1

Red: Linker (optional: any sequence)

Blue: Fc

Green Color: human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)

(Can replace linker 1 with EPKSCDK)

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

- II (B).** A molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:
- (a) said targeting moiety binds a specific target molecule, and
 - (b) said immunomodulatory moiety is a molecule that binds Transforming growth factor-beta receptor (TGF- β R).

Claims: 59-73
SEQ. ID.: 27-34

Said immunomodulatory moiety that binds Transforming growth factor-beta receptor (TGF- β R) may be the following:

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

TGF- β 1 full sequence

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

Green: Mature (active) TGF- β 1 sequence (Ala 279 – Ser 390; 112 aa) = underlined

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

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

Preferred embodiment – Used in SEQ. ID. 27-34

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

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

Protein Sequence:

Heavy chain:

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSV
GRFTISRDNKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGTLLVTVSSASTKGPSV
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
SLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAIEKTIKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGKGGG
SGGGSGGGGSALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSL
DTQYSKVLALYNQHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Black color: anti-TNF α heavy chain variable region

Blue color: IgG1 heavy chain constant region

Pink Color: Linker region

Green Color: Mature (active) human TGF- β ₁

(Note: Options – replace underlined KKA~~E~~ with KRVE or KKVE)

Light Chain:

DIQMTQSPSSLSASVGDRTITCRASQGIRNYLAWYQQLPKGAPKLLIYAASLTQSGVPSRFGS
GSGTDFTLTISLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC

Black color: anti-TNF α light chain variable region

Blue color: IgG1 light chain constant region

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

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

TNFR2 ECD- IgG Cy1- TGF- β 1

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLW
NWVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARP
GTETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLP
QPVSTRSQHTQPTPEPSTAPSTSFLPMGPSPPAEGSTGDEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSVHEALHNH
YTQKSLSLSPGKGGGGSGGGGSGGGGSALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKG
YHANFCLGPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSN
MIVRSCKCS

Black color: TNFR2 ligand-binding domain

Blue Color: human IgG1 heavy chain constant region (IgG Cy1 domain = H, CH2, CH3)

Pink Color: Linker region

Green Color: Mature (active) human TGF- β 1

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

TGF- β 1 - IgG Cy1 -TNFR2 ECD:

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALY
NQHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCSGGGGSGGGGSGGGGSEPK
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSVMSVHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSLPAQVAFTPYAPEPGS
TCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWNWVPECLSCGSRCSDD
QVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGTETSDVVCKPCAPGT
FSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPVSTRSQHTQPTPEP
STAPSTSFLPMGPSPPAEGSTGD

Green Color: Mature (active) human TGF- β 1

Blue Color: human IgG1 heavy chain constant region (IgG Cy1 domain = H, CH2, CH3)

Pink Color: Linker region (optional); any linker

Black color: TNFR2 ligand-binding domain

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

Protein Sequence:

Heavy chain sequence:

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFK
GKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
SLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGSALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPY
IWSLDTQYSKVLALYNQHNP GASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Blue color: anti-CD20 Heavy chain Variable region
Black color: anti-CD20 Heavy chain Constant region
Pink Color: Linker region
Green Color: Mature (active) human TGF- β

Light chain sequence:

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFAQKPGSSPKPWIYATSNLASGVFVRFSGSG
SGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYFREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

Blue color: anti-CD20 Light chain Variable region
Black color: anti-CD20 Light chain Constant region

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

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

(i)

anti-CD25 and TGF- β 1 - Heavy chain

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY
NQKFKGKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LTVSSASTK
GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
LSSVTVPPSS 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 GGGGSGGGGSGGGGSALDTNYCFSSTEKNCCVRQLY
IDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPL
PIVYYVGRKPKVEQLSNMIVRSCKCS

Black: anti-CD25 heavy chain

Red: Linker

Green Color: Mature (active) human TGF- β 1

anti-CD25 - Light chain

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

(ii)

anti-CD25 and TGF- β 1 - Heavy chain

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDIYGY YFDWVGQGT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVTVPPSSS LGTQTYICNV NHKPSNTKVD KRVEPPKSCD KTHTCPPCPA PELLGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK GGGGSGGGGSGGGGSALDTNYCFSSTEKNCCVRQLY
IDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPL
PIVYYVGRKPKVEQLSNMIVRSCKCS

Black: anti-CD25 heavy chain

Red: Linker

Green Color: Mature (active) human TGF- β 1

anti-CD25 – Light chain

QIVSTQSPAI MSASPGKVT MTCSASSSRS YMQWYQQKPG TSPKRWIYDT SKLASGVPAR
FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE

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

(i) Fusion protein: TGF- β 1, Fc, and IL-2

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALY
NQHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCSGGGGSGGGGSGGGGS
THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLTP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRW
QQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGS

(ii) Fusion protein: IL-2, Fc, and TGF- β 1

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIIS
TLTGGGGSGGGGSGGGGSTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLD
SDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGS
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALY
NQHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Black: IL2 fragment

Red: Linker (optional: any sequence)

Blue: Fc

Green Color: Mature (active) human TGF- β 1

(Can replace linker 1 with EPKSCDK)

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

Fusion proteins comprising CTLA-4-Ig and a sequence from transforming growth factor- β (TGF- β)

- 33. Fusion protein 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

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIAFVCEYASPGKATEVRVTVLRQ
ADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYL
GIGNGTQIYVIDPEPCPDSD (Q) EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
EVTCTVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGKGGGGS
GGGGSGGGGSALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLD
TQYSKVLALYNQHNPASAAAPCCVPQALEPLPIVYVGRKPKVEQLSNMIVRSCKCS

Black color: Oncostatin M Signal Peptide (-25 to -1)

Blue color: CTLA-4 extracellular domain (1-125)

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

Pink Color: Optional Linker region (any linker)

Green Color: Mature (active) human TGF- β 1

- 34. Fusion protein 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

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALY
NQHNPGASAAAPCCVPQALEPLPIVYVGRKPKVEQLSNMIVRSCKCSGGGGSGGGSGGGGSEPK
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVD
KSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGKGGGSGGGSGGGGSAMHVAQPAVVLASSRG
IASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQ
GLRAMDTGLYICKVELMYPPPYLIGIGNGTQIYVIDPEPCPDSD

Green Color: Mature (active) human TGF- β 1

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

Pink Color: Optional Linker region (any linker)

Blue color: CTLA-4 extracellular domain (1-125)

Electronic Acknowledgement Receipt

EFS ID:	9295048
Application Number:	61435671
International Application Number:	
Confirmation Number:	7162
Title of Invention:	TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	28213
Filer:	Lisa A. Haile/Cara Grifone
Filer Authorized By:	Lisa A. Haile
Attorney Docket Number:	JHU3260-3
Receipt Date:	24-JAN-2011
Filing Date:	
Time Stamp:	18:34:48
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 110
RAM confirmation Number	5655
Deposit Account	071896
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)	

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		JHU3260-3Provisional.pdf	7399213	yes	132
			b41195ebcc3522c0ac158cc8d9b10d252/8d8ff0		
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Transmittal of New Application		1	2	
	Application Data Sheet		3	4	
	Miscellaneous Incoming Letter		5	5	
	Specification		6	10	
	Claims		11	15	
	Appendix to the Specification		16	54	
	Appendix to the Specification		55	132	
Warnings:					
Information:					
2	Fee Worksheet (PTO-875)	fee-info.pdf	29564	no	2
			262ef09605c653a56c43ae3e4498e0e6a9c9ab77		
Warnings:					
Information:					
Total Files Size (in bytes):			7428777		

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.

**DOCUMENT MADE AVAILABLE UNDER THE
PATENT COOPERATION TREATY (PCT)**

International application number:	PCT/US2011/027317
International filing date:	04 March 2011 (04.03.2011)
Document type:	Certified copy of priority document
Document details:	Country/Office: US
	Number: 61/435,671
	Filing date: 24 January 2011 (24.01.2011)
Date of receipt at the International Bureau:	18 March 2011 (18.03.2011)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a),(b) or (b-bis)

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

PCT/US11/27317

International Application No.

04 MAR 2011 (04.03.11)

International Filing Date

RO/US

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum) JHU3260-4WO

Box No. I TITLE OF INVENTION	
COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	
Box No. II APPLICANT ☐ This person is also inventor	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	
THE JOHNS HOPKINS UNIVERSITY 3400 N. Charles Street Baltimore, Maryland 21218 United States of America	
Telephone No. (410) 516-8300	Facsimile No. (410) 516-4411
Applicant's registration No. with the Office	
E-mail authorization: Marking one of the check-boxes below authorizes the receiving Office, the International Searching Authority, the International Bureau and the International Preliminary Examining Authority to use the e-mail address indicated in this Box to send notifications issued in respect of this international application to that e-mail address if those offices are willing to do so. ☐ as advance copies followed by paper notifications; or ☐ exclusively in electronic form (no paper notifications will be sent). E-mail address:	
State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.	
Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE	
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)	
HAILE, Lisa A. DLA Piper LLP (US) 4365 Executive Drive, Suite 1100 San Diego, California 92121-2133 United States of America	
Telephone No. 858-677-1456	Facsimile No. 858-677-1465
Agent's registration No. with the Office 38,347	
E-mail authorization: Marking one of the check-boxes below authorizes the receiving Office, the International Searching Authority, the International Bureau and the International Preliminary Examining Authority to use the e-mail address indicated in this Box to send notifications issued in respect of this international application to that e-mail address if those offices are willing to do so. ☒ as advance copies followed by paper notifications; or ☐ exclusively in electronic form (no paper notifications will be sent). E-mail address: GTForeign@dlapiper.com	
☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.	

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

BEDI, Atul
2211 Dalewood Road
Timonium, Maryland 21093
United States of America

This person is:

- ☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

Applicant's registration No. with the Office

State (that is, country) of nationality:

US

State (that is, country) of residence:

US

This person is applicant for the purposes of:

- ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

RAVI, Rajani
7810 Ballston Road
Ruxton, Maryland 21204
United States of America

This person is:

- ☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

Applicant's registration No. with the Office

State (that is, country) of nationality:

US

State (that is, country) of residence:

US

This person is applicant for the purposes of:

- ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

- ☐ applicant only
☐ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

Applicant's registration No. with the Office

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of:

- ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

- ☐ applicant only
☐ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

Applicant's registration No. with the Office

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of:

- ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATIONS

The filing of this request **constitutes under Rule 4.9(a) the designation** of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.

However,

- ☐ DE Germany is **not designated** for any kind of national protection
- ☐ JP Japan is **not designated** for any kind of national protection
- ☐ KR Republic of Korea is **not designated** for any kind of national protection

(The check-boxes above may only be used to exclude (irrevocably) the designations concerned if, at the time of filing or subsequently under Rule 26bis.1, the international application contains in Box No. VI a priority claim to an earlier national application filed in the particular State concerned, in order to avoid the ceasing of the effect, under the national law, of this earlier national application.)

Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application: regional Office	international application: receiving Office
item (1) 05 March 2010 (05.03.2010)	61/311,255	US		
item (2) 24 January 2011 (24.01.2011)	61/435,671	US		
item (3)				

☐ Further priority claims are indicated in the Supplemental Box.

☐ The **International Bureau** is requested to obtain from a digital library, a certified copy of the earlier application(s) (if the earlier application(s) is available to it from a digital library) identified above as:*

☐ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ other, see Supplemental Box

☒ The **receiving Office** is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (if the earlier application(s) was filed with the Office which for the purposes of this international application is the receiving Office) or to obtain a certified copy of the earlier application(s) from a digital library and transmit a copy of it to the International Bureau (if the earlier application(s) is available to the receiving Office from a digital library), identified above as:*

☒ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ other, see Supplemental Box

* Where the certified copy of the earlier application(s) is not stored in a digital library under the number of the earlier application indicated above but under the application number of another application which also claims priority from it, indicate that number in the supplemental sheet (item 4).

Restore the right of priority: the receiving Office is requested to restore the right of priority for the earlier application(s) identified above or in the Supplemental Box as item(s) (_____). (See also the Notes to Box No. VI; further information **must** be provided to support a request to restore the right of priority.)

Incorporation by reference: where an element of the international application referred to in Article 11(1)(iii)(d) or (e) or a part of the description, claims or drawings referred to in Rule 20.5(a) is not otherwise contained in this international application but is completely contained in an earlier application whose priority is claimed on the date on which one or more elements referred to in Article 11(1)(iii) were first received by the receiving Office, that element or part is, subject to confirmation under Rule 20.6, incorporated by reference in this international application for the purposes of Rule 20.6.

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) (if more than one International Searching Authority is competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):

ISA/ KR

Box No. IX CHECK LIST for EFS-Web filings - this sheet is only to be used when filing an international application with RO/US via EFS-Web			
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<p>Figure of the drawings which should accompany the abstract: 53G</p>	<p>Language of filing of the international application: English</p>		

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Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

DLA Piper LLP (US)

Lisa Haile

Lisa A. Haile, J.D., Ph.D., Reg. No. 38,347

04-03-2011

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APPLICATION NUMBER: 61/311,255

FILING DATE: *March 05, 2010*

RELATED PCT APPLICATION NUMBER: *PCT/US11/27317*

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US61/311,255*



Certified by

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET – Page 1 of 2

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Electronically Filed, March 5, 2010

INVENTOR(S)		
Given Name (first and middle (if any))	Family Name or Surname	Residence (City and either State or Foreign Country)
Atul	Bedi	Timonium, Maryland
Additional inventors are being named on the _____ separately numbered sheets attached hereto		
TITLE OF THE INVENTION (500 characters max):		
TUMOR TARGETING COMPOSITIONS		
Direct all correspondence to: CORRESPONDENCE ADDRESS		
☒ The address corresponding to Customer Number: 021971		
OR		
☐ Firm or Individual Name		
Address		
City	State	Zip
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ENCLOSED APPLICATION PARTS (check all that apply)		
☐ Application Data Sheet. See 37 CFR 1.76		
☐ CD(s), Number of CDs _____		
☒ Drawing(s) Number of Sheets 7		
☐ Other (specify) _____		
☒ Specification (e.g., description of the invention) Number of Pages 33		
Fees Due: Filing Fee of \$220 (\$110 for small entity). If the specification and drawings exceed 100 sheets of paper, an application size fee is also due, which is \$270 (\$135 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).		
METHOD OF PAYMENT OF THE FILING FEE AND APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT		
☒ Applicant claims small entity status. See 37 CFR 1.27.		
☐ A check or money order is enclosed to cover the filing fee and application size fee (if applicable).		
☐ Payment by credit card. Form PTO-2038 is attached		
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Account Number: 23-2415 (35575-709.101)		
TOTAL FEE AMOUNT (\$)		
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PROVISIONAL APPLICATION COVER SHEET
Page 2 of 2

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SIGNATURE _____

Date March 5, 2010

TYPED or PRINTED NAME Angie S. Mah, Ph.D.

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Docket Number: 35575-709.101

PROVISIONAL PATENT APPLICATION
TUMOR TARGETING COMPOSITIONS

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TUMOR TARGETING COMPOSITIONS

BACKGROUND

[0001] 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

[0002] In one embodiment, a composition disclosed herein comprises an immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells. In one embodiment, the targeting moiety binds a component of a tumor cell, tumor cell surface molecule, tumor-associated cell, tumor microenvironment, tumor-associated immune cell, tumor antigen, tumor-associated antigen, tumor-antigen-containing fusion protein, or tumor vaccine. In another embodiment, the targeting moiety or moiety that inhibits the production or function of regulatory T cells binds a surface molecule on regulatory T cells. In one embodiment, the surface molecule is CD4, CD25, CTLA-4 or LAG-3. In another embodiment, the moiety that inhibits the production or function of regulatory T cells is a molecule that inhibits expression or function of STAT3. In yet another embodiment, the molecule that inhibits expression or function of STAT3 is a small interfering RNA. In one embodiment, the moiety that inhibits the production or function of regulatory T cells is a molecule that binds transforming growth factor β (TGF- β) or antagonizes the function of TGF- β . In one embodiment, the moiety that inhibits the production or function of regulatory T cells is a molecule that inhibits expression or function of Foxp3. In yet another embodiment, the molecule that inhibits expression or function of FOXP3 is a small interfering RNA. In one embodiment, the moiety that inhibits the production or function of regulatory T cells is a molecule that binds CTLA-4.

[0003] Also provided herein is a composition comprising a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β). In one embodiment, the tumor targeting moiety is conjugated or fused to said molecule that binds transforming growth factor beta (TGF- β). In another embodiment, the tumor targeting moiety is an antibody, antibody fragment, peptide, aptamer, ligand, or a combination thereof. In one embodiment, the tumor targeting moiety is an antibody or scFv. In yet another 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 or tumor antigen-containing fusion protein. In one embodiment, the tumor targeting moiety binds a growth factor receptor, growth factor, cytokine receptor, cytokine, or cell surface molecule. In another embodiment, the tumor targeting moiety binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), Insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, Vascular endothelial growth factor, CD20, CD30, CD33, CD40, or a combination thereof. In another embodiment, the tumor targeting moiety binds a regulatory T cell or a suppressor T cell. In yet another embodiment, the tumor targeting moiety binds CD4, CD25, CTLA-4, or a combination thereof. In one embodiment, the tumor targeting moiety is fused to the molecule that binds transforming growth factor beta (TGF- β), wherein said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence derived from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII). In one embodiment, the polypeptide sequence comprises SEQ ID NO. 1, 2 or 3.

[0004] In one embodiment, the tumor targeting moiety is an antibody or scFv that binds EGFR1. In yet another embodiment, the tumor targeting moiety is an antibody or scFv that binds CD20. In one embodiment, the tumor targeting moiety is an antibody or scFv that binds CD4. In another embodiment, the tumor targeting moiety is an antibody or scFv that binds CD25. In another embodiment, the tumor targeting moiety is an antibody or scFv that binds CTLA-4.

[0005] Also provided herein, is a composition as described herein, further comprising a tumor cell, tumor antigen, or tumor-associated antigen, immune cell, dendritic cell, or any combination thereof. In one embodiment, the composition further comprises a PAMP or DAMP. In another embodiment, the composition further comprises a tetanus antigen or a pDOM, or both. In yet another embodiment, the composition comprises a pDOM fused to a cationic polypeptide. In yet another embodiment, the composition further comprises a pDOM fused to arginine or histidine enriched polypeptide sequence. In one embodiment, the composition comprises a pDOM fused to polyarginine or polyhistidine polypeptide. In one embodiment, the pDOM fused to 1 to 20 arginines. In one embodiment, the pDOM is fused to 9 arginines. Also provided herein, is a composition as described herein, further comprising a chemotherapeutic agent. In one embodiment, the chemotherapeutic agent is doxorubicin.

[0006] In another embodiment, in a composition comprising a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β), the tumor targeting moiety is an antibody that binds to EGFR1. In another embodiment the tumor targeting moiety is cetuximab. In another embodiment, the tumor targeting moiety is antibody that binds to HER2/Neu. In one embodiment, the tumor targeting moiety is trastuzumab. In yet another embodiment, the tumor targeting moiety is antibody that binds to CD20. In one embodiment, the tumor targeting moiety is rituximab.

[0007] Also provided herein is a method of preventing or treating a neoplastic condition or disorder in a subject in need thereof comprising administering a composition as described herein. In another embodiment, the method of preventing or treating a neoplastic disorder in a subject in need thereof comprises administering an immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells. In yet another embodiment, a method of preventing or treating a neoplastic disorder in a subject in need thereof comprises administering a composition comprising a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β).

[0008] In one embodiment, a method of preventing or treating a neoplastic disorder in a subject in need thereof comprises administering a combination comprising: a cytotoxic anticancer agent and a moiety that reduces the number or function of a CD4⁺ regulatory T cell, wherein the cytotoxic anticancer agent is a chemotherapeutic agent, a kinase inhibitor, a tumor targeted antibody, or any combination thereof. In one embodiment, the neoplastic disorder is not a CD4-expressing T-cell malignancy. In another embodiment, the moiety that reduces the number or function of CD4⁺ regulatory T cells is an antibody or other moiety that binds CD4 or CD25.

[0009] In one embodiment, the method of preventing or treating a neoplastic disorder in a subject in need thereof further comprises administering to the subject an anticancer agent. In one embodiment, the anticancer agent is a chemotherapeutic agent, antibody, tyrosine kinase inhibitor, cytokine, targeted therapeutic agent, immunotherapeutic agent, hormonal therapeutic agent, or antiangiogenic agent. In yet another embodiment, the method of preventing or treating a neoplastic disorder in a subject in need thereof further comprises administering to the subject a vaccine, wherein the vaccine comprises a tumor cell, tumor antigen, tumor-associated antigen, dendritic cell, or any combination thereof. In yet another embodiment, the method of preventing or treating a neoplastic disorder in a subject in need thereof further comprises administering to the subject a PAMP or DAMP.

[0010] In one embodiment, the method of preventing or treating a neoplastic disorder in a subject in need thereof further comprises administering to the subject a tetanus antigen. In another embodiment, the method of preventing or treating a neoplastic disorder in a subject in need thereof further comprises administering to the subject pDOM. In one embodiment, the pDOM is fused to a cationic polypeptide. In yet another embodiment, the pDOM is fused to arginine or histidine enriched polypeptide sequence. In yet another embodiment, the pDOM is fused to polyarginine or polyhistidine polypeptide. pDOM fused to 1 to 20 arginines. In yet another embodiment, the pDOM fused to 9 arginines.

INCORPORATION BY REFERENCE

[0011] 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0013] **FIG. 1** illustrates immune cells play an instrumental role in the antitumor efficacy of cytotoxic anticancer agents.

[0014] **FIG. 2** illustrates regulatory T cells inhibit the antitumor efficacy of cytotoxic anticancer agents.

[0015] **FIG. 3** illustrates depletion of CD4+ T cells improves the efficacy of cytotoxic anticancer agents.

[0016] **FIG. 4** illustrates depletion of CD4+ T cells improves the efficacy of chemo-immunotherapy.

DETAILED DESCRIPTION

[0001] Before the present composition, methods, and methodologies are described, it is to be understood that the disclosure is not limited to particular compositions, methods, and experimental conditions described, as such compositions, methods, and conditions may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting.

[0002] 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 “a nucleic acid” includes one or more nucleic acids, and/or compositions of the type described herein which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0003] As used herein “immune effector cells” include T cells, NK cells, B cells, monocytes, macrophages, and dendritic cells (DC).

[0004] 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.

[0005] As used herein, “neoplasm,” 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, melanoma, sarcoma, liver, kidney, lung, testicular, breast, ovarian, pancreatic, brain, head and neck, melanoma, leukemia, lymphoma cancer, and the like.

[0006] 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.

[0007] As used herein “conjugation,” including grammatical variations thereof, means directly or indirectly linking, coupling, binding and the like of the foreign DNA or RNA with target-specific antibodies and/or peptides and/or tumor targeting moieties, either chemically, electrostatically, non-covalently, or by other techniques. For example, an isolated antibody-nucleic acid conjugate or peptide-nucleic acid conjugate as presently disclosed

would fall under this definition.

[0008] An “immunostimulatory nucleic acid sequence” (INAS) refers to a nucleic acid molecule that is a pathogen-associated molecular pattern (PAMP) or other motif that can activate immune cells, including, but not limited to, double stranded DNA (ds DNA), single stranded DNA (ss DNA), CpG DNA (CpG), herpes simplex virus (HSV) DNA, double stranded RNA (dsRNA), and single stranded RNA (ssRNA). In a related aspect, the INAS may be a coding or non-coding sequence. As illustrative examples, an INAS may be DNA or RNA.

[0009] 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.

[0010] 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.

[0011] 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. 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.

[0012] 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.

Targeting Moiety

[0013] Provided herein is a composition comprising a targeting moiety. Also provided herein is an immunoconjugate or fusion protein comprising a targeting moiety. In one embodiment, an immunoconjugate or

fusion protein comprises a targeting moiety and a moiety that inhibits the production or function of regulatory T cells. In another embodiment, a composition comprises a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β).

[0014] A targeting moiety refers to a molecule that has the ability to localize and bind to a molecule present on a cell or tissue, such as a normal cell or tissue, or a diseased cell or tissue, such as a cancer cell or tumor. In one embodiment, a targeting moiety can bind to a ligand (directly or indirectly), which is present on a cell. The targeting moiety can be an antibody, antibody fragment, polypeptide, peptide, aptamer, ligand, nucleic acid, or any combination thereof, that can bind a component of a target cell or molecule.

[0015] In one embodiment, the targeting moiety facilitates delivery of a biologically active agent to a target cell (e.g. via receptor-mediated endocytosis of antibodies binding target cell receptors). For example, a targeting moiety can facilitate delivery of the biologically active agent(s), immunogenic apoptotic material from antibody-bound tumor targets, or both, to an immune cell via interactions between their Fc and Fc receptors (on immune cells); this promotes internalization of nucleic acid via endocytosis and activation of endosomal pattern recognition receptors (e.g. Toll-like receptors).

[0016] In one embodiment, a targeting moiety is selected which is specific for a non-cancerous cell or tissue. For example, a targeting moiety can be specific for a molecule present normally on a particular cell or tissue. Furthermore, in some embodiments, the same molecule can be present on normal and cancer cells. Various cellular components and molecules are known. For example, if a targeting moiety is specific for EGFR, the resulting conjugate of the invention can target cancer cells expressing EGFR as well as normal skin epidermal cells expressing EGFR. Therefore, in some embodiments, a conjugate of the invention can operate by two separate mechanisms (targeting cancer and non-cancer cells), as further discussed herein.

[0017] In one embodiment, the targeting moiety binds a surface molecule on a regulatory T cell. In one embodiment, the surface molecule is CD4, CD25, CTLA-4 or LAG-3.

[0018] In another embodiment, the targeting moiety is a tumor targeting moiety. The tumor targeting moiety can bind 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.

[0019] The tumor targeting moiety can bind to a receptor or ligand on the surface of the cancer cell or may bind to an intracellular target of cancer cell provided that the target is accessible to the molecule. Accessibility to one or more intracellular cancer cell targets may arise in cancer cells that have a compromised plasma membrane such as cells which are undergoing apoptosis, necrosis, and the like. In one embodiment, a cancer targeting molecule can bind intracellular a portion of a cell that does not have a compromised plasma membrane.

[0020] In yet another embodiment, the targeting moiety, such as a tumor targeting moiety, binds a growth factor

receptor, growth factor, cytokine receptor, cytokine, or cell surface molecule. For example, the targeting moiety can bind epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), Insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, Vascular endothelial growth factor, CD20, CD30, CD33, CD40, or a combination thereof.

[0021] In another embodiment, the tumor targeting moiety binds a regulatory T cell or a suppressor T cell, such as CD4, CD25, CTLA-4, or a combination thereof.

[0022] For example, in various embodiments, a targeting moiety is specific for or binds to a 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), IGF ligand family (IGF-1R); platelet derived growth factor receptor (PDGFR) family, PDGFR ligand family; fibroblast growth factor receptor (FGFR) family, FGFR ligand family, vascular endothelial growth factor receptor (VEGFR) family, VEGF family; HGF receptor family; TRK receptor family; ephrin (EPH) receptor family; AXL receptor family; leukocyte tyrosine kinase (LTK) receptor family; TIE receptor family, angiopoietin 1,2; receptor tyrosine kinase-like orphan receptor (ROR) receptor family; discoidin domain receptor (DDR) family; RET receptor family; KLG receptor family; RYK receptor family; MuSK receptor family; Transforming growth factor alpha (TGF-alpha) receptors, TGF-beta; Cytokine receptors, Class I (hematopoietin family) and Class II (interferon/IL-10 family) receptors, tumor necrosis factor (TNF) receptor superfamily (TNFRSF), death receptor family; 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), HLA-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 I, NFYC, OGT, OS-9, pml-RARalpha fusion protein, PRDX5, PTPRK, K-ras (KRAS2), N-ras (NRAS), HRAS, RBAF600, SIRT2, SNRPD1, SYT-SSX1 or -SSX2 fusion protein, Triosephosphate Isomerase, BAGE, BAGE-1, BAGE-2,3,4,5, GAGE-1,2,3,4,5,6,7,8, GnT-V (aberrant N-acetyl glucosaminyl transferase V, MGAT5), HERV-K-MEL, KK-LC, KM-HN-1, LAGE, LAGE-1, CTL-recognized antigen on melanoma (CAMEL), MAGE-A1 (MAGE-1), MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, 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), EphA3, fibroblast growth factor-5 (FGF-5), glycoprotein 250 (gp250), EGFR (ERBB1), HER-2/neu (ERBB2), interleukin 13 receptor alpha2 chain (IL13Ralpha2), IL-6 receptor, intestinal carboxyl esterase (iCE), alpha-feto protein (AFP), M-CSF, mdm-2, MUC1, p53 (TP53), PBF, PRAME, 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, LIPI, CTAGE-1, CSAGE, MMA1, CAGE, BORIS, HOM-TES-85, AF15q14, HCA661, LDHC, MORC, SGY-1, SPO11, TPX1, NY-SAR-35, FTHL17, NXF2, TDRD1, TEX15, FATE, TPTE, immunoglobulin idiotypes, Bence-Jones protein, estrogen receptors (ER), androgen receptors (AR), CD40, CD30, CD20, CD19, CD33, 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, I-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), carcinoembryogenic antigen peptide-1 (CAP-1), calcium-activated chloride channel-2 (CLCA2), cyclophilin B (Cyp-B), human signet ring tumor-2 (HST-2), Human papilloma virus (HPV) proteins (HPV-E6, HPV-E7, major or minor capsid antigens, others), Epstein-Barr virus (EBV) proteins (EBV latent membrane proteins—LMP1, LMP2; others), Hepatitis B or C virus proteins, and HIV proteins. A conjugate can further comprise the foregoing as a peptide/polypeptide and/or encoding the same.

Antibodies

[0023] In one embodiment, the targeting moiety 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.

[0024] 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 comprising either a VL or VH domain. In one embodiment, the targeting moiety is an antibody or scFv.

[0025] An antigen-binding antibody fragment, including single-chain antibody, 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. An antigen-binding fragment can also comprise any combination of variable region(s) with a

hinge region, CH1, CH2, and CH3 domains. Also included is an 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.

[0026] 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 comprising variable domain antigen-binding sequences derived from a non-human primate (e.g., Old World Monkey, Ape etc) and human constant region sequences.

[0027] 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.

[0028] In one embodiment, a hybridoma can produce a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β). For example, a hybridoma can produce an antibody, such as an anti-EGFR or anti-HER2 antibody linked to a molecule that binds TGF- β , such as a polypeptide comprising SEQ ID NO. 1, 2, or 3.

[0029] An antibody fragment can comprise a portion of an intact antibody, e.g. comprising 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).

[0030] An intact antibody is one which comprises 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 or any other modified Fc (e.g., glycosylation or other engineered Fc).

[0031] 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), etc.

[0032] Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different “classes.” There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into “subclasses” (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called alpha, Δ , ϵ , γ , and μ respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0033] Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as cetuximab (chimeric monoclonal antibody to epidermal growth factor receptor EGFR), panitumumab (anti-EGFR), nimotuzumab (anti-EGFR), B8, Rituximab (chimeric murine/human anti-CD20 MAb); Herceptin, trastuzumab (anti-Her2 hMAb); Panorex™ (17-1A) (murine monoclonal antibody); Panorex @ (17-1A) (chimeric murine monoclonal antibody); IDEC-Y2B8 (murine, anti-CD20 MAb); BEC2 (anti-idiotypic MAb, mimics the GD epitope) (with BCG); Oncolym (Lym-1 monoclonal antibody); SMART M195 Ab, humanized 13' I LYM-1 (Oncolym), Ovarex (B43.13, anti-idiotypic mouse MAb); MDX-210 (humanized anti-HER-2 bispecific antibody); 3622W94 MAb that binds to EGP40 (17-1A) pancreatic carcinoma antigen on adenocarcinomas; Anti-VEGF, RhuMAb (Avastin; inhibits angiogenesis); Zenapax (SMART Anti-Tac (IL-2 receptor); SMART M195 Ab, humanized Ab, humanized); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-447 (humanized anti-EGF receptor bispecific antibody); 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 LL2 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.

[0034] In one aspect, an antibody or fragment thereof (e.g., scFv) is coupled, conjugated, linked or fused, either directly or via a linker, to a molecule that binds TGF- β , such as a polypeptide sequence derived from a TGF- β receptor. On one embodiment, the antibody or scFv fused or coupled to the molecule that binds TGF- β can be an antibody or scFv that binds HER2/neu, CD20, CD4, CD25, or CTLA-4. The linker can be an amino acid linker. In one embodiment, a linker is GGGGSGGGGSGGGGS (SEQ ID NO: 31). In another embodiment, a linker is GGGGS (SEQ ID NO: 32). In one embodiment, the TGF- β receptor is TGF- β receptor type II (TGF- β RII), or a

variant thereof. In another embodiment, the TGF- β receptor is TGF- β receptor type III (TGF- β RIII), or a variant thereof. The polypeptide derived from a TGF- β receptor can be any portion of the receptor. In one embodiment, the polypeptide is derived from the extracellular domain of a TGF- β receptor, such as the extracellular domain of TGF- β RII or TGF- β RIII, or a variant thereof. In one embodiment, the polypeptide sequence comprises SEQ. ID. NO: 1. In another embodiment, the polypeptide sequence comprises SEQ. ID. NO: 2. In another embodiment, the polypeptide sequence comprises SEQ. ID. 3.

[0035] In one embodiment, an antibody or fragment thereof is linked or fused to a polypeptide that binds TGF- β , with a linker or without a linker. In one embodiment, the conjugate comprises Cetuximab heavy chain linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Cetuximab heavy chain linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Cetuximab light chain linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Cetuximab light chain linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Cetuximab scFv linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Cetuximab scFv linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Trastuzumab heavy chain linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Trastuzumab heavy chain linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Trastuzumab light chain linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Trastuzumab light chain linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Trastuzumab scFv linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Trastuzumab scFv linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Rituximab heavy chain linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Rituximab heavy chain linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Rituximab light chain linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Rituximab light chain linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Rituximab scFv linked or fused to TGF β -RII ECD. In another embodiment, the conjugate comprises Rituximab scFv linked or fused to TGF β -RII ECD.

[0036] In another embodiment, the conjugate comprises Cetuximab heavy chain linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Cetuximab heavy chain linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Cetuximab light chain linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Cetuximab light chain linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Cetuximab scFv linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Cetuximab scFv linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Trastuzumab heavy chain linked or fused to TGF β -RIII ECD. In another embodiment, the conjugate comprises Trastuzumab heavy chain linked or fused to TGF β -RIII ECD. In another

embodiment, the conjugate comprises Trastuzumab light chain linked or fused to TGFβ-RIII ECD. In another embodiment, the conjugate comprises Trastuzumab light chain linked or fused to TGFβ-RIII ECD. In another embodiment, the conjugate comprises Trastuzumab scFv linked or fused to TGFβ-RIII ECD. In another embodiment, the conjugate comprises Trastuzumab scFv linked or fused to TGFβ-RIII ECD. In another embodiment, the conjugate comprises Rituximab heavy chain linked or fused to TGFβ-RIII ECD. In another embodiment, the conjugate comprises Rituximab heavy chain linked or fused to TGFβ-RIII ECD. In another embodiment, the conjugate comprises Rituximab light chain linked or fused to TGFβ-RIII ECD. In another embodiment, the conjugate comprises Rituximab light chain linked or fused to TGFβ-RIII ECD. In another embodiment, the conjugate comprises Rituximab scFv linked or fused to TGFβ-RIII ECD. In another embodiment, the conjugate comprises Rituximab scFv linked or fused to TGFβ-RIII ECD.

Aptamers

[0037] In one aspect of the invention, the targeting moiety is an aptamer. For example, in some embodiments, the aptamer is comprised of a nucleic acid that functions as a targeting moiety, which is coupled to a molecule that binds transforming growth factor beta (TGF-β), such as a polypeptide sequence derived from a TGF-β receptor. In one embodiment, the TGF-β receptor is TGF-β receptor type II (TGF-β RII), or a variant thereof. In another embodiment, the TGF-β receptor is TGF-β receptor type III (TGF-β RIII), or a variant thereof. The polypeptide derived from a TGF-β receptor can be any portion of the receptor. In one embodiment, the polypeptide is derived from the extracellular domain of a TGF-β receptor, such as the extracellular domain of TGF-β RII or TGF-β RIII, or a variant thereof. In one embodiment, the polypeptide sequence comprises SEQ. ID. NO: 1. In another embodiment, the polypeptide sequence comprises SEQ. ID. NO: 2. In another embodiment, the polypeptide sequence comprises SEQ. ID. 3.

[0038] 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 or 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.

[0039] 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-methylg and g (Haller and Samow, Proc. Natl. Acad. Sci. USA 94:8521-8526 (1997))

or between D and L-tryptophan (supra, Gold et al.).

[0040] 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.

Peptides

[0041] In some aspects of the invention the targeting moiety is a peptide or polypeptide. For example, in some embodiments, the targeting polypeptide is coupled to a molecule that binds transforming growth factor beta (TGF- β), such as a polypeptide sequence derived from a TGF- β receptor. In one embodiment, the TGF- β receptor is TGF- β receptor type II (TGF- β RII), or a variant thereof. In another embodiment, the TGF- β receptor is TGF- β receptor type III (TGF- β RIII), or a variant thereof. The polypeptide derived from a TGF- β receptor can be any portion of the receptor. In one embodiment, the polypeptide is derived from the extracellular domain of a TGF- β receptor, such as the extracellular domain of TGF- β RII or TGF- β RIII, or a variant thereof. In one embodiment, the polypeptide sequence comprises SEQ. ID. NO: 1. In another embodiment, the polypeptide sequence comprises SEQ. ID. NO: 2. In another embodiment, the polypeptide sequence comprises SEQ. ID. NO: 3.

[0042] 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, an alphavbeta3 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 an alphavbeta3 peptide in one or more of the assays.

[0043] 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 alphavbeta3 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.

[0044] 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.

[0045] 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.

[0046] 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.

Inhibition of Regulatory T Cell Function or Production

[0047] Also provided herein is a moiety that reduces the number or function of a CD4⁺ regulatory T cell. The moiety that reduces the number or function of a CD4⁺ regulatory T cell can be in a composition with a cytotoxic anticancer agent. The cytotoxic anticancer agent can be a chemotherapeutic agent, a kinase inhibitor, a tumor targeted antibody, or any combination thereof.

[0048] Without being bound by theory, immune cells can play a role in the antitumor efficacy of cytotoxic anticancer agents (chemotherapeutic agents, tumor targeted antibodies). Activated NK cells and antigen-specific CD4⁺ helper T cells and CD8⁺ cytotoxic T cells can induce or promote death of tumor cells via cytolytic products: Death ligands (TRAIL, FasL) which engage death receptors (TRAIL-R1, TRAIL-R2, Fas/CD95), granule proteases (Granzyme A, Granzyme B), Perforin, and Interferon- γ , which are believed to be repressed by TGF- β . Immune cells can cooperate with cytotoxic anticancer agents or tumor cell-targeted antibodies to induce tumor cell death: (i) Immune cell-mediated 'extrinsic' death signals cooperate with chemotherapeutic agent-induced 'intrinsic' death signals to induce tumor cell death; (ii) Immune cells promote antibody dependent cellular cytotoxicity. Depletion of CD8⁺ T cells can impair the *in vivo* response of tumors to cytotoxic anticancer agents (chemotherapeutic agents). Depletion of NK cells can also impair ADCC of tumor cells by tumor targeted antibodies.

[0049] Without being bound by theory, regulatory T cells can inhibit the antitumor efficacy of cytotoxic anticancer agents (chemotherapeutic agents, tumor targeted antibodies). Naïve CD4⁺T cells may differentiate into helper T cells (T_H1, T_H2) or regulatory T cells (T_{regs}). CD4⁺ T helper cells have a role in the induction and maintenance of immune responses and memory. CD4⁺ T cell help is typically used for priming and secondary expansion of CD8⁺ T cells. However, tumor cells can actively suppress immune responses by producing immunosuppressive cytokines (such as TGF- β) and increasing the number or function of regulatory T cells (CD4⁺ CD25⁺ Foxp3⁺). TGF- β can promote the number and function of regulatory T cells, and inhibit the cytotoxic ability of CD8⁺ T cells and NK cells. Regulatory T cells can express TGF- β and inhibit the generation or activation of CD4⁺ T helper cells or CD8⁺ cytotoxic T cells.

[0050] In one embodiment, the moiety that reduces the number or function of a CD4⁺ regulatory T cell is fused

or conjugated to a cytotoxic anticancer agent. In another embodiment, the moiety that reduces the number or function of a CD4⁺ regulatory T cell is fused to a targeting moiety.

[0051] In one embodiment, the moiety that reduces the number or function of a CD4⁺ regulatory T cell binds a surface molecule on a regulatory T cell. In one embodiment, the surface molecule is CD4, CD25, CTLA-4 or LAG-3. In one embodiment, the moiety that reduces the number or function of CD4⁺ regulatory T cells is an antibody or other moiety that binds CD4 or CD25. In another embodiment, the moiety that inhibits the production or function of regulatory T cells is a molecule that inhibits or antagonizes the expression or function of STAT3, Foxp3, CTLA-4, or TGF- β . In one embodiment, the molecule that inhibits or antagonizes the expression or function of STAT3, Foxp3, CTLA-4, or TGF- β is a small interfering RNA.

Compositions

[0052] Also provided herein is a composition comprising a cytotoxic anticancer agent and a moiety that reduces the number or function of a CD4⁺ regulatory T cell, wherein said cytotoxic anticancer agent is a chemotherapeutic agent, a kinase inhibitor, a tumor targeted antibody, or any combination thereof. In another embodiment, a composition comprises an immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells.

[0053] In yet another embodiment, a composition comprises a tumor targeting moiety and a molecule that binds TGF- β . In one embodiment, the targeting moiety is a tumor targeting moiety and the molecule that binds TGF- β is a polypeptide sequence derived from a TGF- β receptor. In one embodiment, the TGF- β receptor is TGF- β receptor type II (TGF- β RII), or a variant thereof. In another embodiment, the TGF- β receptor is TGF- β receptor type III (TGF- β RIII), or a variant thereof. The polypeptide derived from a TGF- β receptor can be any portion of the receptor. In one embodiment, the polypeptide is derived from the extracellular domain of a TGF- β receptor, such as the extracellular domain of TGF- β RII or TGF- β RIII, or a variant thereof. In one embodiment, the polypeptide sequence comprises SEQ. ID. NO: 1. In another embodiment, the polypeptide sequence comprises SEQ. ID. NO: 2. In another embodiment, the polypeptide sequence comprises SEQ. ID. 3.

[0054] The one or more compositions disclosed herein can further comprise a cytotoxic anticancer agent. In one embodiment, a cytotoxic anticancer agent can induce ‘immunogenic’ death of tumor cells. An anticancer agent can induce cellular stress or damage of tumor cells. Damaged tumor cells release tumor cell antigens and endogenous “danger signals” termed a “Damage-associated molecular patterns” (DAMPs/Alarmins). DAMPs/Alarmins alert the immune system by the following mechanisms: (i) Promoting uptake and cross-presentation of antigens from tumor cells by antigen presenting cells (APC) or dendritic cells (DCs); (ii) Inducing activation or maturation of DCs, leading to upregulation of co-stimulatory molecules and cytokines which promote activation of innate and adaptive immune responses: (i) Activation of Natural Killer (NK) Cells; and (ii) Activation of antigen-specific CD4⁺ T cells and CD8⁺ T cells.

[0055] In another embodiment, the one or more compositions disclosed herein can further comprise a

chemotherapeutic agent. The chemotherapeutic agent can be doxorubicin. In another embodiment, the chemotherapeutic agent is a topoisomerase-interacting agent, anthracycline, mitoxantrone, camptothecin, camptothecin analogue, epipodophyllotoxin, etoposide, alkylating agent, cyclophosphamide, cisplatin, cisplatin analogue, antimetabolite, fluoropyrimidine analogue, 5-fluorouracil, gemcitabine, azacytidine, antimicrotubule agent, taxane, paclitaxel, or docetaxel.

[0056] In one embodiment, the anticancer cytotoxic agent can be fused or conjugated to a moiety that reduces the number or function of a CD4⁺ regulatory T cell, an immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells, or a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β).

[0057] In another embodiment, the one or more compositions disclosed herein can further comprise a tumor cell, tumor antigen, or tumor-associated antigen, immune cell, dendritic cell, or any combination thereof. In yet another embodiment, the one or more compositions disclosed herein can further comprise a tetanus antigen. In yet another embodiment, the one or more compositions disclosed herein further comprises a pDOM. In one embodiment, the pDOM is fused to a cationic polypeptide. In another embodiment, the pDOM is fused to an arginine or histidine enriched polypeptide sequence. In yet another embodiment, the pDOM is fused to polyarginine or polyhistidine polypeptide. In one embodiment, the pDOM is fused to 1 to 20 arginines, such as to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 arginines.

[0058] Also provided herein is a method of preventing or treating a neoplastic disorder in a subject in need thereof comprising administering a compositions disclosed herein. In one embodiment, a cytotoxic anticancer agent and a moiety that reduces the number or function of a CD4⁺ regulatory T cell, wherein said cytotoxic anticancer agent is a chemotherapeutic agent, a kinase inhibitor, a tumor targeted antibody, or any combination thereof, is administered to the subject. In another embodiment, a composition comprising an immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells is administered to the subject. In yet another embodiment, a composition comprises a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β) is administered to the subject. In one embodiment, the neoplastic disorder is not a CD4-expressing T-cell malignancy.

[0059] In one embodiment, the method comprises administering a composition comprising a targeting moiety, which is coupled to a molecule that binds transforming growth factor beta (TGF- β), such as a polypeptide sequence derived from a TGF- β receptor. In one embodiment, the TGF- β receptor is TGF- β receptor type II (TGF- β RII), or a variant thereof. In another embodiment, the TGF- β receptor is TGF- β receptor type III (TGF- β RIII), or a variant thereof. The polypeptide derived from a TGF- β receptor can be any portion of the receptor. In one embodiment, the polypeptide is derived from the extracellular domain of a TGF- β receptor, such as the extracellular domain of TGF- β RII or TGF- β RIII, or a variant thereof. In one embodiment, the polypeptide sequence comprises SEQ. ID. NO: 1. In another embodiment, the polypeptide sequence comprises SEQ. ID. NO:

2. In another embodiment, the polypeptide sequence comprises SEQ. ID. 3.

[0060] In one embodiment, the method further comprises administering to the subject an anticancer agent, wherein said anticancer agent is a chemotherapeutic agent, antibody, tyrosine kinase inhibitor, cytokine, targeted therapeutic agent, immunotherapeutic agent, hormonal therapeutic agent, or antiangiogenic agent. The chemotherapeutic agent can be doxorubicin. In another embodiment, the chemotherapeutic agent is a topoisomerase-interacting agent, anthracycline, mitoxantrone, camptothecin, camptothecin analogue, epipodophyllotoxin, etoposide, alkylating agent, cyclophosphamide, cisplatin, cisplatin analogue, antimetabolite, fluoropyrimidine analogue, 5-fluorouracil, gemcitabine, azacytidine, antimicrotubule agent, taxane, paclitaxel, or docetaxel.

[0061] In another embodiment, the method further comprises administering to the subject a vaccine, wherein the vaccine comprises a tumor cell, tumor antigen, tumor-associated antigen, dendritic cell, or any combination thereof. In another embodiment, the method further comprises administering to the subject a PAMP or DAMP.

[0062] In another embodiment, the method further comprises administering to the subject a tetanus antigen. In yet another embodiment, In another embodiment, the method further comprises administering to the subject a pDOM. In one embodiment, the pDOM is fused to a cationic polypeptide. In another embodiment, the pDOM is fused to an arginine or histidine enriched polypeptide sequence. In yet another embodiment, the pDOM fused to polyarginine or polyhistidine polypeptide. In one embodiment, the pDOM is fused to 1 to 20 arginines, such as to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 arginines.

[0063] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

EXAMPLES

[0064] Example 1: Depletion of CD8⁺ T cells impairs the *in vivo* response of tumors to cytotoxic anticancer agents.

[0065] To determine whether CD4⁺ T cells help or inhibit the antitumor efficacy of cytotoxic anticancer agents *in vivo*, immunocompetent mice bearing syngeneic tumors were treated with cytotoxic anticancer agents with or without administration of an anti-CD4 antibody that depletes CD4⁺ T cells *in vivo*. Our studies indicate that the depletion of CD4⁺ regulatory T cells with an antibody targeting CD4 augments the response of tumors to cytotoxic chemotherapy or tumor-targeted antibodies. Improvement of the antitumor response to chemotherapy by depletion of CD4⁺ T cells is mediated via CD8⁺ T cells since combined depletion of CD8⁺ T cells and CD4⁺ T

cells results in loss of the antitumor effect of chemotherapy.

[0066] BALB/c mice were injected subcutaneously with syngeneic CT-26 mouse colon cancer cells (2×10^5). CT26 tumor-bearing BALB/c mice were treated with monoclonal antibodies to deplete specific lymphocyte sub-populations *in vivo* [anti-CD4 antibody (GK1.5), anti-CD8 antibody (GK2.43), and/or anti-NK cell antibody (asialo-GM)], and then treated with irinotecan (i.p. 50 mg/kg weekly x 3 weeks) or left untreated (**FIG. 1**). Analysis of tumor volume indicated that the antitumor effect of irinotecan was significantly impaired in animals depleted of NK cells and CD8⁺ T cells; in mice depleted of CD8⁺ T cells, tumors failed to respond to a dose of irinotecan that inhibited tumor growth in immunocompetent animals. These data indicate that the cellular immune system (CD8⁺ T cells) is instrumental in promoting the antitumor effects of chemotherapy *in vivo*.

[0067] Example 2: Depletion of CD4⁺ T cells improves the response of colorectal cancers to cytotoxic anticancer agents.

[0068] BALB/c mice bearing syngeneic CT-26 mouse colon cancer cells (2×10^5) were treated with irinotecan (i.p. 50 mg/kg weekly x 3 weeks) with or without administration of antibodies to deplete specific lymphocyte sub-populations *in vivo* [anti-CD4 antibody (GK1.5) and/or anti-CD8 antibody (GK2.43)]. Depletion of CD4⁺CD25⁺Foxp3⁺ regulatory T cells with the anti-CD4 antibody (confirmed by flow cytometry-**FIG. 2C**) improved the response of tumors to cytotoxic chemotherapy (**FIG. 2 A, B**). Improvement of the antitumor response to chemotherapy by depletion of CD4⁺ T cells was mediated via CD8⁺ T cells since combined depletion of CD8⁺ T cells and CD4⁺ T cells resulted in loss of the antitumor effect of chemotherapy (**A, B**).

[0069] Example 3: Depletion of CD4⁺ T cells improves the response of urothelial cancers to cytotoxic anticancer agents.

[0070] BALB/c mice bearing syngeneic MB49 mouse bladder cancer cells (5×10^5) were treated with cisplatin (i.p. 5 mg/kg weekly x 4) with or without administration of antibodies to deplete specific lymphocyte sub-populations *in vivo* [anti-CD4 antibody (GK1.5) and/or anti-CD8 antibody (GK2.43)]. Whereas depletion of CD8⁺ T cells resulted in loss of the efficacy of chemotherapy (**FIG. 3 A, B**), depletion of CD4⁺CD25⁺Foxp3⁺ regulatory T cells with the anti-CD4 antibody (confirmed by flow cytometry - **FIG. 3 C**) improved the antitumor efficacy of chemotherapy.

[0071] Example 4: Depletion of CD4⁺ regulatory T cells improves the response of urothelial cancers to chemo-immunotherapy.

[0072] BALB/c mice bearing syngeneic MB49 mouse bladder cancer cells (5×10^5) were treated with Doxorubicin (intratumoral 7.5 mg/kg weekly x 3), a cell permeable pathogen antigen-containing fusion protein (Arg9-pDOM)(intratumoral 10 μ g/dose weekly x3), or a combination of doxorubicin and Arg9-pDOM with or without administration of antibodies to deplete specific lymphocyte sub-populations *in vivo* [anti-CD4 antibody (GK1.5) and/or anti-CD8 antibody (GK2.43)]. Whereas depletion of CD8⁺ T cells resulted in loss of the antitumor efficacy of chemo-immunotherapy (**FIG. 4 A, B**), depletion of CD4⁺CD25⁺Foxp3⁺ regulatory T cells with the anti-CD4 antibody (confirmed by flow cytometry - **FIG. 4 D**) increased the percentage of CD8⁺ T cells

expressing interferon- γ (**FIG. 4 C**) and improved the response of tumors to chemimmunotherapy. These data indicate that strategies to decrease the number or function of CD4⁺ regulatory T cells 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 chemo-immunotherapy (combination of chemotherapeutic agent with immunotherapeutic agents).

[0073] Example 5: A fusion protein comprising a tumor targeting antibody and a sequence from the extracellular domain of TGFBR2

[0074] A targeting moiety, such as an antibody, is coupled to a polypeptide derived from the extracellular domain of TGFBR2. The polypeptide sequence is selected from **Table 1**. The antibody, or a portion of antibody utilized for coupling with the polypeptide derived from TGFBR2 comprises sequences selected from **Table 2**. Examples of a fusion protein comprising a tumor targeting antibody and a sequence from the extracellular domain of TGFBR2 are shown in **Table 3**.

[0075] Table 1: Polypeptide Sequences Derived from the Extracellular Domain of TGF- β

SEQ. ID.	Sequence
1	MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDL LLVIFQ
2	TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD
3	PQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD

[0076] Table 2: Antibody Sequences for Coupling with A Polypeptide Derived from TGF- β RII

SEQ. ID.	Sequence
4	QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKLEWLGVIWSGGNTD YNTPTFSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTVT VSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
5	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRITNGSPRLLIKYASESISGIPSRFS

	GSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
6	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRNGSPRLIKYASESISGIPSRFS GSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKGGGGSGGGGSGGGG SQVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGNT DYNTPTFSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGTLV TVSAGGGGS
7	EVQLVESGGGLVQPGGSLRLSCAASGFTNIKDTYIHWRQAPGKGLEWVARIYPTNGYT RYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF LYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
8	DIQMTQSPSSLSASVGDRVITICRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVP SRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYYTPPTFGQGTKVEIKRTVAAPSVFIFPPS DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTL TLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
9	DIQMTQSPSSLSASVGDRVITICRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVP SRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYYTPPTFGQGTKVEIKGGGGSGGGGSGG GGSEVQLVESGGGLVQPGGSLRLSCAASGFTNIKDTYIHWRQAPGKGLEWVARIYPTN GYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWG QGT LTVSSGGGGGS
10	QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGD TSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAG TTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
11	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWYQQKPGSSPKPWYATSNLASGVPVR

	FSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
12	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFAQKPGSSPKPWYATSNLASGVPVR FSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKGGGSGGGSGGG GSQVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGN GDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWG AGTTVTVSAGGGGS

[0077] **Table 3. Examples of a Fusion Protein.**

SEQ. ID.	Sequence
13	QVQLKQSGPGLVQPSSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSGGNT DYNTPTFSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGT VTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCP APPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSTIPPHVQKSV NNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAVWR KNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNIIF SEEYNTSNPD
14	QVQLKQSGPGLVQPSSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSGGNT DYNTPTFSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGT VTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCP APPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSPQLCKFCDFR FSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAA APKCMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
15	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQRTNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRVAAPSVFIFPPSD

	EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTL TLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSTIPPHVQKSVNNDMIVT DNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITL ETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSN PD
16	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRRTNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTL TLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSPQLCKFCDFRSTCDNQ KSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIM KEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
17	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRRTNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKGGGGSGGGGSGGG GSQVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWISGG NTDYNTPTFSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQG TLVTVSAGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSC MSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEK KKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
18	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRRTNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKGGGGSGGGGSGGG GSQVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWISGG NTDYNTPTFSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQG TLVTVSAGGGGSPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDEN ITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEY NTSNPD
19	EVQLVESGGGLVQPGGSLRLSCAASGFINIKDTYIHWRQAPGKGLEWVARIYPTNGY TRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQ GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLD SDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSTIPPH VQKSVNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAV

	AVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
20	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYITLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
21	DIQMTQSPSSLSASVGDRVITTCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
22	DIQMTQSPSSLSASVGDRVITTCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
23	DIQMTQSPSSLSASVGDRVITTCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGGGSGGGGS GGGGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVSSGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
24	DIQMTQSPSSLSASVGDRVITTCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGGGSGGGGS

	GGGGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIY PTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMD YWGQGTLLTVSSGGGGSPQLCKFCDFRFSTCDNQKSCMSNCSITSICEKPQEVCAV WRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECND NIIFSEYNTSNPD
25	QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNG DTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWG AGTTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSTIPP HVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRFSTCDNQKSCMSNCSITSICEKPQEVC VAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDE CNDNIIFSEYNTSNPD
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28	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWYATSNLASGVPVR

	FSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPS DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSST LTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSPQLCKFCDFRSTCDN QKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCI MKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
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30	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVR FSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKGGGGSGGGSGG GGSQVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYP GNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFN VWGAGTTVTVSAGGGGSPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAV WRKNDENITLETVCHDPKLPYHDFILEDAAAPKCI MKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

[0017] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of preventing or treating a neoplastic disorder in a subject in need thereof comprising administering a combination comprising:
 - a. a cytotoxic anticancer agent and
 - b. a moiety that reduces the number or function of a CD4⁺ regulatory T cell,wherein said cytotoxic anticancer agent is a chemotherapeutic agent, a kinase inhibitor, a tumor targeted antibody, or any combination thereof.
2. The method of claim 1, wherein said neoplastic disorder is not a CD4-expressing T-cell malignancy.
3. The method of claim 1, wherein said moiety that reduces the number or function of CD4⁺ regulatory T cells is an antibody or other moiety that binds CD4 or CD25.
4. A method of preventing or treating a neoplastic disorder in a subject in need thereof comprising administering an immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells.
5. A method of preventing or treating a neoplastic disorder in a subject in need thereof comprising administering a composition comprising a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β).
6. The method of claims 1, 4 or 5, further comprising administering to said subject an anticancer agent, wherein said anticancer agent is a chemotherapeutic agent, antibody, tyrosine kinase inhibitor, cytokine, targeted therapeutic agent, immunotherapeutic agent, hormonal therapeutic agent, or antiangiogenic agent.
7. A method of claims 1 or 6, further comprising administering to a subject a vaccine, wherein said vaccine comprises a tumor cell, tumor antigen, tumor-associated antigen, dendritic cell, or any combination thereof.
8. The method of claims 1, 4 or 5, further comprising administering to said subject a PAMP or a DAMP.
9. The method of claims 1, 4 or 5, further comprising administering to said subject a tetanus antigen.
10. The method of claims 1, 4 or 5, further comprising administering to said subject pDOM.

11. The method of claims 1, 4 or 5, further comprising administering to said subject pDOM fused to a cationic polypeptide..
12. The method of claims 1, 4 or 5, further comprising administering to said subject pDOM fused to arginine or histidine enriched polypeptide sequence.
13. The method of claims 1, 4 or 5, further comprising administering to said subject pDOM fused to polyarginine or polyhistidine polypeptide.
14. The method of claims 1, 4 or 5, further comprising administering to said subject pDOM fused to 1 to 20 arginines.
15. The method of claims 1, 4 or 5, further comprising administering to said subject pDOM fused to 9 arginines.
16. An immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells.
17. The immunoconjugate or fusion protein of claim 16, wherein said targeting moiety binds a component of a tumor cell, tumor cell surface molecule, tumor-associated cell, tumor microenvironment, tumor-associated immune cell, tumor antigen, tumor-associated antigen, tumor-antigen-containing fusion protein, or tumor vaccine.
18. The immunoconjugate or fusion protein of claim 16, wherein said targeting moiety or moiety that inhibits the production or function of regulatory T cells binds a surface molecule on regulatory T cells.
19. The immunoconjugate or fusion protein of claim 18, wherein said surface molecule is CD4, CD25, CTLA-4 or LAG-3.
20. The immunoconjugate or fusion protein of claim 16, wherein said moiety that inhibits the production or function of regulatory T cells is a molecule that inhibits expression or function of STAT3.
21. The immunoconjugate or fusion protein of claim 20, wherein said molecule that inhibits expression or function of STAT3 is a small interfering RNA.

22. The immunoconjugate or fusion protein of claim 16, wherein said moiety that inhibits the production or function of regulatory T cells is a molecule that binds Transforming growth factor β (TGF- β) or antagonizes the function of TGF- β .
23. The immunoconjugate or fusion protein of claim 16, wherein said moiety that inhibits the production or function of regulatory T cells is a molecule that inhibits expression or function of Foxp3.
24. The immunoconjugate or fusion protein of claim 23, wherein said molecule that inhibits expression or function of FOXP3 is a small interfering RNA.
25. The immunoconjugate or fusion protein of claim 16, wherein said moiety that inhibits the production or function of regulatory T cells is a molecule that binds CTLA-4.
26. A composition comprising a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β).
27. The composition of claim 26, wherein said tumor targeting moiety is conjugated or fused to said molecule that binds transforming growth factor beta (TGF- β).
28. The composition of claim 26, wherein said tumor targeting moiety is an antibody, antibody fragment, peptide, aptamer, ligand, or a combination thereof.
29. The composition of claim 26, wherein said tumor targeting moiety is an antibody or scFv.
30. The composition of claim 26, wherein said 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 or tumor antigen-containing fusion protein.
31. The composition of claim 26, wherein said tumor targeting moiety binds a growth factor receptor, growth factor, cytokine receptor, cytokine, or cell surface molecule.
32. The composition of claim 26, wherein said tumor targeting moiety binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), Insulin-like growth factor receptor (IGF-1R), TRAIL-receptor,

epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, Vascular endothelial growth factor, CD20, CD30, CD33, CD40, or a combination thereof.

33. The composition of claim 26, wherein said tumor targeting moiety binds a regulatory T cell or a suppressor T cell.
34. The composition of claim 33, wherein said tumor targeting moiety binds CD4, CD25, CTLA-4, or a combination thereof.
35. The composition of claim 27, wherein said tumor targeting moiety is fused to said molecule that binds transforming growth factor beta (TGF- β), wherein said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence derived from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII).
36. The composition of claim 35, wherein said polypeptide sequence comprises SEQ ID NO: 1
37. The composition of claim 35, wherein said polypeptide sequence comprises: SEQ ID NO: 2.
38. The composition of claim 35, wherein said polypeptide sequence comprises: SEQ ID NO: 3
39. The composition of claim 26, wherein said tumor targeting moiety is an antibody or scFv that binds EGFR1 and said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII).
40. The composition of claim 26, wherein said tumor targeting moiety is an antibody or scFv that binds HER2/neu and said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII).
41. The composition of claim 26, wherein said tumor targeting moiety is an antibody or scFv that binds CD20

and said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII).

42. The composition of claim 26, wherein said tumor targeting moiety is an antibody or scFv that binds CD4 and said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII).
43. The composition of claim 26, wherein said tumor targeting moiety is an antibody or scFv that binds CD25 and said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII).
44. The composition of claim 26, wherein said tumor targeting moiety is an antibody or scFv that binds CTLA-4 and said molecule that binds transforming growth factor beta (TGF- β) comprises a polypeptide sequence from the extracellular domain of transforming growth factor beta receptor type I (TGF- β RI), transforming growth factor beta receptor type II (TGF- β RII) or transforming growth factor beta receptor type III (TGF- β RIII).
45. A composition comprising the immunoconjugate of claim 16.
46. The composition of claim 26 or 45, further comprising a tumor cell, tumor antigen, or tumor-associated antigen, immune cell, dendritic cell, or any combination thereof.
47. The composition of claim 26 or 45,, further comprising a PAMP or a DAMP.
48. The composition of claim 26 or 45,, further comprising a tetanus antigen.
49. The composition of claim 26 or 45,, further comprising a pDOM.

50. The composition of claim 26 or 45,, further comprising a pDOM fused to a cationic polypeptide.
51. The composition of claim 26 or 45,, further comprising a pDOM fused to arginine or histidine enriched polypeptide sequence.
52. The composition of claim 26 or 45,, further comprising a pDOM fused to polyarginine or polyhistidine polypeptide.
53. The composition of claim 26 or 45, further comprising a pDOM fused to 1 to 20 arginines.
54. The composition of claim 26 or 45, further comprising a pDOM fused to 9 arginines.
55. The composition of claim 26 or 45, further comprising a chemotherapeutic agent.
56. The composition of claim 26 or 45, further comprising doxorubicin.
57. The composition of claim 26 or 45, wherein said tumor targeting moiety is antibody that binds to EGFR1.
58. The composition of claim 26 or 45, wherein said tumor targeting moiety is cetuximab.
59. The composition of claim 26 or 45, wherein said tumor targeting moiety is antibody that binds to HER2/Neu.
60. The composition of claim 26 or 45, wherein said tumor targeting moiety is trastuzumab.
61. The composition of claim 26 or 45, wherein said tumor targeting moiety is antibody that binds to CD20.
62. The composition of claim 26 or 45, wherein said tumor targeting moiety is rituximab.

TUMOR TARGETING COMPOSITIONS

ABSTRACT

Disclosed herein are methods and compositions for treating or preventing neoplastic diseases or other disorders. In one embodiment, a composition comprising an immunoconjugate or fusion protein comprising a targeting moiety and a moiety that inhibits the production or function of regulatory T cells is provided. In another embodiment, a composition comprising a tumor targeting moiety and a molecule that binds transforming growth factor beta (TGF- β) is provided. Also provided herein are methods of producing and using said compositions.

FIGURE 1

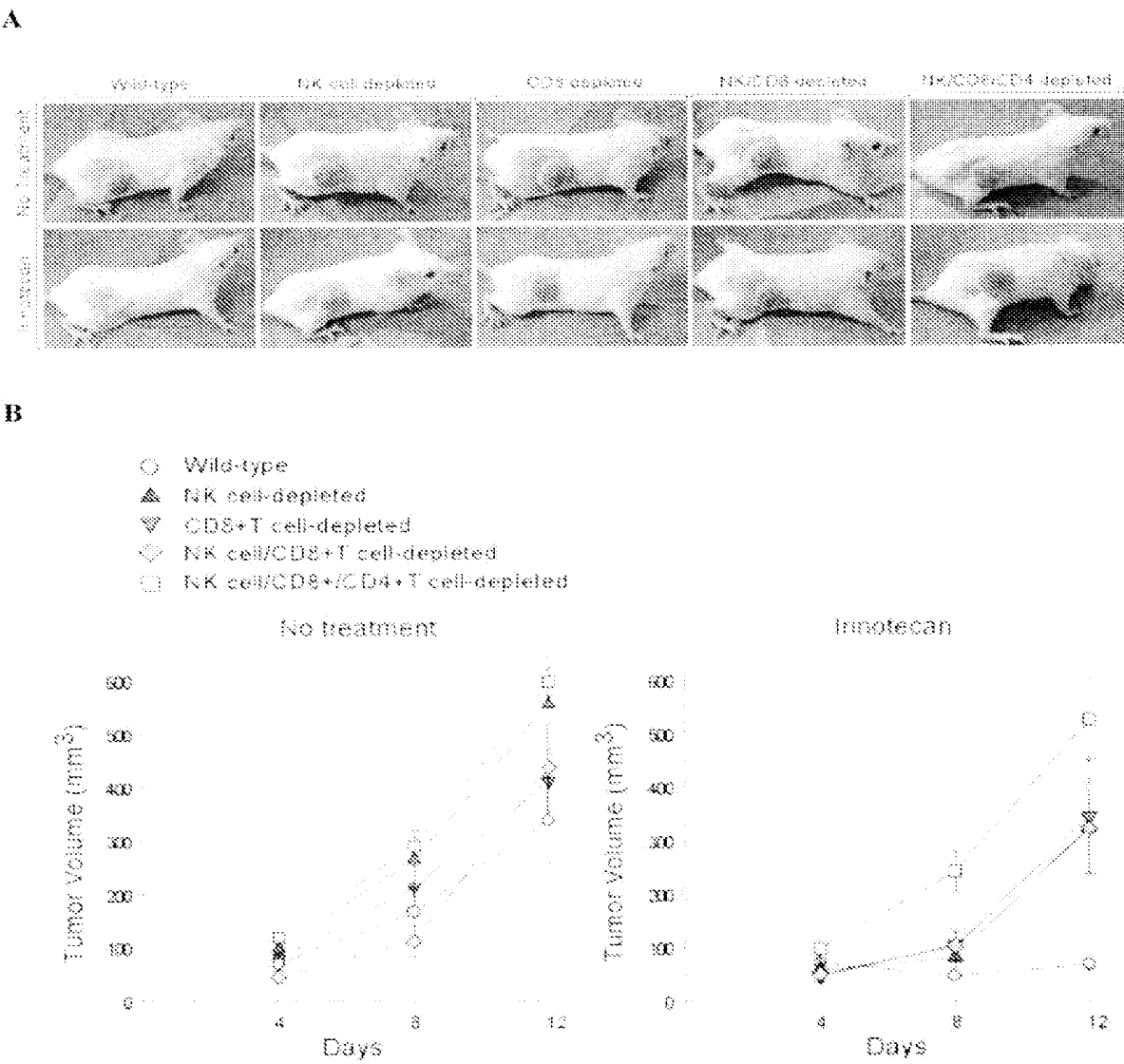
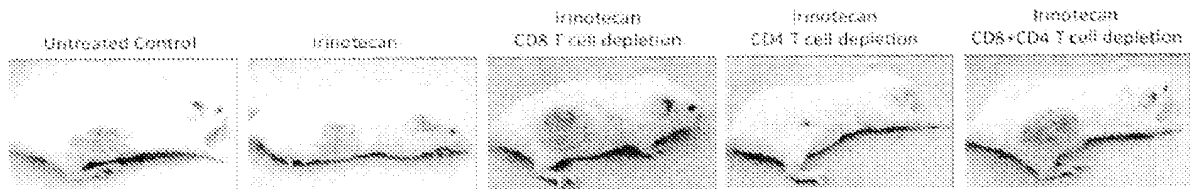
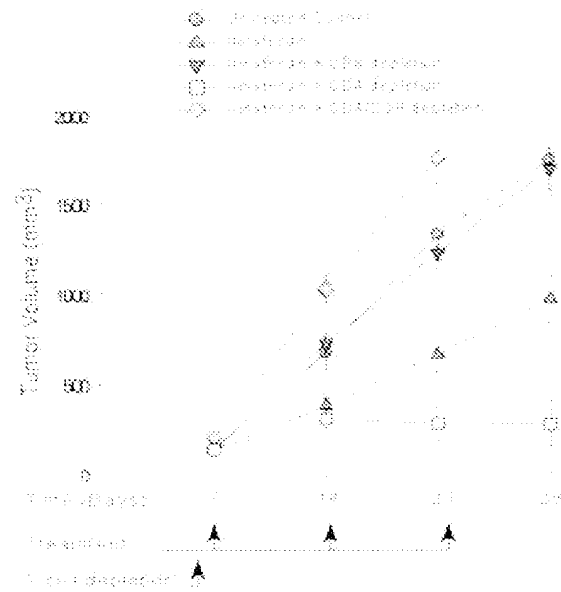


FIGURE 2

A



B



C.

FIGURE 2

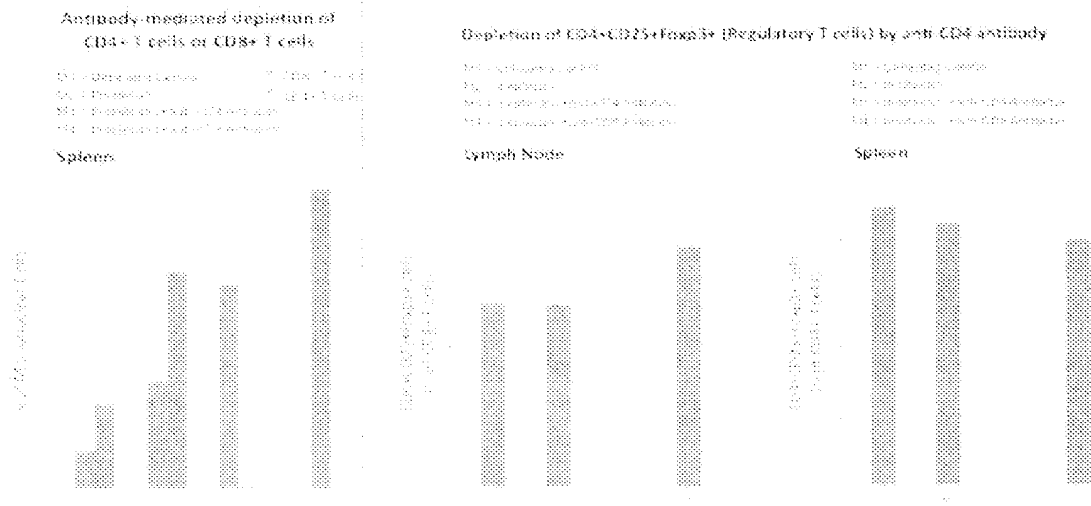


FIGURE 3

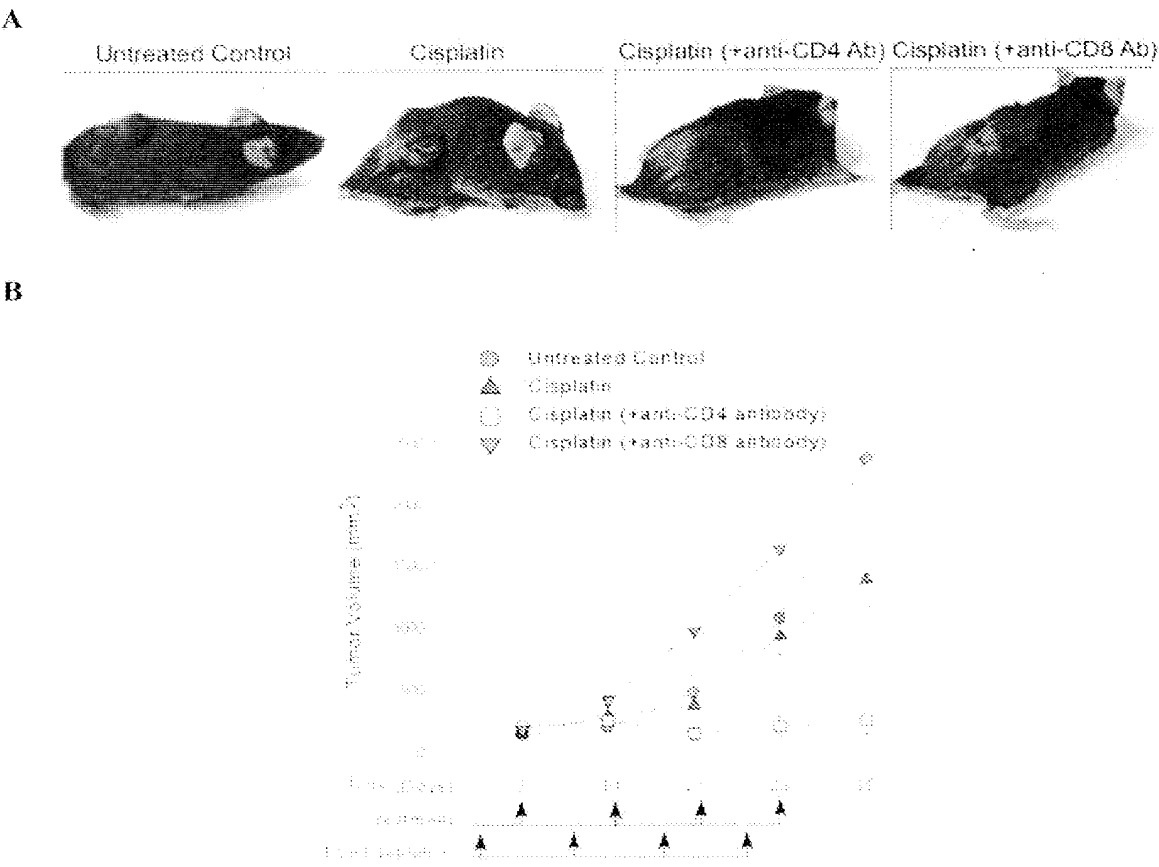


FIGURE 3

C

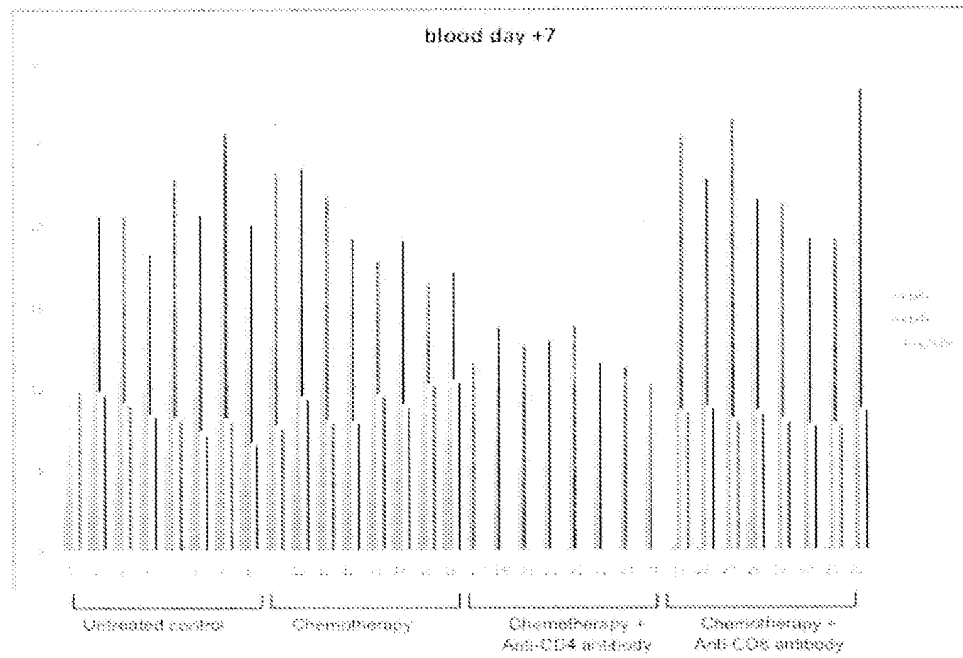
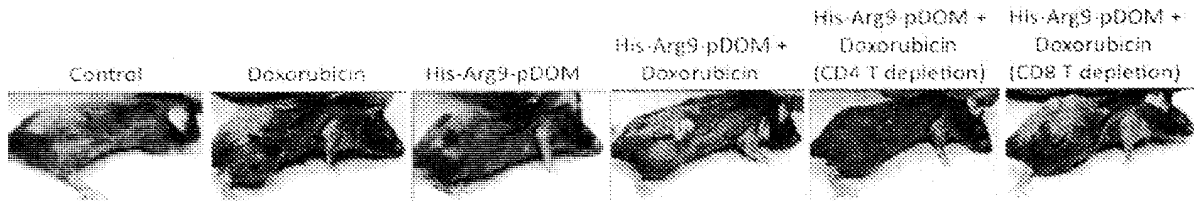
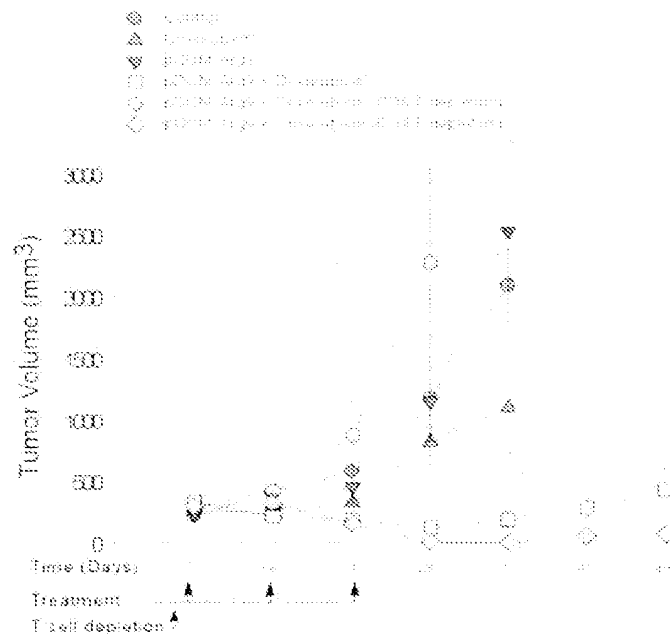


FIGURE 4

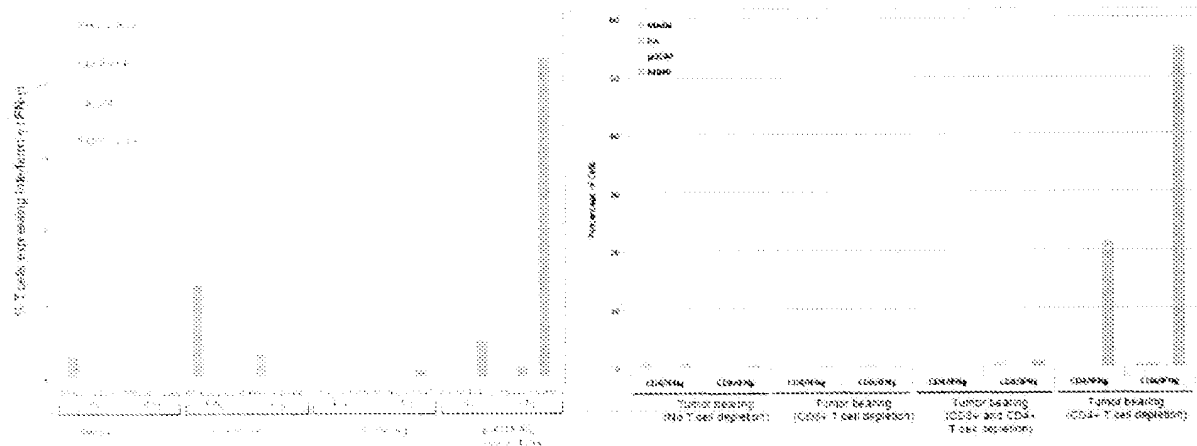
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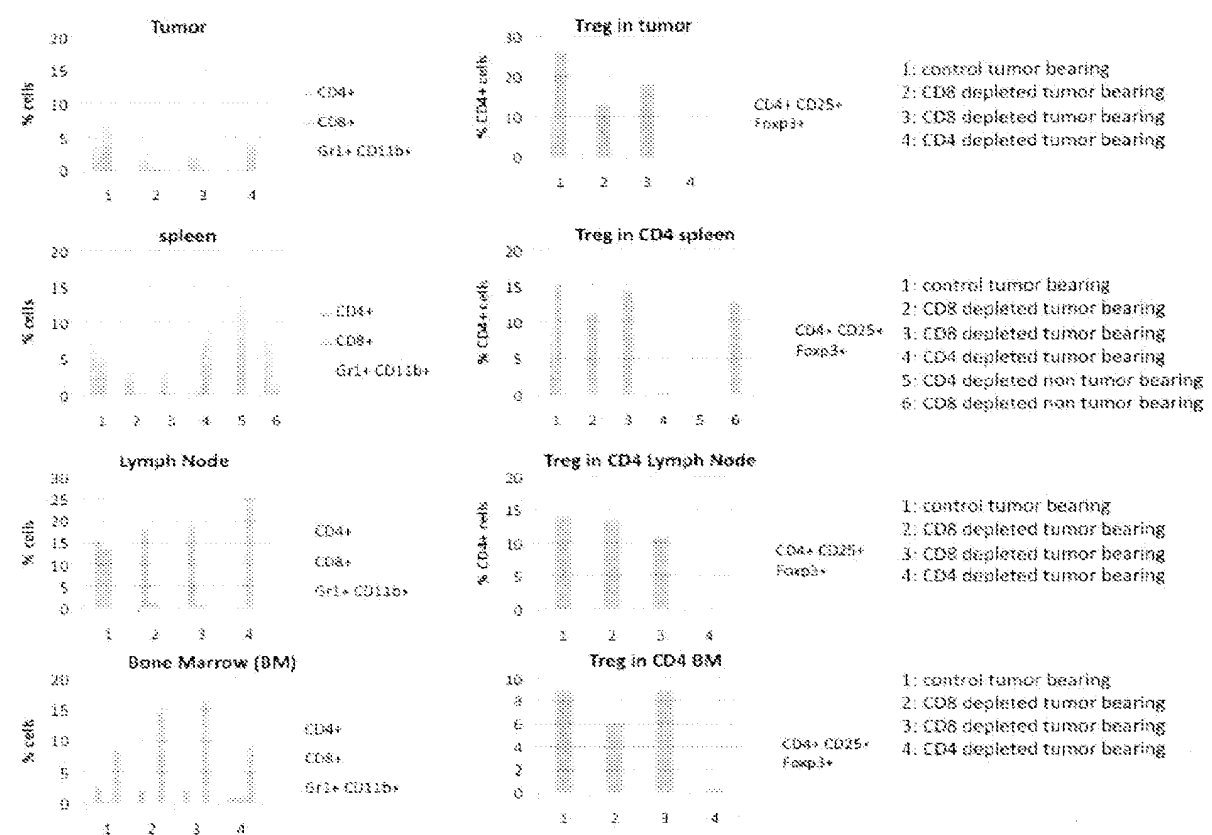
B



C



D



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[Continued on next page]

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

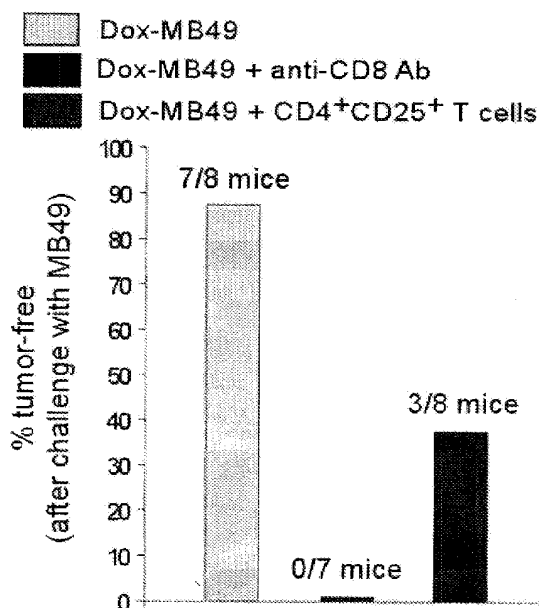


Fig. 53G

(57) Abstract: The present invention is based on the seminal discovery that targeted immunomodulatory antibodies and fusion proteins can counter act or reverse immune tolerance of cancer cells. Cancer cells are able to escape elimination by chemotherapeutic agents or tumor-targeted antibodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. Such immunosuppressive mechanisms include immunosuppressive cytokines (for example, Transforming growth factor beta (TGF-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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COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

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

BACKGROUND INFORMATION

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

SUMMARY OF THE INVENTION

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

[0004] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule, and the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF- 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) Transforming growth factor-beta receptor (TGF- β R); (v) Programmed death-1 (PD-1); and (vi) Receptor activator of nuclear factor- κ B (RANK).

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

[0006] In one aspect, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: (i) CD4; (ii) CD25 (IL-2 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).

[0007] 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).

[0008] 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 .

[0009] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 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

[0010] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Programmed Death-1 (PD-1), Programmed death-1 ligand 1 (PD-L1), or Programmed death-1 ligand 2 (PD-L2). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- 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.

[0011] 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.

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

[0013] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 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.

[0014] 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.

[0015] 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).

[0016] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 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.

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

[0018] In one aspect, the targeting moiety specifically binds to Tumor Necrosis Factor- 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.

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

[0020] In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C 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.

[0021] 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.

[0022] 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.

[0023] 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.

[0024] 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.

[0025] 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.

[0026] 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.

[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 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.

[0028] In one aspect, the targeting moiety includes 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.

[0029] 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.

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

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

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

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

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

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

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

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

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

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] Fig. 1 shows exemplary amino acid sequences of transforming growth factor beta receptor type II (TGF- 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).

[0040] Also shown in Figure 1 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).

[0041] In addition, Figure 1 also shows 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).

[0042] 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).

[0043] 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).

[0044] 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).

[0045] 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).

[0046] 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).

[0047] 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 optional and can be replaced with EPKSCDK (SEQ ID NO: 105) or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0048] Figure 8 shows exemplary fusion proteins including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGF 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) (Figure 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).

[0049] 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)

[0050] 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.

[0051] Figure 11 shows exemplary fusion proteins including Receptor activator of nuclear factor-kB (RANK) Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGF 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.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[0069] Figure 29 shows exemplary fusion proteins including IL-2, Fc, and RANK ectodomain, including IL-2 + Fc + RANK ectodomain (SEQ ID NO: 34) and RANK ectodomain + Fc + IL-2 (SEQ ID NO: 35). The linker 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.

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

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

[0072] Figure 32 shows exemplary fusion proteins including anti-tumor necrosis factor (TNF 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).

[0073] 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).

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

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

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

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

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

[0078] Figure 38 shows exemplary fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG C 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.

[0079] 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.

[0080] 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).

[0081] 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).

[0082] 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).

[0083] 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).

[0084] Figure 44 shows 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) (Figure 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) (Figure 44B).

[0085] 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.

[0086] 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)

[0087] 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.

[0088] 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).

[0089] 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).

[0090] 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.

[0091] 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).

[0092] 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).

[0093] Figure 53 shows Regulatory T cells (Treg) accumulate in the tumor microenvironment and counteract the ability of chemotherapy to activate CD8⁺ T cell-mediated antitumor immunity. (a) Surface exposure of calreticulin (CRT) in response to treatment of human (SW780) and murine (MB49) cancer cells with doxorubicin (10 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. (b) 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. (c) 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. (d) 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. (e) 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. (f) Tregs infiltrating the tumor microenvironment suppress priming of tumor-reactive immune responses by doxorubicin-treated tumor cells. Naïve C57BL/6 mice were vaccinated with 5×10^6 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. (g) Tregs infiltrating the tumor microenvironment suppress the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death. Naïve C57BL/6 mice were vaccinated with 5×10^6 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.

[0094] Figure 54 shows inhibition of TGF- β in the tumor microenvironment reduces 'adaptive' FoxP3⁺ regulatory T cells and enhances the antitumor efficacy chemotherapy. (a) 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. (b) 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. (c) 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. (d) 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. (e, f) 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 (e), 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 (f).

[0095] Figure 55 shows 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. (a) *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. (b) 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. (c) 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. (d)

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 5×10^5 MB49 tumor cells were left untreated or treated with doxorubicin (5mg/kg i.p. weekly x 3) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting either CD4⁺ T cells or CD8⁺ T cells on the *in vivo* antitumor efficacy of doxorubicin.

[0096] Figure 56 shows 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. (a) Surface exposure of calreticulin (CRT) in response to treatment of MB49 cancer cells with either cisplatin or the combination of cisplatin and gemcitabine for 4h. The surface exposure of CRT was determined by immunofluorescence cytometry of untreated control or chemotherapy-treated cells stained with Dylight-labeled anti-CRT antibody or an isotype control (IgG1) antibody. (b,c) Depletion of CD4⁺ 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 5×10^5 MB49 tumor cells were left untreated or treated with cisplatin (0.5mg/kg i.p. weekly x 4) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of IFN- γ expression in CD8⁺ T cells from the tumor and draining lymph node (DLN) in response to *in vitro* stimulation with MB49 cell lysates (b). The percentage of effector memory T_{EM} cells was determined by flow cytometric analyses of CD8⁺CD62L⁻ cells (c). (d,e,f) 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 5×10^5 MB49 tumor cells were left untreated or treated with either cisplatin (0.5mg/kg) or the combination of cisplatin and gemcitabine (i.p. weekly x 4) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting either CD4⁺ T cells or CD8⁺ T cells on the *in vivo* antitumor efficacy of chemotherapy and the percentage of mice exhibiting complete tumor-regression by d50 following tumor inoculation. Establishment of adaptive antitumor

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

[0097] Figure 57 shows 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. (a) 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)]. (b) Genotoxic chemotherapeutic agents induce p53-independent cell surface expression of human NKG2D ligands (MHC-I-related A and B molecules – MICA/MICB) on cancer cells. Isogenic p53-proficient (p53^{+/+}) 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 MAb (R&D Systems). (c) & (d) Induction of NKG2D ligands contributes to the antitumor effect of chemotherapy *in vivo*. Immunocompetent Balb/C mice injected s.c. with 2 x10⁵ syngeneic CT26 tumor cells were treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d5 following tumor inoculation, with or without pre-treatment with an NKG2D blocking antibody (CX5, eBIOscience) (200 µg i.p.) at 16h before each dose of chemotherapy. Tumor volume was monitored to determine the effect of NKG2D blockade on the *in vivo* antitumor efficacy of irinotecan. (e) *In vivo* depletion of CD4⁺CD25⁺FoxP3⁺ T cells by treatment of tumor-bearing mice with anti-CD4 antibody. Balb/C mice injected with 2 x10⁵ 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. (f) Depletion of CD4⁺ regulatory T cells facilitates irinotecan-induced activation of tumor-reactive IFN-γ⁺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- γ expression in CD8 $^+$ T cells from the tumor and draining lymph node (DLN) in response to *in vitro* stimulation with either CT26 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone. (g) & (h) Chemotherapy-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD4 $^+$ regulatory T cells to stimulate CD8 $^+$ T cell-mediated tumor regression. Balb/C mice injected with 2×10^5 CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) and/or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting CD4 $^+$ T cells and/or CD8 $^+$ T cells on the *in vivo* antitumor efficacy of irinotecan.

DETAILED DESCRIPTION OF THE INVENTION

[0098] Targeted immunostimulatory antibodies and/or fusion proteins for prevention or treatment of cancer: Chemotherapy is a cornerstone of systemic treatment of patients with most common types of advanced cancers. The vast majority of human cancers harbor genetic alterations and signaling mechanisms that impair the direct death signaling pathways entrained by chemotherapeutic agents. Although chemotherapeutic agents employ diverse mechanisms to directly kill tumor cells, the present invention provides that these agents have immuno-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.

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

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

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

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

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

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

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

[0104] The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to disrupt immunosuppressive networks in the tumor microenvironment. Tumors employ a wide array of regulatory mechanisms to avoid or suppress the immune response. Cancer cells actively promote immune tolerance in the tumor microenvironment via the expression of cytokines and molecules that inhibit the differentiation and maturation of antigen-presenting dendritic cells. The immunosuppressive cytokines and ligands produced by tumor cells include the following: (i) Transforming growth factor-beta (TGF- 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.

[0105] The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to inhibit the development or function of Tregs and myeloid suppressor cells (DCs or MDSC) within the tumor microenvironment. Tregs (CD4+CD25+FoxP3+ cells) express an array of immunosuppressive cytokines and molecules which act in concert to induce immune tolerance and promote tumor progression and metastases. These include: (i) Cytotoxic T-lymphocyte associated protein 4 (CTLA-4; CD152), a co-inhibitory receptor that binds to the ligands CD80 (B7-1) or CD86 (B7-2) on the antigen presenting cell (APC) and inhibits co-stimulation of T cells; (ii) Programmed death-1 ligand 1 (PD-L1; B7-H1), a ligand which engages the co-inhibitory receptor Programmed death-1 (PD-1) and inhibits T cell activation and proliferation. (iii) Transforming growth factor-beta (TGF- 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.

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

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

[0108] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule, and the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF- 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).

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

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

[0111] 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).

[0112] 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 .

[0113] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 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.

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

[0115] 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.

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

[0117] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 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.

[0118] 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.

[0119] 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).

[0120] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 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.

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

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

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

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

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

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

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

[0126] In one aspect, the targeting moiety specifically binds to Tumor Necrosis Factor- 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.

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

[0128] In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C 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.

[0129] 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.

[0130] 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.

[0131] 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.

[0132] 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.

[0133] 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.

[0134] 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.

[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 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.

[0136] In one aspect, the targeting moiety includes 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.

[0137] 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.

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

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

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

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

[0142] In another embodiment, the method includes administration to a subject in need thereof an antibody or molecule that targets and depletes CD4+ regulatory T cells (Tregs) in combination with an immunostimulatory antibody, fusion protein, peptide or ligand that targets CTLA-4, PD1, PD-1L, RANKL, TGF- 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.

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

antibody, fusion protein, peptide or ligand that targets CTLA-4, PD1, PD-1L, RANKL, TGF- 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.

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

[0145] In another embodiment, the subject is administered one or more molecule of the invention in combination with any vaccine. In another aspect, the vaccine includes a tumor antigen, tumor-associated antigen, tumor epitope, tumor antigen-containing fusion protein, tumor cell, or dendritic cell. In another aspect, the vaccine includes a pathogen antigen, pathogen-associated antigen, pathogen epitope, or pathogen antigen-containing fusion protein. In one aspect, the vaccine includes a surrogate CD4+ 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.

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

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

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

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

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

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

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

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

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

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

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

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

[0157] For example, in various embodiments, a targeting moiety is specific for or binds to a molecule or component, which includes but is not limited to, epidermal growth factor receptor (EGFR, EGFR1, ErbB-1, HER1), ErbB-2 (HER2/neu), ErbB-3/HER3, ErbB-4/HER4, EGFR ligand family; insulin-like growth factor receptor (IGFR) family, IGF-binding proteins (IGFBPs), IGF 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 (TNFRSF), death receptor family, TRAIL-receptor; cancer-testis (CT) antigens, lineage-specific antigens, differentiation antigens, alpha-actinin-4, ARTC1, breakpoint cluster region-Abelson (Bcr-abl) fusion products, B-RAF, caspase-5 (CASP-5), caspase-8 (CASP-8), beta-catenin (CTNNB1), cell division cycle 27 (CDC27), cyclin-dependent kinase 4 (CDK4), CDKN2A, COA-1, dek-can fusion protein, EFTUD-2, Elongation factor 2 (ELF2), Ets variant gene 6/acute myeloid leukemia 1 gene ETS (ETC6-AML1) fusion protein, fibronectin (FN), GPNMB, low density lipid receptor/GDP-L fucose: beta-Dgalactose 2-alpha-Lfucosyltransferase (LDLR/FUT) fusion protein, HLA-A2, arginine to isoleucine exchange at residue 170 of the alpha-helix of the alpha2-domain in the HLA-A2 gene (HLA-A*201-R170I), MLA-A11, heat shock protein 70-2 mutated (HSP70-2M), KIAA0205, MART2, melanoma ubiquitous mutated 1,2, 3 (MUM-1, 2,3), prostatic acid phosphatase (PAP), neo-PAP, Myosin class 1, NFYC, OGT, OS-9, pml-RARalpha fusion protein, PRDX5, PTPRK, K-ras (KRAS2), N-ras (NRAS), HRAS, RBAF600, SIRT2, SNRPD1, SYT-SSX1 or -SSX2 fusion protein, Triosephosphate Isomerase, BAGE, BAGE-1, BAGE-2,3,4,5, GAGE-1,2,3,4,5,6,7,8, GnT-V (aberrant N-acetyl glucosaminyl transferase V, MGAT5), HERV-K-MEL, KK-LC, KM-HN-1, LAGE, LAGE-1, CTL-recognized antigen on melanoma (CAMEL), MAGE-A1 (MAGE-1), MAGE-A2, MAGE-A3, MAGE-A4, MAGE-AS, MAGE-A6, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-3, MAGE-B1, MAGE-B2, MAGE-B5, MAGE-B6, MAGE-C1, MAGE-C2, mucin 1 (MUC1), MART-1/Melan-A (MLANA), gp100, gp100/Pmel17 (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 (BIRCS), human telomerase reverse transcriptase (hTERT), telomerase, Wilms' tumor gene (WT1), SYCP1, BRDT, SPANX, XAGE, ADAM2, PAGE-5, LIP1, CTAGE-1, CSAGE, MMA1, CAGE, BORIS, HOM-TES-85, AF15q14, HCA66I, LDHC, MORC, SGY-1, SPO11, TPX1, NY-SAR-35, 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, I-2 microglobulin, squamous cell carcinoma antigen, neuron-specific enolase, heat shock protein gp96, GM2, sargramostim, CTLA-4, 707 alanine proline (707-AP), adenocarcinoma antigen recognized by T cells 4 (ART-4), carcinoembryonic antigen peptide-1 (CAP-1), calcium-activated chloride channel-2 (CLCA2), cyclophilin B (Cyp-B), human signet ring tumor-2 (HST-2), Human papilloma virus (HPV) proteins (HPV-E6, HPV-E7, major or minor capsid antigens, others), Epstein-Barr virus (EBV) proteins (EBV latent membrane proteins—LMP1, LMP2; others), Hepatitis B or C virus proteins, and HIV proteins. A composition of the invention can further include the foregoing as a peptide/polypeptide and/or encoding the same.

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

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

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

[0160] In one embodiment, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: CD4; CD25 (IL-2 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.

[0161] In one aspect, the targeting moiety binds a specific cytokine, cytokine receptor, co-stimulatory molecule, co-inhibitory molecule, or immunomodulatory receptor that modulates the immune system. In another aspect, the targeting moiety specifically binds one of the following molecules: tumor necrosis factor (TNF) superfamily; tumor necrosis factor- 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.

[0162] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide, or peptide that specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a cytokine, cytokine receptor, co-stimulatory molecule, or co-inhibitory molecule that modulates the immune system. In another aspect, the targeting moiety specifically binds one of the following molecules: CD4; CD25 (IL-2 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.

[0163] Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as Zanolimumab (anti-CD4 mAb), Keliximab (anti-CD4 mAb); Ipilimumab (MDX-101; anti-CTLA-4 mAb); Tremilimumab (anti-CTLA-4 mAb); (Daclizumab (anti-CD25/IL-2R mAb); Basiliximab (anti-CD25/IL-2R mAb); MDX-1106 (anti-PD1 mAb); antibody to GITR; GC1008 (anti-TGF- 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).

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

aspect, the “immunomodulatory moiety” specifically binds a cytokine, cytokine receptor, co-stimulatory molecule, or co-inhibitory molecule that modulates the immune system. In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: Transforming growth factor-beta receptor (TGF- 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; TNR4); OX40 ligand (OX40L); B7-H3; B7-H4; BTLA; CD40; CD40 ligand (CD40L); CD70; CD27; LIGHT; or HVEM. In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: tumor necrosis factor- α (TNF- α); Interleukin-12 (IL-12); IL-12R; Interleukin-10 (IL-10); IL-10R. In another aspect, the immunomodulatory moiety comprises an extracellular domain of CTLA-4. In one aspect, the immunomodulatory moiety is an agonist that increases the function of the bound molecule. In another aspect, the immunomodulatory moiety is an antagonist that inhibits the function of the targeted molecule.

[0165] In another aspect, the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF- 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; TNR4); CD40; IL-12R; or glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR; TNFRSF18). In an additional aspect, the extracellular domain of the specific receptor binds the cognate ligand and inhibits the interaction of the ligand with its native receptor.

[0166] In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: Transforming growth factor-beta (TGF- 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.

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

[0168] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell, and the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF- 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.

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

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

Transforming growth factor-beta receptor (TGF- 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).

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

[0172] In another aspect, the targeting moiety and immunomodulatory moiety are two different molecules selected from any of the following: an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds TGF- β , CTLA-4, PD-1, 4-1BB (CD137), OX40 (CD134; TNR4), CD40; IL-12R, or GITR/AITR (TNFRSF18), or Toll-like receptor (TLR); an extracellular domain or ligand-binding sequence of Transforming growth factor-beta receptor (TGF- 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.

[0173] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds one of the following molecules: CD4; CD25 (IL-2 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.

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

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

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

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

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

[0178] An antibody as disclosed herein includes an antibody fragment, such as, but not limited to, Fab, Fab' and F(ab')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.

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

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

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

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

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

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

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

[0184] An antibody fragment can include a portion of an intact antibody, e.g. including the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')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).

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

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

[0187] Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different “classes.” There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into “subclasses” (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, 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.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

EXAMPLE 1

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

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

were injected into one flank of syngeneic immunocompetent C57BL/6 mice. Unlike mice injected with live tumor cells, mice injected with doxorubicin-treated tumor cells exhibited increased production of IFN- γ by draining lymph node (DLN) cells in response to *in vitro* re-challenge with MB49 cell lysates (Fig. 1b). Vaccination with doxorubicin-killed MB49 cells generated a tumor-specific immune response since no corresponding increase in IFN- 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. 1c). 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.

[0203] 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. 1b). Whereas treatment with doxorubicin on d3 following tumor inoculation was able to arrest tumor growth, delayed administration of the same dose of doxorubicin on d10 failed to inhibit the progressive growth of established MB49 tumors (Fig. 1d). These results indicate that tumor-induced immune tolerance in the microenvironment of established cancers counteracts the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death.

[0204] Regulatory T cells (Treg) accumulate in the tumor microenvironment and counteract the ability of chemotherapy to activate CD8⁺ 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. 1e). To investigate whether Tregs infiltrating the tumor microenvironment can suppress the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death, $CD4^+CD25^+$ 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. 1f). 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. 1g). 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.

[0205] 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. 2a). 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. 2b). 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. 2b). 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. 2c). 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. 2d) and a dramatic reduction of CD4⁺CD25⁺FoxP3⁺ Tregs in tumor tissue (Fig. 2e). 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.

[0206] 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. 3a). 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 (Fig. 3a, b). 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. 3c). 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. 3c). 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. 3d).

[0207] 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. 4a). Whereas tumor-reactive IFN- γ ⁺CD8⁺ T cells were evident in tumors of MB49 tumor-bearing mice treated with doxorubicin (Fig. 3c), treatment with cisplatin was unable to induce a corresponding elevation of IFN- γ expression in CD8⁺ T cells in response to *in vitro* re-challenge with MB49 cell lysates (Fig 4b). To examine whether counteraction of Treg-mediated immune tolerance enables the activation of antitumor immunity by cisplatin, immunocompetent tumor-bearing mice were treated with cisplatin following depletion of Tregs with anti-CD4 antibody. Antibody-mediated depletion of CD4⁺ T cells enhanced the percentage of tumor-reactive IFN- γ ⁺CD8⁺ T cells as well as CD8⁺CD62L⁻ T cells in cisplatin-treated animals (Fig. 4b,c). Treatment of MB49 tumor-bearing mice with cisplatin partially inhibited tumor growth but failed to arrest tumor progression. Whereas depletion of CD8⁺ 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. 4d). Although treatment of tumor-bearing mice with the combination of cisplatin and gemcitabine was also able to arrest tumor growth, tumor growth rapidly resumed following termination of therapy with none of the animals (0/8) being tumor-free at d50 following tumor inoculation (Fig. 4e). In contrast, mice depleted of CD4⁺ T cells exhibited a more sustained response to either single agent or combination chemotherapy, with 7/16 mice exhibiting complete tumor regression. The complete regression of tumors was attended with establishment of adaptive antitumor immunity since none of the cured mice (7/7) developed tumors when re-challenged with live MB49 tumor cells in the opposite flank.

[0208] Chemotherapy-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD4⁺ 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^{R+} T cells and $\alpha\beta$ -TCR^{R+} T cells for immune surveillance of tumors. Expression of mouse and human ligands for NKG2D is upregulated in transformed epithelial cell lines in response to genotoxic stress or stalled DNA replication, via activation of a DNA damage checkpoint pathway initiated by ATM (ataxia telangiectasia, mutated) or ATR (ATM- and Rad3-related) protein kinases. Treatment of CT26 mouse colon cancer cells with genotoxic chemotherapeutic agents resulted in upregulation of mouse NKG2D ligands of the

retinoic acid inducible gene family (Rae1)(Fig. 5a). RT-PCR showed that Rae1 mRNA was induced in CT26 cells by 2-4h, reached a plateau after 16-24h, and began to decline after 48h of treatment with either the irinotecan or oxaliplatin (Fig. 5a). Flow cytometric analysis demonstrated that cell surface expression of human NKG2D ligands (MHC-I-related A and B molecules – MICA, MICB) was also upregulated on human colorectal cancer cells (HCT116) in response to treatment with irinotecan (Fig. 1b). Isogenic HCT116 cells that differ only in their p53 status demonstrated that p53 is not required for irinotecan-induced upregulation of MICA/B (Fig. 1b). To examine whether the induction of NKG2D ligands contributes to the antitumor effect of chemotherapy *in vivo*, immunocompetent Balb/C mice inoculated with syngeneic CT26 tumor cells were treated with irinotecan (50 mg/kg i.p) with or without pre-treatment with an NKG2D blocking antibody (200 µg i.p.). Whereas treatment with irinotecan alone inhibited the growth of CT26 tumors, the antitumor effect of irinotecan was negated by pre-treatment with the NKG2D blocking antibody (Fig. 1c). Since engagement of NKG2D by its ligands provides a costimulatory signal for the activation of 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. 5d). Antibody-mediated depletion of CD⁴⁺ T cells enhanced the percentage of tumor-reactive IFN- γ ⁺CD⁸⁺ T cells in irinotecan-treated animals (Fig. 5e). 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. 5f). 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. 5f).

[0209] 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

[0210] Exemplary Targeted Immunomodulatory Antibodies & Fusion Proteins

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

[0212] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety specifically binds to Transforming growth factor-beta (TGF- 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) (Figure 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) (Figure 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) (Figure 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) (Figure 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) (Figure 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) (Figure 7). SEQ ID NO: 7 provides a fusion protein including Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD), Fc, and IL-2 (Figure 7). SEQ ID NO: 8 provides a fusion protein including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD) (Figure 8A). SEQ ID NO: 9 provides a fusion protein including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD) (Figure 8B). SEQ ID NO: 10 provides a fusion protein including anti-CD4 antibody and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ECD) (Figure 9). SEQ ID NO: 11 provides a fusion protein including PD-1 Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ectodomain) (PD-1 ectodomain + Fc + TGF β RII ectodomain; Figure 10). SEQ ID NO: 12 provides a fusion protein including Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ectodomain), Fc, and PD-1 Ectodomain (TGF β RII ectodomain + Fc + PD-1 ectodomain; Figure 10). SEQ ID NO: 13 provides a fusion protein including RANK Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ectodomain) (RANK ectodomain + Fc + TGF β RII ectodomain; Figure 11). SEQ ID NO: 14 provides a fusion protein including Transforming growth factor-beta receptor II (TGF β -RII) Extracellular domain (ectodomain), Fc, and RANK Ectodomain (TGF β RII ectodomain + Fc + RANK ectodomain; Figure 11).

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

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

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

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

[0216] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety includes a molecule that specifically binds to Transforming growth factor-beta receptor (TGF- 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) (Figure 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 ; Figure 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; Figure 42). SEQ ID NO: 53 provides a fusion protein including anti-CD20 antibody and a sequence from Transforming growth factor- β (TGF- β) (Figure 43). SEQ ID NO: 54 provides a fusion protein including anti-CD25 antibody and a sequence from transforming growth factor- β (TGF- β) (Figure 44A). SEQ ID NO: 55 provides a fusion protein including anti-CD25 antibody and a sequence from transforming growth factor- β (TGF- β) (Figure 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; Figure 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 ; Figure 45). SEQ ID NO: 58 provides a fusion protein including anti-CD4 antibody and a sequence from transforming growth factor- β (TGF- β) (Figure 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; Figure 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) (Figure 47).

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

domain: (RANK Ectodomain + IgG C γ 1 + TNFR2 ECD; Figure 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; Figure 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) (Figure 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; Figure 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) (Figure 51). SEQ ID NO: 68 provides a fusion protein including a sequence from Programmed death-1 ligand 1 (PD-L1), immunoglobulin Fc region (IgG C 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; Figure 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) (Figure 52).

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

What is claimed is:

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

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

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

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

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

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

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

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

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

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

[*Italic* – Extracellular domain of Transforming growth factor beta Receptor II (TGF 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* *NIIFSEEYNT*
SNPDL *LLVIF* *QVTGISLLPP* *LGVAISVII* *FYCYRVNRQQ* *KLSTWETGK* *TRKLMEFSEH*
CAIILEDERS DISSTCANNI NHNTPELLIE LDTLVGKGRF AEVYKAKLKQ NTSEQFETVA VKI FP
YEEYASWKTE KDIFSDINLK HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH
VISWEDLRKL GSSLARGIAH LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL
SLRLDPTLSV DDLANSQGQVG TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR
CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC
WDHDPEARLT AQCVAERFSE LEHLDRLSGR SCSEEKIPED GSLNTTK

[*Italic* – Extracellular domain of Transforming growth factor beta Receptor IIB (TGF 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* *QEVCAVWRK* *NDENITLET* *CHDPKLPYHD* *FILEDAASPK*
CIMKEKKKPG *ETFFMCSCSS* *DECNDNIIFS* *EEYNTSNPDL* *LLVIFQVTGI* *SLLPPLGVAI*
SVIIIFYCYR VNRQQKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQGQVG
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 ICEKPQEV CV AVWRKNDENI
 TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT
 SNPDLLLIVIF QVTGISLLPP LGVAISVIII FYCYRVNRQQ KLSSTWETGK TRKLMEFSEH
 CAIILEDERS DISSTCANNI NHNTELLPIE LDTLVGKGRF AEVYKAKLKQ NTSEQFETVA VKI FP
 YEEYASWKTE KDIFSDINLK HENILQFLTA EERKTELKQ YWLITAFHAK GNLQEYLTRH
 VISWEDLRKL GSSLARGIAH LHSHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL
 SLRLDPTLSV DDLANSQVVG TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR
 CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC
 WDHDPEARL

(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 NDENITLET V CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD LLLVIFQVTGI 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 ICEKPQEV CV AVWRKNDENI
 TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT
SNPDLLLIVIF 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 NDENITLET V CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD LLLVIFQ

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 ICEKPQEV CV AVWRKNDENI
 TLETVCHDPK LPYHDFILED AASPKCIMKE KKKPGETFFM CSCSSDECND NIIFSEEYNT
SNPDLLLIVIF 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 NDENITLET V 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 ICEKPQEV CV 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 NDENITLET V 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 VNRQQKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT
 LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVRIFP YEEYASWKTE KDIFSDINLK
 HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
 LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQGQVG
 TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
 LEHLDRLSGR SCSEKIPED 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 VNRQQKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT
 LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
 HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
 LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQGQVG
 TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
 HPCVESMKDA SGIQMVCE TL TECWDHDPEA RLTAQCVAER FSELEHLDR L 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):

GGGGSGGGSGGGGS

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
PSSKSTSGGTAALGCLVKDYFPEPVTSVNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **TIPPHVQK** **SVMNDMIVTD** **NNGAVKFPQL** **CKFCDVRFST**
CDNQKSCMSN **CSITSICEKP** **QEVCAVWRK** **NDENITLETV** **CHDPKLPYHD** **FILEDAASPK**
CIMKEKKKPG **ETFFMCSSS** **DECNDNIIFS** **EEYNTSNPD**

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

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSSGSR
SGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
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):

QVQLKQSGPGLVQPSSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSGGNTDYNTPTSR
LSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLVTVSAASTKGPSVFPLAP
 SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
 TYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
 VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
 PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
 PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHENHHTQKSLSLSPGK
 GGGGSGGGGSGGGGS 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
SGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKR
 TQVAAPSVPFI FPPSDEQLKSGTASV
 VCLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYEEKHKVYACEVTH
 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
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST**
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQOKPGSSPKPWYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSLTLSKADYEEKHKVYACEVTHQ
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):

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFKR
RFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGLTVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSS
LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST**
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD

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

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSRFSGSG
SGTDFTLTITSSLPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSKADYEKHKVYACEVTH
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 VVQPGRLRL SCAASGFTFS SYTMHWVRQA PGKGLEWVTF ISYDGNNKYY
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGQ GTLVTVSSAS
 TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL
 YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPELLGGPS
 VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ
 GNVFSCSVMH EALHNHYTQK SLSLSPGK **GGGSGGGSGGGGS** **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
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPWTFG QGTKVEIKRT VAAPSVFIFP
 PSDEQLKSGT ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYSLSSTL
 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 (TGF β -RII) Extracellular domain (ECD).IL-2 + Fc + TGF β -RII Extracellular domain (SEQ ID NO: 6):

APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T GGGGSGGGGSGGGGS
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS TIPPHVQK SVNNDMIVTD
 NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV
 CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFSEEYNTSNPD

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

TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP
 QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS
 DECNDNIIFS EEEYNTSNPD GGGGSGGGGSGGGGS
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T

Bold: TGF β RII ectodomain*Italic: Linker (Optional; other linker sequences)*

Plain: Fc

Underlined: IL-2 fragment

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

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

Fig. 7

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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
NQKFQDKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LVTVSSASTK
GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK GGGGSGGGGSGGGGS TIPPHVQK SVNNDMIVTD
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV
CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

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

DIQMTQSPST LSASVGDRVT ITCSASSSIS YMHWYQQKPG KAPKLLIYTT SNLASGVPAR
FSGSGSGTEF TLTISSLQPD DFATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS
DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSLTTL
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 WMHWIKQRPG 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** **TIPPHVQK SVNNDMIVTD**
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV
CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD

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

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

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
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
 TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRF**
STCDNQKSCMSNCSITSICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMK
EKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPD

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
 SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
 THQGLSSPVTKSFNRGEC

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

Italic: Linker

Bold: TGF 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):

PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGYLCAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV *GGGSGGGSGGGGS*
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*
TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVW
RKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGGETFFMCSCSSDECNDNIIFSEEYN
TSNPD

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

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVW
RKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGGETFFMCSCSSDECNDNIIFSEEYN
TSNPD *GGGSGGGSGGGGS*
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*
PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGYLCAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV

Underlined: PD-1 ectodomain*Italic: Linkers*

Plain: IgG1 Fc

Bold: TGF β RII ectodomain

(Note: The first linker sequence *GGGSGGGSGGGGS* 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
 RKNDENITLETVCHDPKLPYHDFILEDAAAPKICIMKEKKKPGETFFMCSSSDECNDNIIFSEEYN
 TSNPDGGGGSGGGSGGGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
 EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
 SFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK GGGGSGGGSGGGGS QI
APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDCKR 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 RCACTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDCKR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
 GGGSGGGSGGGSGGGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
 KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
 SFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGGSTIPPHVQKS
 VNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKN
 DENITLETVCHDPKLPYHDFILEDAAAPKICIMKEKKKPGETFFMCSSSDECNDNIIFSEEYNTSNPD

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 VWA~~V~~LQLGWR PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS
 61 ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT
 121 YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV VGVVGGLLGS
 181 LVLLVWVLAV ICSRAARGTI GARRTGQPLK EDPSAVPVFS VDYGELDFQW REKTPEPFVP
 241 CVPEQTEYAT IVFPSGMGTS SPARRGSADG PRSAQPLRPE DGHCSWPL

*Italic: PD-1 ectodomain*Underlined Italic: ligand-binding domain

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

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

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

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

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

Fig. 12

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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
PSSKSTSGGTAALGCLVKDYFPEPVTSVNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **PGWFLDSPDR** **PWNPPTFSPA** **LLVVTEGDNA** **TFTCSFSNTS**
ESFVLNWMYRM **SPSNQTDKLA** **AFPEDRSQPG** **QDCRFRVTQL** **PNGRDFHMSV** **VRARRNDSGT**
YLCGAISLAP **KAQIKESLRA** **ELRVTERRAE** **VPTAHPSPPSP** **RPAGQFQTLV**

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

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSSGSR
SGTDFTLTITSSLPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSKADYEKHKVYACEVTH
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):

QVQLKQSGPGLVQPSLISITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPFTSR
LSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYDYEFAYWGQGTLVTVSAASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPTVTSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQ
TYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PFVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **PGWFLDSPDR** **PWNPPTFSPA** **LLVVTEGDNA** **TFTCSFSNTS**
ESFVLNWMYRM **SPSNQTDKLA** **AFPEDRSQPG** **QDCRFRVTQL** **PNGRDFHMSV** **VRARRNDSGT**
YLCGAISLAP **KAQIKESLRA** **ELRVTERRAE** **VPTAHPSPPSP** **RPAGQFQTLV**

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

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGSG
SGTDFTLSINSVESEDIADYYCQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSKADYEKKHKVYACEVTH
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
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
 TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS**
ESFVLNWMYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT
YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV

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

QIVLSQSPAILLSASPGEKVTMTCRASSSVSYIHWFQOKPGSSPKPWIYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
 CLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEEKHKVYACEVTHQ
 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):

EVQLVESGGGLVQPGGSLRLSCAASGYFTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFKR
RFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSSASTKGPSVF
 PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSS
 LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
 VTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
 NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
 YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS PGWFLDSPDR PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS
 ESFVLNWMYRM SPSNQTDKLA AFPEDRSQPG QDCRFRTQL PNGRDFHMSV VRARRNDSGT
 YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV

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

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSRFSGSG
SGTDFTLTITSSLPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
 VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTH
 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 ISYDGNNKYY
 ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGO GTLVTVSSAS
 TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL
 YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDKTHTCPFC PAPELLGGPS
 VFLFPPKPKD TLMISRTPEV TCVVDVDSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ
 GNVFSCSVMH EALHNHYTQKSLSLSPGK **GGGSGGGSGGGGS** **PGWFLDSPDR**
 PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG
 QDCRFRTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA ELRVTERRAE
 VPTAHPSPSP RPAGQFQTLV

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

EIVLTQSPGT LSLSPGERAT LSCRASQSVG SSYLAWYQQK PGQAPRLLIY GAFSRATGIP
 DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPWTFG QGTKVEIKRT VAAPSVFIFP
 PSDEQLKSGT ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYSLSTL
 TLSKADYEKH KVYACEVTHQ 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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Fusion proteins comprising anti-CD25 antibody and PD-1 Ectodomain.

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

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

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

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

Plain: Anti-CD25 antibody

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

Fig. 18A

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

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPG 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*
PGWFLDSPDRPWNPPTFSPALLVTEGDNATFTCSFSNT
SESFVLNWMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRAR
RNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLV

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

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

Plain: Anti-CD25 antibody

Italic: Linker

Bold: PD-1 ectodomain

Fig. 18B

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

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

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T *GGGSGGGSGGGSGGGGS*
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGSGGGGS*
 PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
 SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGYLCGAISLAPKAQIKESLRAELRVTERRAEVP
 TAHPSPSPRPAGQFQTLV

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

PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
 SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGYLCGAISLAPKAQIKESLRAELRVTERRAEVP
 TAHPSPSPRPAGQFQTLV *GGGSGGGSGGGSGGGGS*
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGK *GGGSGGGSGGGSGGGGS*
APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T

Underlined: IL-2 fragment*Italic: Linker*

Plain: Fc

Bold: human PD-1 ectodomain(Note: Can replace optional linker 1 *GGGSGGGSGGGSGGGGS* SEQ ID NO: 104 with*EPKSCDK* SEQ ID NO: 105)(Note: Can replace underlined aa in Fc: E with D and M with L)

Fig. 19

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

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

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSISIDTSKNLFSCLKLRSVTAADTAVYYCASNILKYLHWLLYWGQGLVTVSSASTKGPSVFPL
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
 TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
 PGWFLDSPDRPWNPPTFSPALLVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
 SQPGQDCRFRTQLPNGRDFHMSVVRARRNDSGYLCAISLAPKAQIKESLRAELRVTERRAEVP
 TAHPSPSPRPAGQFQTLV

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
 SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEV
 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 SQDCECCRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
GGGSGGGSGGGGS
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSLKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGK GGGSGGGSGGGGS
 PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
 SQPGQDCRFRTQLPNGRDFHMSVVRARRNDSGYLCAISLAPKAQIKESLRAELRVTERRAEVP
 TAHPSPSPRPAGQFQTLV

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

PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
 SQPGQDCRFRTQLPNGRDFHMSVVRARRNDSGYLCAISLAPKAQIKESLRAELRVTERRAEVP
 TAHPSPSPRPAGQFQTLVGGGSGGGSGGGGS
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSLKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGK GGGSGGGSGGGGS QI APPCTSEKHY EHLGRCCNKC
EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR
RACTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR
PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Bold: PD-1 ectodomain

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

Plain: IgG1 Fc

Underlined: RANK ectodomain

Fig. 21

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

1 MAPRARRRRP LFAALLLCAL LARLQVALQI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC
 61 TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW
 121 SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDCKR PWTNCTFLGK
 181 RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPGLIILLLF ASVALVAII FGVCYRKKGK
 241 ALTANLWHWI NEACGRLSGD KBSSGDSVSV THTANFGQQG ACEGVLLLTLL EEKTFPEDMC
 301 YPDQGGVCQG TCVGGGPY AQ GEDARMLSLV SKTEIEEDSF RQMPTEDEYM DRPSQPTDQL
 361 LFLTEPGSKS TPPFSEPLEV GENDSLSQCF TGTQSTVGSE SCNCTEPLCR TDWTPMSSSEN
 421 YLQKEVD SGH CPHWAASPSP NWADVCTGCR NPPGEDCEPL VGSPKRGPLP QCAYGMGLPP
 481 EEEASRTEAR DQPEDGADGR LPSSARAGAG SGSSPGGQSP ASGNVTGNSN STFISSGQVM
 541 NFKGDIIIVVY VSQTSQEGAA AAAEPMGRPV QEETLARRDS FAGNGPRFPD PCGGPEGLRE
 601 PEKASRPVQE QGGAKA

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

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA
 VVAGNSTTPR RACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDCKR
 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 LFAALLLCAL LARLQVALQI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC
 61 TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW
 121 SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDCKR 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 CDKCPPGTYL KQHCTAKWKT
 61 VCAPCPDHY TDSWHTSDEC LYCSPVCKEL QYVKQECNRT HNRVCECKEG RYLEIEFCLK
 121 HRSCPPGFGV VQAGTPERN T VCKRCPDGFF SNETSSKAPC RKHTNCSVFG LLLTQKGNAT
 181 HDNICS GNSE STQKCGIDVT LCEEAFRRFA VPTKFTPNWL SVLVDNLP GT KVNAESVERI
 241 KRQHSSQEQT FQLKLWKHQ NKAQDIVKKI IQDIDLCENS VQRHIGHANL TFEQLRSLME
 301 SLP GKVGAE 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):

EVQLVESGGGLVQPGGSLRLSCAASG FNIKDTYIHWRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISA
DTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAA
 LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDK
 KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH
 NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
 MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMH
 EALHNHYTQKSLSLSPGK **GGGGSGGGGSGGGGS** **QI** **APPCTSEKHY** **EHLGRCCNKC** **EPGKYMSSKC**
TTTSDSVCLP **CGPDEYLDW** **NEEDKCLLHK** **VCDTGKALVA** **VVAGNSTTPR** **RCACTAGYHW**
SQDCECCRRN **TECAPGLGAQ** **HPLQLNKDTV** **CKPCLAGYFS** **DAFSSTDKCR** **PWTNCTFLGK**
RVEHHGTEKS **DAVCSSSLPA** **RKPPNEPHVY** **LPG**

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

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFT
LTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
 KVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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):

QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGNTDYNTPFTSRLSINKD
NSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLVTVSAASTKGPSVFPLAPSSKSTSGGTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKR
VEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVME
ALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
TTTSDSVCLP CGPDEYLD SW 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):

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGSGSGTDFT
LSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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
 ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD
 KKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
 ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVDFCSVM
 HEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS* **QI** APPCTSEKHY EHLGRCCNKC EPGKYMSSKC
 TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RACTAGYHW
 SQDCECCRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
 RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFOQKPGSSPKPWIYATSNLASGVPVRFSGSGSGTSYSL
TISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
 VQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC

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
KRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWFYFDVWGQGTLLTVSSASTKG
 PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
 SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTF
 EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
 CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
 ENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS **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):

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSRFS
SGSG
TDFLTISLQPEDFATYYCQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
 NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEEKHKVYACEVTHQGL
 SSPVTKSFNRGEC

Underlined: Anti-VEGF antibody variable region

Plain: Anti-VEGF antibody constant region

Italics: Linker

Bold: RANK ectodomain

Fig. 26

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

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

QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYTMHWVRQA PGKGLEWVTF ISYDGNNKYY
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGQ GTLVTVSSAS
 TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL
 YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPELLGGPS
 VFLEPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
 KNQVSLTCLV KGFYPSTIAV EWESNGQOPEN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ
 GNVFSCSVMH EALHNHYTQK SLSLSPGK **GGGSGGGSGGGGS** **QI APPCTSEKHY EHLGRCCNKC**
EPGKYMSSKC **TTTSDSVCLP** **CGPDEYLD SW** **NEEDKCLLHK** **VCDTGKALVA** **VVAGNSTTPR**
RCACTAGYHW **SQDCECCRRN** **TECAPGLGAQ** **HPLQLNKDTV** **CKPCLAGYFS** **DAFSSTDKCR**
PWTNCTFLGK **RVEHHGTEKS** **DAVCSSSLPA** **RKPPNEPHVY** **LPG**

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

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

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 KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK *GGGSGGGSGGGGS* **QI** APPCTSEKHY EHLGRCCNKC
EPGKYMSSKC TTTSDSVCLP CGPDEYLDSE NEEDKCLLHK VCDTGKALVA VVAGNSTTPR
RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR
PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

DIQMTQSPST LSASVGDRVT ITCSASSSIS YMHWYQQKPG KAPKLLIYTT SNLASGVPAR
FSGSGSGTEF TLTISSLQPD DFATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS
DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLT
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 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 WLNQKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTL PPSRDELTKNQ
 VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
 FSCSVMEAL HNHYTQKSL LSPGK GGGGSGGGGSGGGGS **QI** APPCTSEKHY EHLGRCCNKC
 EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR
 RACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR
 PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

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

Plain: Anti-CD25 antibody

Italics: Linker

Bold: RANK ectodomain

Fig. 28B

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

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

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEEFLNRWITFCQSIISTLTGGGGSGGGSG
 GGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
 PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ
 VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHN
 HYTQKSLSLSPGK GGGGSGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR R**CACTAGYHW**
SQDCECCRRN **TECAPGLGAQ** **HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK**
RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR R**CACTAGYHW SQDCECCRRN** **TECAPGLGAQ HPLQLNKDTV**
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
 GGGSGGGSGGGSGGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
 PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV
 SCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGGSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKN
PKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMC
EYADETATIVEEFLNRWITFCQSIISTLT

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 or IEGRDMD)

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

Fig. 29

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

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

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSISIDTSKNLFLSLKLRSVTAADTAVYYCASNILKYLHWLLYWGQGVLTIVSSASTKGPSVFPL
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
 TQTYICNVNHKPSNTKVDKRVKPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
 GGGGSGGGGSGGGGS 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
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTLVLRGRTVAAPSVFIFPPSDEQLKSGTA
 SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEKHKVYACEV
 THQGLSSPVTKSFNRGEC

Underlined: Anti-CD4 antibody variable region

Plain: Anti-CD4 antibody constant region

Italic: Linker

Bold: PD-1 ectodomain

Fig. 30

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

MRIFAVFIFM TYWHLNNAFT VTPKDLVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME
DKNIIQFVHG EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG
ADYKRITVKV NAPYNKINQR ILVDPVTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT
TTNSKREEKL FNVSTLRIN TTTNEIFYCT FRRLDPEENH TAEVIPPEL LAHPPNERTH
LVILGAILLC LGVALTFIFR LRKGRMDVK KCGIQDTNSK KQSDTHLEET

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

AFT VTPKDLVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG
EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV
NAPYNKINQR ILVDPVTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL
FNVSTLRIN TTTNEIFYCT FRRLDPEENH TAEVI

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

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

RIFAVFIFM TYWHLNNAFT VTPKDLVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME
DKNIIQFVHG EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG
ADYKRITVKV NAPYNKINQR ILVDPVTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT
TTNSKREEKL FNVSTLRIN TTTNEIFYCT FRRLDPEENH TAEVIPPEL LAHPPNERTH
LVILGAILLC LGVALTFIFR LRKGRMDVK KCGIQDTNSK KQSDTHLEET

Bold: PD-L1 extracellular domain (ectodomain)

Underlined Bold: Ligand Binding domain

Fig. 31

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

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

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSVEG
RFTISRDNAKNSLYLQMNLSRAEDTAVYYCALVSYLSTASSLDYWGQGTLLVTVSSASTKGPSVFPL
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
 TQTYICNVNHKPSNTKVD**KKAE**PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
 GGGGSGGGGSGGGGS
 RIFAVFIFMTYWHLLNAFTVTVPKDLVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF
 VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
 YNKINQRILVVDVPTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
 NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR
 MMDVKKCGIQDTNSKKQSDTHLEET

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

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLPGKAPKLLIYAASLTQSGVPSRFS
SGTDFTLTISLQPEDVATYYCQRYNRPYTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASV
 VCLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSSTLTLSKADYEEKHKVYACEVTH
 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: 106 can be replaced with **KRVE** SEQ ID NO: 107
 or **KKVE** SEQ ID NO: 108)

Fig. 32

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

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

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSSDQVETQACTREQNRICTRPGWYCALSKEGCRCLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPMGPSPPAEGSTGD *EPKSCDK*
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
RIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
YNKINQRIILVVDVPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR
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):

MRIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQ
FVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
PYNKINQRIILVVDVPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFR
LRKGRMMDVKKCGIQDTNSKKQSDTHLEET *GGGGSGGGSGGGGS* *EPKSCDK*
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSSDQVETQACTREQNRICTRPGWYCALSKEGCRCLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPMGPSPPAEGSTGD

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 or GGGSGGGSGGGGS)

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

Fig. 33

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

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

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKG
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPL
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
 TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK
GGGSGGGSGGGGS
 RIFAVFIFMTYWHLLNAFTVTVPKDLVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF
 VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
 YNKINQRILVVDPVPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
 NTTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR
 MMDVKKCGIQDTNSKKQSDTHLEET

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFAQKPGSSPKPWIYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
 CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEEKHKVYACEVTHQ
 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 KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN
 VFSCSVMHEA LHNHYTQKSL SLSPGK *GGGGSGGGSGGGGS*
RIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECK
FPVEKQLDLAALIVWEMEDKNIIQFVHGEEEDLKVQHSSYRQRARLLKDQLSLGNA
ALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHEL
TCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYC
TFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

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

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

Plain: anti-CD25 antibody

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

Fig. 35A

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

QLQQSGTVLA RPGASVKMSC KASGYSFTRY 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 PREPQVYTL PPSRDELTKNQ
 VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
 FSCSVMEAL HNHYTQKSLS LSPGK *GGGGSGGGSGGGGS*

RIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECK

FPVEKQLDLAALIVYWEMEDKNIIQFVHGEEEDLKVQHSSYRQRARLLKDQLSLGNA

ALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPTSEHEL

TCQAEQYPAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYC

TFRRLDPEENHTAELVPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR

MMDVKKCGIQDTNSKKQSDTHLEET

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

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

Plain: anti-CD25 antibody

Italic: Linker

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

Fig. 35B

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

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

MRIFAVFIFMTYWHLLNAFTVTVPKDLVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQFVHGEE
DLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKV
NAPYNKINQRILVVDPTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTT
TNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFR
 LRKGRMMDVKKCGIQDTNSKKQSDTHLEET GGGGSGGGGSGGGGS
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
 CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMHEALHNHYTQ
 KSLSLSPGK GGGGSGGGGSGGGGS
APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

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

APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
 GGGGSGGGGSGGGGS
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
 CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMHEALHNHYTQ
 KSLSLSPGK GGGGSGGGGSGGGGS
RIFAVFIFMTYWHLLNAFTVTVPKDLVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQFVHGEE
LKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD
PVTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEE

T

Underlined: IL-2 fragment

Italic: Linker (optional)

Plain: Fc

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

(Note: Can replace linker 1 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
NRVSISIDTSKNLFSCLKRSVTAADTAVYYCASNILKYLHWLLYWQGVLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
GGGSGGGSGGGGS

RIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
YNKINQRILVVDVPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRITVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEV
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):

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQA
DSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLGI
GNGTQIYVIDPEPCPDS QEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
 ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
 YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
 GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
 GGGGSGGGGSGGGGS
 RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF
 VHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
 YNKINQRIILVVDVPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
 NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGR
 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):

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQ
FVHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
PYNKINQRIILVVDVPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFR
LRKGRMMDVKKCGIQDTNSKKQSDTHLEET GGGGSGGGGSGGGGS
 QEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
 QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV
 DKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSI
CTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLGIGNGTQIYVIDPEPCPDS

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

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

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

Italic: Linker (optional)

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

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

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

Fig. 38

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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
QHNPAGASAAPCCVPQALEPLPIVYVGRKPKVEQLSNMIVRSCKCS *GGGGSGGGSGGGGS*
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMHREALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
 RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF
 VHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
 YNKINQRIILVDPVTSEHELTCQAEYGPKEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
 NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTTFIFRLRKGR
 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):

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQ
FVHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNA
PYNKINQRIILVDPVTSEHELTCQAEYGPKEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTTFIFRLRKGR
RMMDVKKCGIQDTNSKKQSDTHLEET *GGGGSGGGSGGGGS*
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMHREALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPAGASAAPCCVPQALEPLPIVYVGRKPKVEQLSNMIVRSCKCS

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 *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. 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 VKRKRIEAIR GQILSKLRLS
61  SPPSQGEVPP VPLPEAVLAL YNSTRDRVAG ESAEPEPEPE ADYYAKEVTR VLMVENTNKI
121 YEKVKKSPHS IYMLFNTSEL REAVPEPVLL SRAELRLLRL KLKAEQHVEL YQKYSNDSWR
181 YLSNRLLAPS DTPEWLSFDV TGVVRQWLSH GGEVEGFRLS AHCSCDSKDN TLQVDINGFS
241 SSRRGDLATI HGMNRPFLLL MATPLERAQH LHSSRQRRAL DTNYCFSSTE KNCCVRQLYI
301 DFRKDLGWKW IHEPKGYHAN FCLGPCPYIW SLDTQYSKVL 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):

EVQLVESGGGLVQPGRSRLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSV
EG
RFTISRDNANKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
TQTYICNVNHKPSNTKVD**KKAE**PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGS
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Anti-TNF α light chain (SEQ ID NO: 77):

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLPQKAPKLLIYAASLTQSGVPSRFS
SGSG
SGTDFTLTISSLQPEDVATYYCQRYNRPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEVTH
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
WVPECLSCGSRCSDDQVETQACTREQNRICRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPMGPSPPAEGSTGD *EPKSCDK*
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMHREALHNHYTQKSLSLSPGK *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*
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED
 DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
 ISKAKGQPREPQVYTLPPSR**EEM**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD
 GSFFLYSKLTVDKSRWQQGNVFSCSVMHREALHNHYTQKSLSLSPGK *GGGSGGGSGGGGS*
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSDDQVETQACTREQNRICRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLPMGPSPPAEGSTGD

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

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
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
 TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
 ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
 ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
 QHNPGASAAPCCVFPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFAQKPGSSPKPWIYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASV
 CLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEEKHKVYACEVTHQ
 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
 NQKFQDKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LVTVSSASTK
 GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
 LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF
 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR
 VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
 QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN
 VFSCSVMHEA LHNHYTQKSL SLSPGK **GGGGSGGGSGGGGS** **ALDTNYCFSSTEKNCCVRQLY**
IDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPLP
IVYYVGRKPKVEQLSNMIVRSCKCS

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

DIQMTQSPST LSASVGDRVT ITCSASSSIS YMHWYQQKPG KAPKLLIYTT SNLASGVPAR
 FSGSGSGTEF TLTISLQPD DFATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS
 DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTTL
 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 VYYCSRDYGY YPFDWGQGT LTSSASTKG
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
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHAN
FCLGPCPYIWSLDTQYSKVLALYNQHNPASAPCCVPQALEPLPIVYYYVGRKPKVEQ
LSNMIVRSCKCS

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

QIVSTQSPAI MSASPGEKVT MTCSASSSRYSMQWYQQKPG TSPKRWIYDT SKLASGVPAR
FSGSGSGTSSLTSSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE
QLKSGTASVV CLLNMFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK
ADYEKHKVYA CEVTHQGLSS PVTKSFNRGEC

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
 QHNPASAAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS *GGGGSGGGSGGGGS*
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T

IL-2 + Fc + TGF- β 1 (SEQ ID NO: 57):

APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPL
EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTL
T *GGGGSGGGSGGGGS*
 THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
 ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
 QHNPASAAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

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:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNP
SLN
NRVSISIDTSKNLFSLKLRSVTAADTAVYYCASNILKYLHWLLYWGQGVLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGSGGGSGGGGS
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSKADYEKKHVKYACEV
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):

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLAASSRGIA SFVCEYASPGKATEVRVTVLRQA
DSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLGI
GNGTQIYVIDPEPCPDSDQEPKSCDK THTCPPCPAPELLGGPSVFLFPPKPKDTLM
 ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
 YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
 GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHYTQKSLSLSPGK
 GGGGSGGGGSGGGGS
 ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
 QHNP GASAAPCCVPQALEPLPIVYVGRKPKVEQLSNMIVRSCKCS

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 GASAAPCCVPQALEPLPIVYVGRKPKVEQLSNMIVRSCKCS EPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSVMSHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
AMHVAQPAVVLAASSRGIA SFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSI
CTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLGIGNGTQIYVIDPEPCPDSD

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

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

Plain: human IgG1 heavy chain constant region (IgG C 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):

EVQLVESGGGLVQPGRSRLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSV
EG
RFTISRDNKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG
TQTYICNVNHKPSNTKVD**KKAE****PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT**
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **QI** **APPCTSEKHY** **EHLGRCCNKC** **EPGKYMSSKC** **TTTSDSVCLP**
CGPDEYLDW **NEEDKCLLHK** **VCDTGKALVA** **VVAGNSTTPR** **RCACTAGYHW** **SQDCECCRRN**
TECAPGLGAQ **HPLQLNKDTV** **CKPCLAGYFS** **DAFSSTDKCR** **PWTNCTFLGK** **RVEHHGTEKS**
DAVCSSSLPA **RKPPNEPHVY** **LPG**

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

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLP GKAPKLLIYAAS
TLQSGVPSRFS
SGSG
SGTDFTLTIS
SLQPEDVATYYCQRYN
RAPYTFGQGTKVEIKRTVAAPS
VFIFPPSDEQLKSGTASV
VCLLNNFY
PREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYSL
SSTLTLSKADYEKHKVYACEVTH
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- κ B (RANK)
TNFR2 ECD + IgG C γ 1 + RANK ectodomain (SEQ ID NO: 62):

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLLLPMGPSPPAEGSTGD *EPKSCDK*
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMH~~EA~~LHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS* **QI** APPCTSEKHY
EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA
VVAGNSTTPR R~~CA~~CTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS
DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Fusion proteins comprising a RANKL-binding sequence of receptor activator of nuclear factor- κ B (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 CGPDEYLD SW
NEEDKCLLHK VCDTGKALVA VVAGNSTTPR R~~CA~~CTAGYHW SQDCECCRN TECAPGLGAQ
HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA
RKPPNEPHVY LPG
GGGGSGGGSGGGGS THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPS**REEM**TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFS~~CS~~VMHEALHNHYTQKSLSLSPGK *GGGGSGGGSGGGGS*
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWN
WVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGT
ETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPV
STRSQHTQPTPEPSTAPSTSFLLLPMGPSPPAEGSTGD

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):

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLAASSRGIA SFVCEYASPGKATEVRVTVL RQA
DSQVTEVCAATYMMGNELTFLDDSI CTGTSSGNQVNLT IQGLRAMDTGLYICKVELMYPPPYLGI
GNGTQIYVIDPEPCPDS DQEPKSCDK **THTCPPCPAPELLGGPSVFLFPPKPKDTLM**
 ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
 YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
 GQPENNYKTTTPVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK
 GGGGSGGGGSGGGGS **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP**
CGPDEYLD SW **NEEDKCLLHK VCDTGKALVA VVAGNSTTPR R** **CACTAGYHW S** **QDCECCRN**
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 R **CACTAGYHW S** **QDCECCRN** **TECAPGLGAQ**
HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA
RKPPNEPHVY LPG EPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYSKLTVDKSRWQQG
NV FSCSV MHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
AMHVAQPAVVLAASSRGIA SFVCEYASPGKATEVRVTVL RQADSQVTEVCAATYMMGNELTFLDDSI
CTGTSSGNQVNLT IQGLRAMDTGLYICKVELMYPPPYLGI 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)

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 GGGGSGGGGSGGGGS
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSV MHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS **QI** APPCTSEKHY
 EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW NEEDKCLLHK VCDTGKALVA
 VVAGNSTTPR RCTAGYHW SQDCECCRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS
 DAFSSTDCKR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD SW
 NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCTAGYHW SQDCECCRN TECAPGLGAQ
 HPLQLNKDTV CKPCLAGYFS DAFSSTDCKR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA
 RKPPNEPHVY LPG GGGGSGGGGSGGGGS
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSV MHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNP GASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

Underlined: Mature (active) human TGF- β 1

Italic: Linker (optional)

Plain: Fc

Bold: RANK ectodomain

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

EPKSCDK SEQ ID NO: 105)

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

Fig. 51

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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 PD-1 ligand 1

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

RIFAVFIFMTYWHL~~LLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEED~~
~~LKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD~~
~~PVTSEHELTCQAE~~GY~~PKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE~~
~~ENHTAELV~~~~IP~~~~EL~~~~PLA~~~~HPP~~~~NERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEE~~
 T ~~GGGGSGGGSGGGGS~~

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR~~EEM~~TKNQVSLT
 CLVKGFPYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS~~CS~~VMHEALHNHYTQ
 KSLSLSPGK ~~GGGGSGGGSGGGGS~~ QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP
 CGPDEYLD~~SW~~ NEEDKCLLHK VCDTGKALVA VVAGNSTTPR R~~ACTAGY~~HW ~~SQDCECCRRN~~
 TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTD~~KCR~~ PWTNCTFLGK RVEHHGTEKS
 DAVCSSSLPA RKPPNEPHVY LPG

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

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLD~~SW~~ NEEDKCLLHK
 VCDTGKALVA VVAGNSTTPR R~~ACTAGY~~HW ~~SQDCECCRRN~~ TECAPGLGAQ HPLQLNKDTV
 CKPCLAGYFS DAFSSTD~~KCR~~ PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
 GGGSGGGSGGGGS

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR~~EEM~~TKNQVSLT
 CLVKGFPYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS~~CS~~VMHEALHNHYTQ
 KSLSLSPGK ~~GGGGSGGGSGGGGS~~

RIFAVFIFMTYWHL~~LLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEED~~
~~LKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD~~
~~PVTSEHELTCQAE~~GY~~PKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE~~
~~ENHTAELV~~~~IP~~~~EL~~~~PLA~~~~HPP~~~~NERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEE~~
 T

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

Italic: Linker (optional)

Plain: Fc

Underlined: 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. 52

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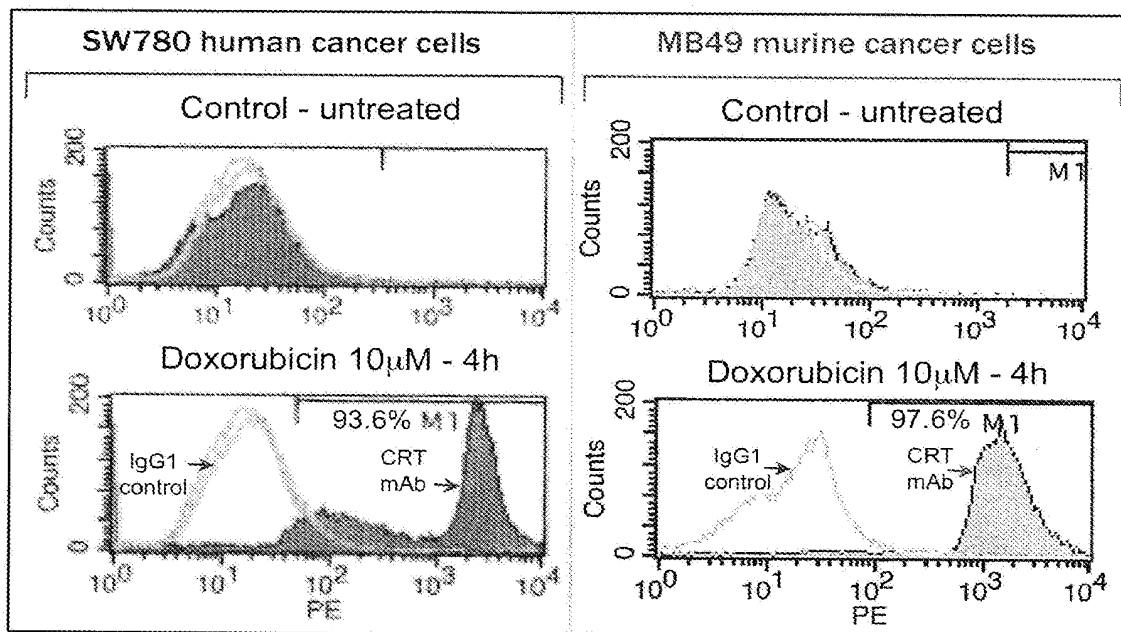
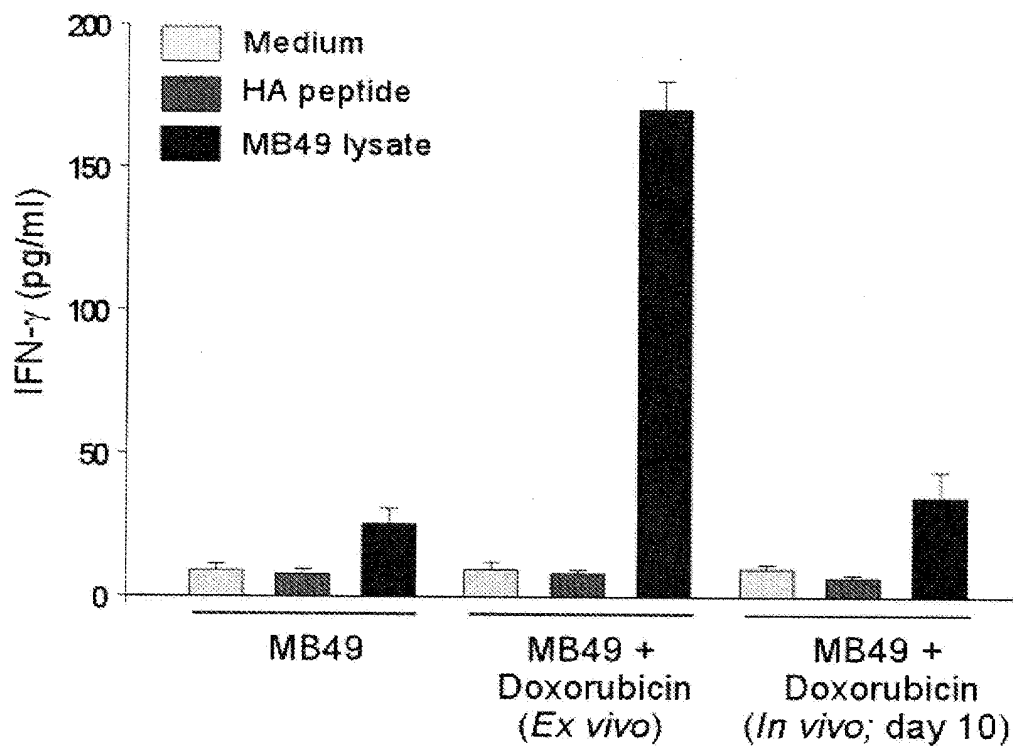
A**B**

Fig. 53 A & B

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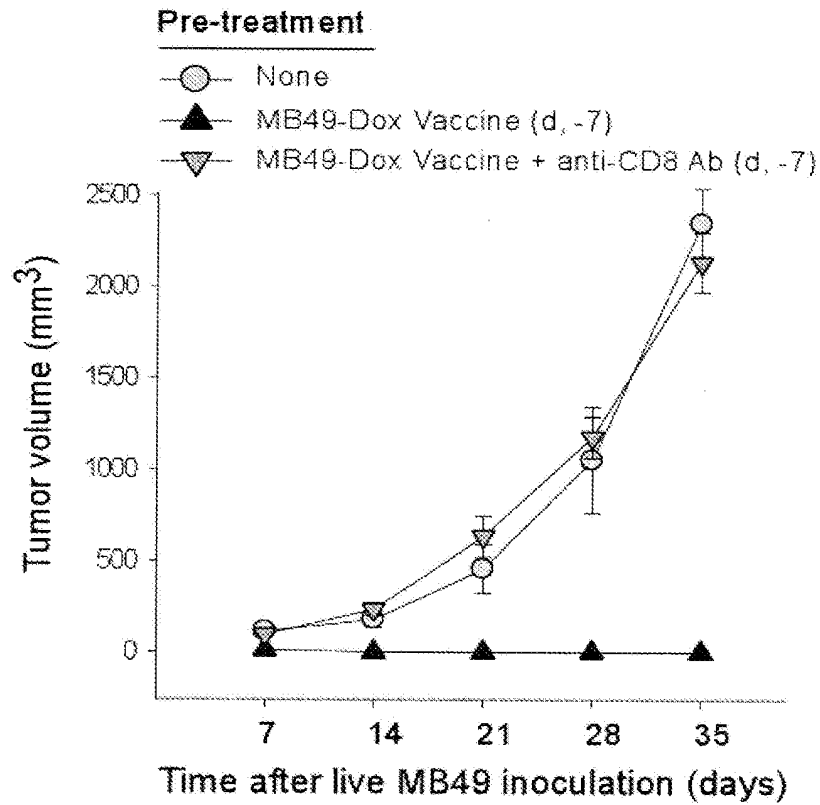
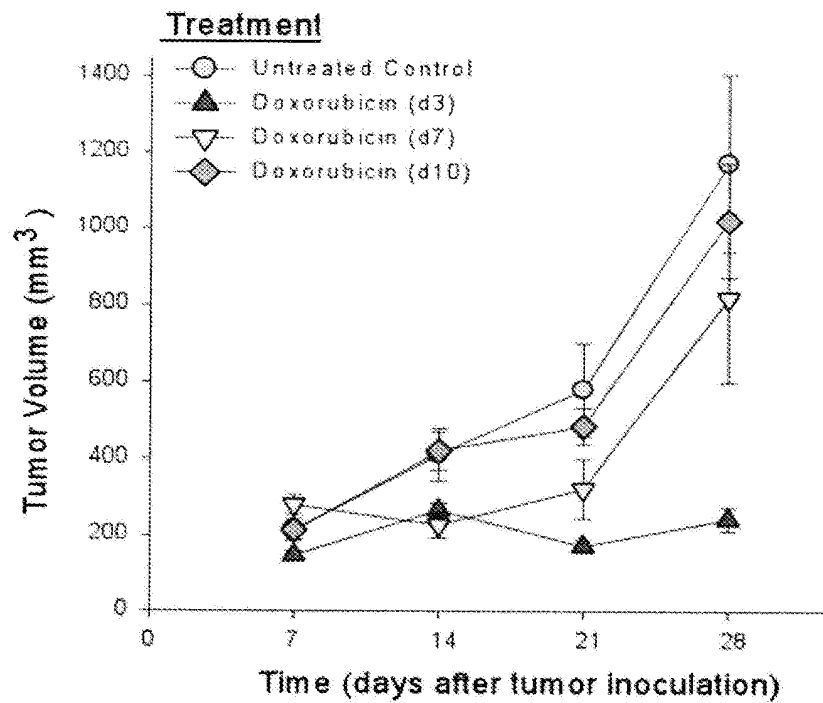
C**D**

Fig. 53 C & D

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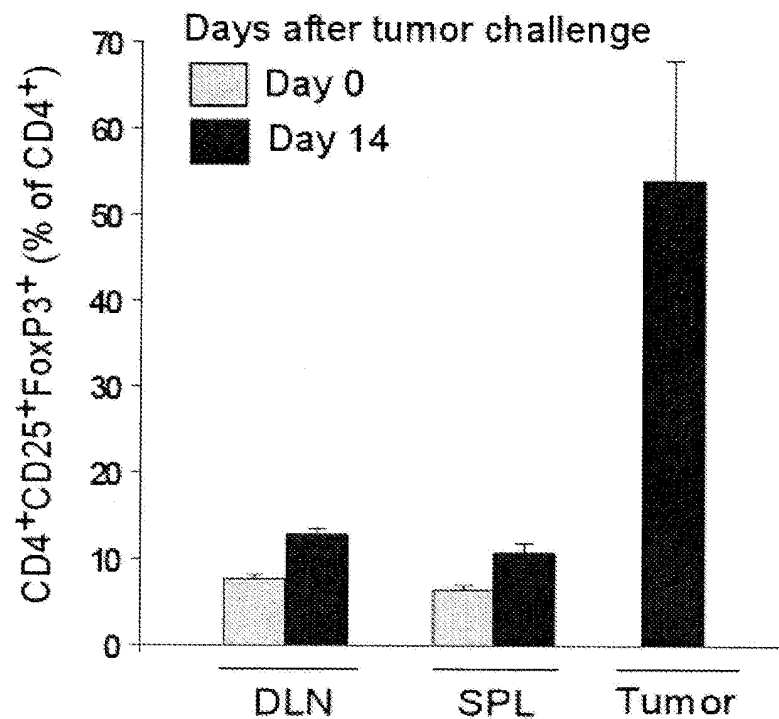
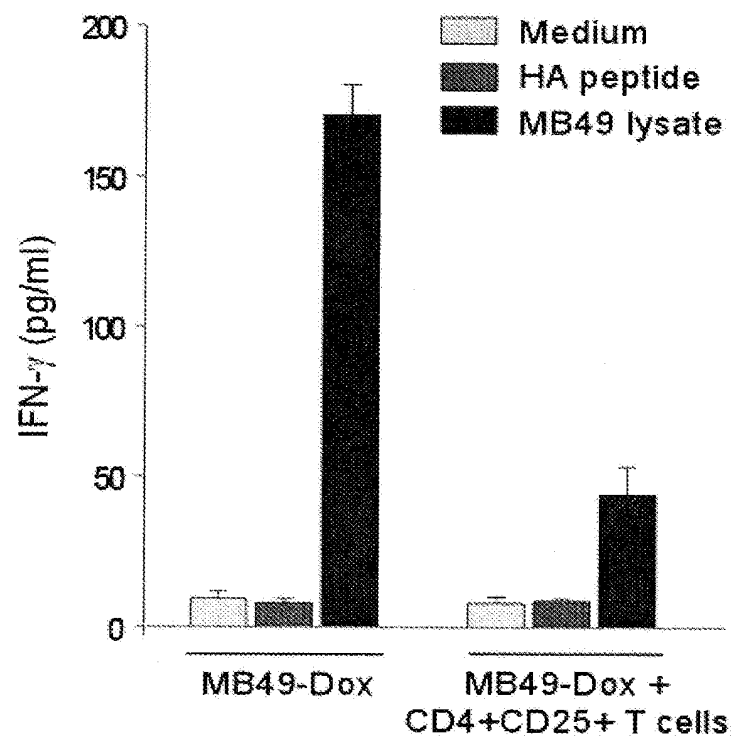
E**F**

Fig. 53 E & F

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G

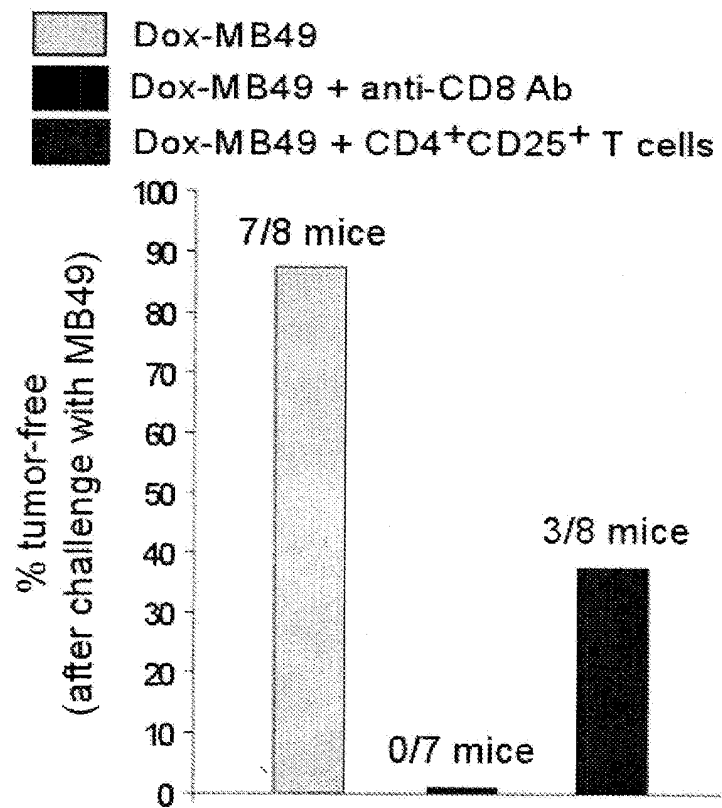


Fig. 53G

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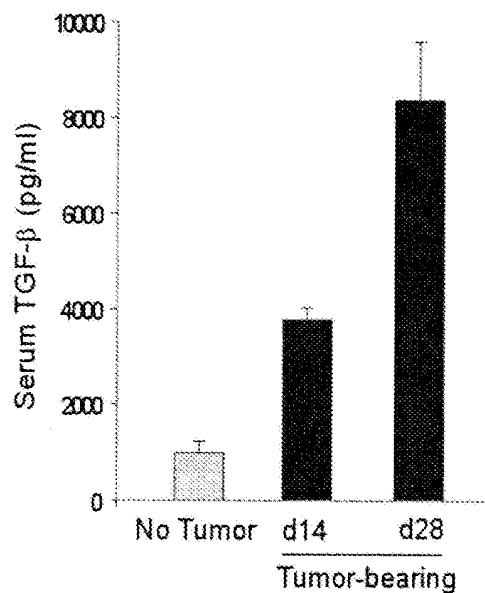
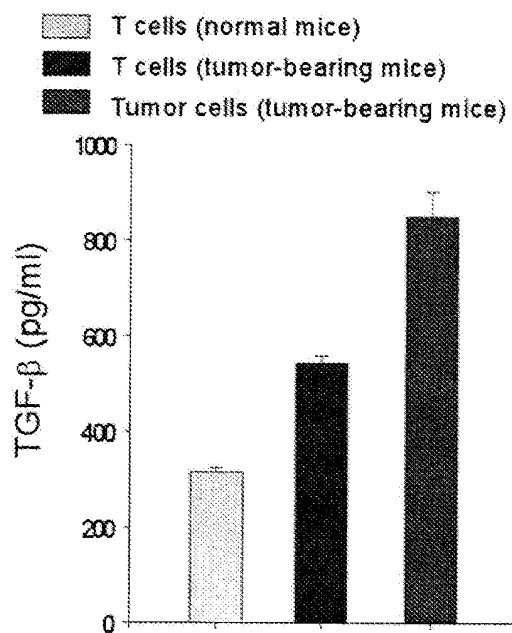
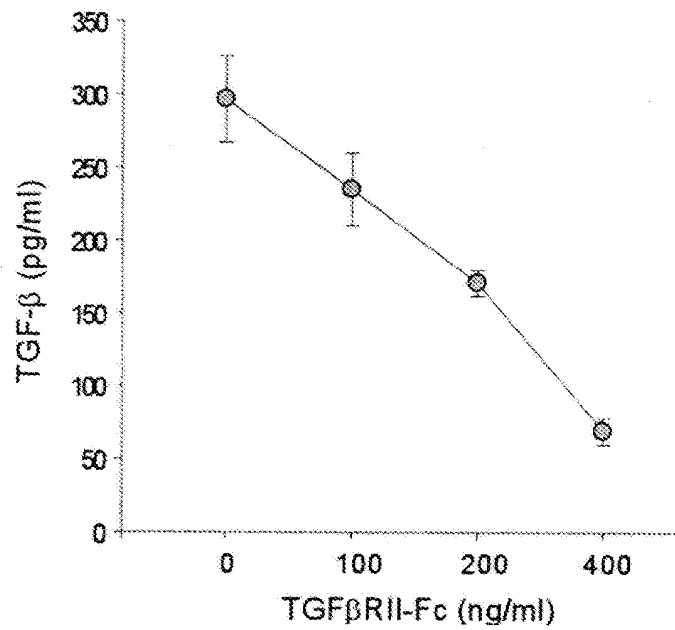
A**B**

Fig. 54 A & B

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C



D

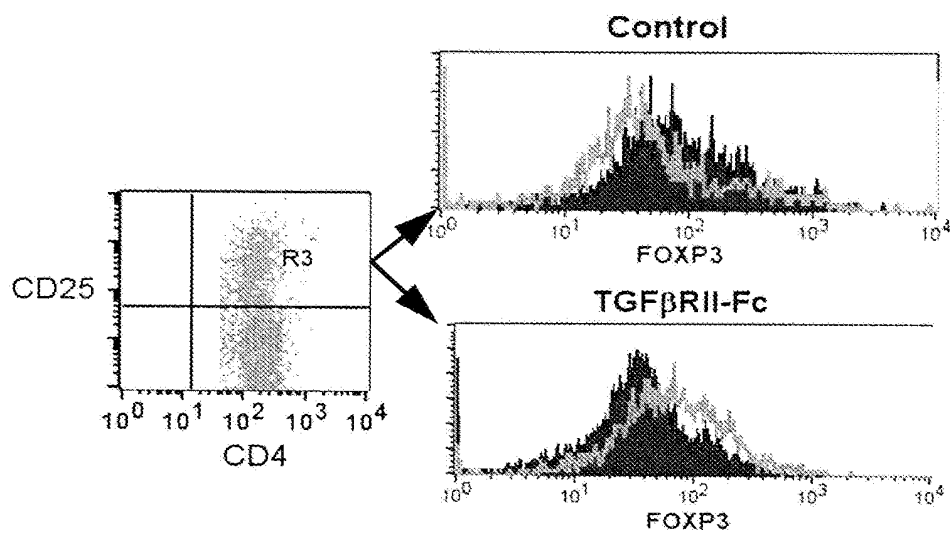


Fig. 54 C & D

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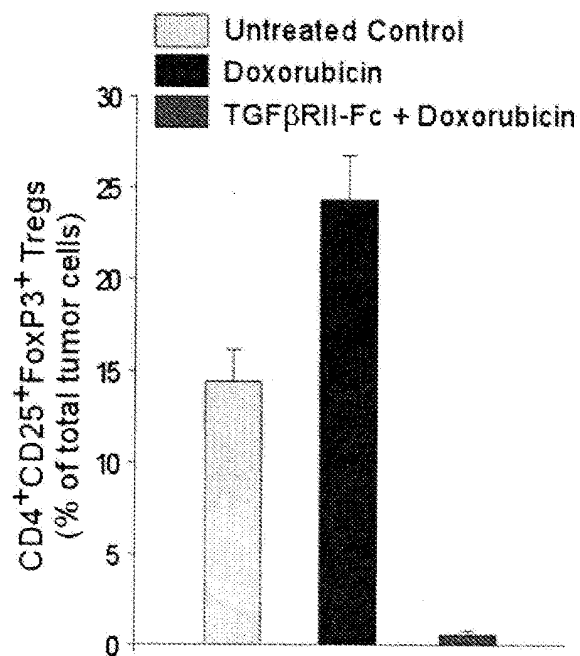
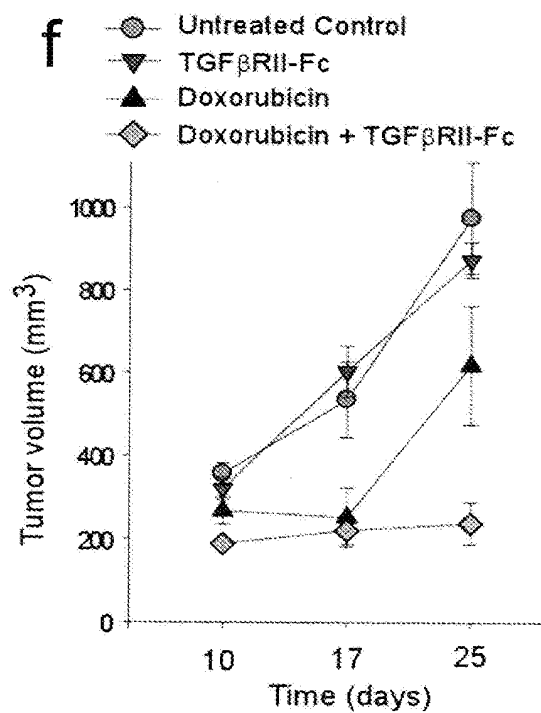
E**F**

Fig. 54 E & F

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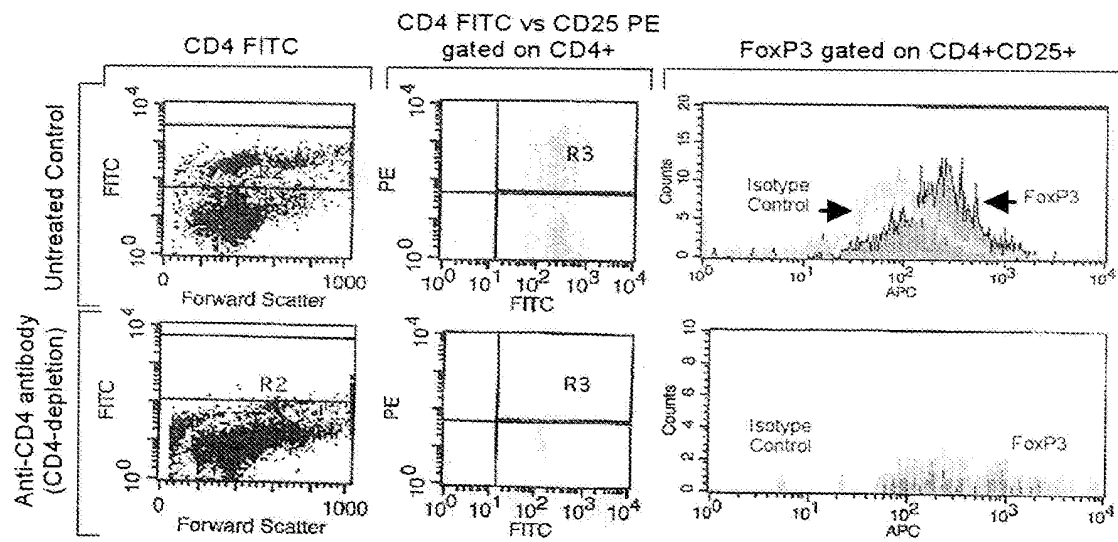
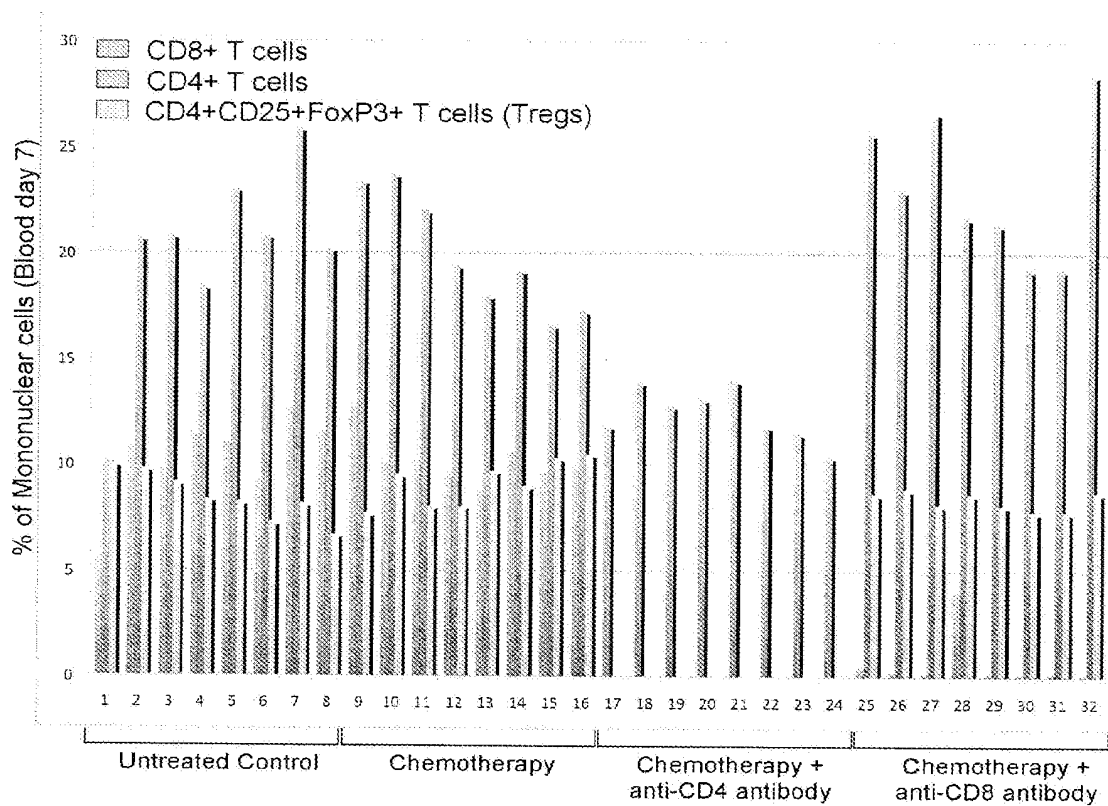
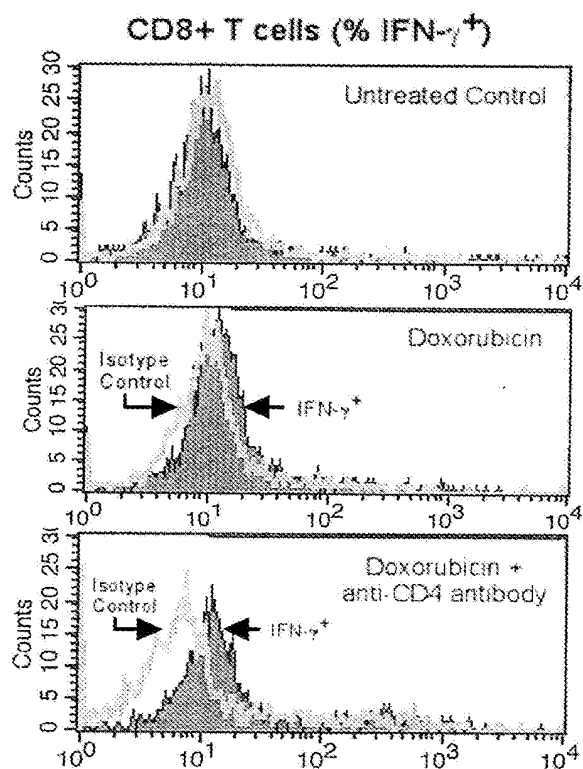
A**B**

Fig. 55 A & B

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C



D

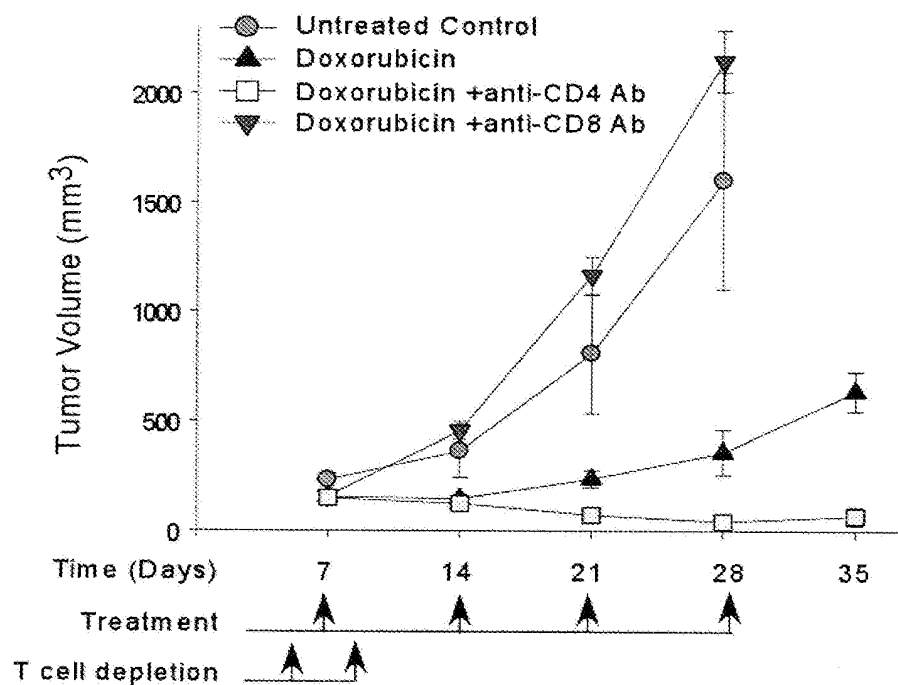
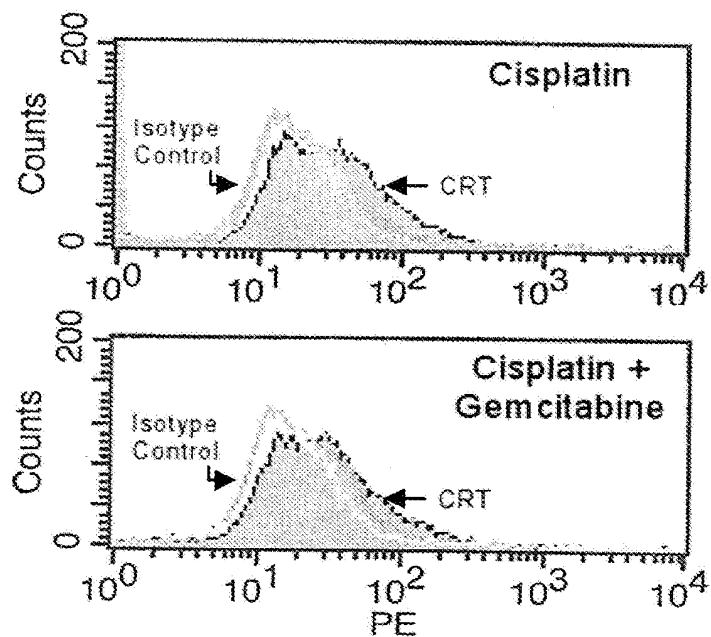


Fig. 55 C & D

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A



B

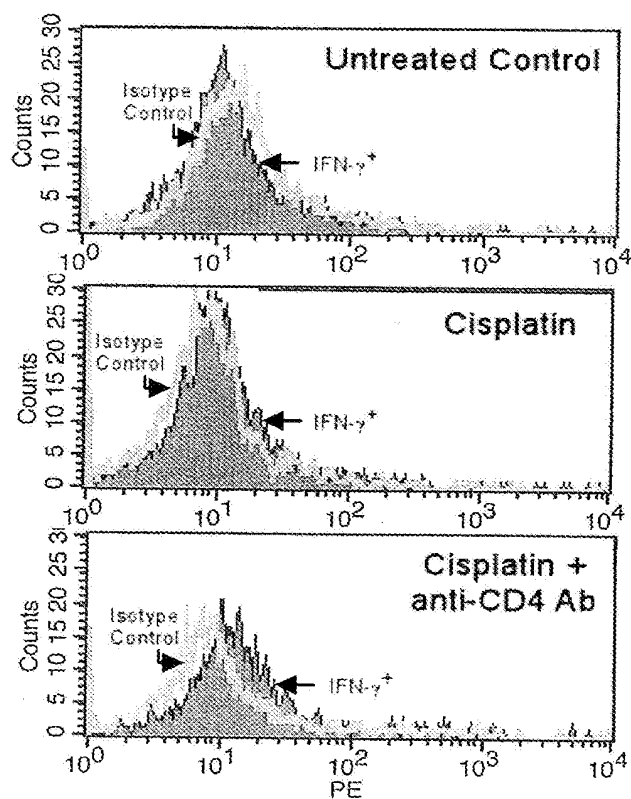


Fig. 56 A & B

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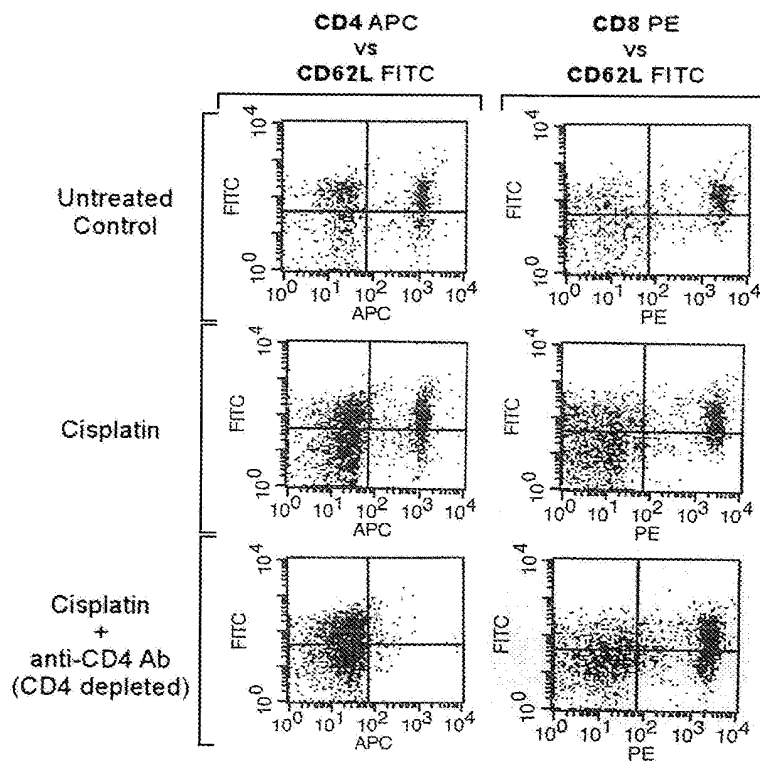
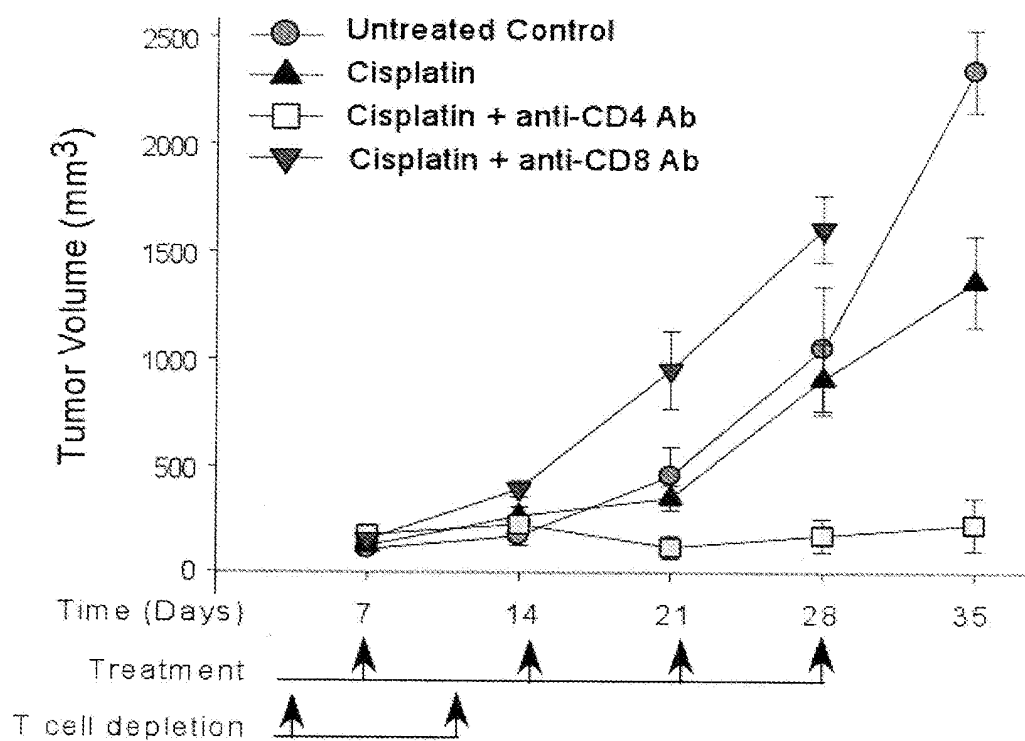
C**D**

Fig. 56 C & D

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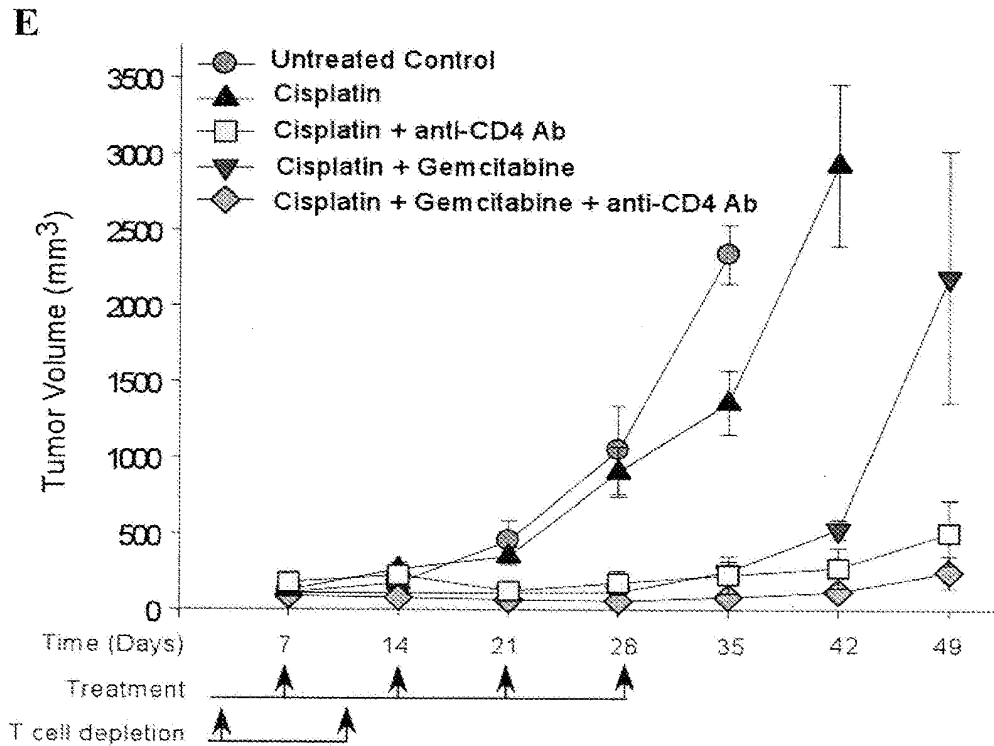
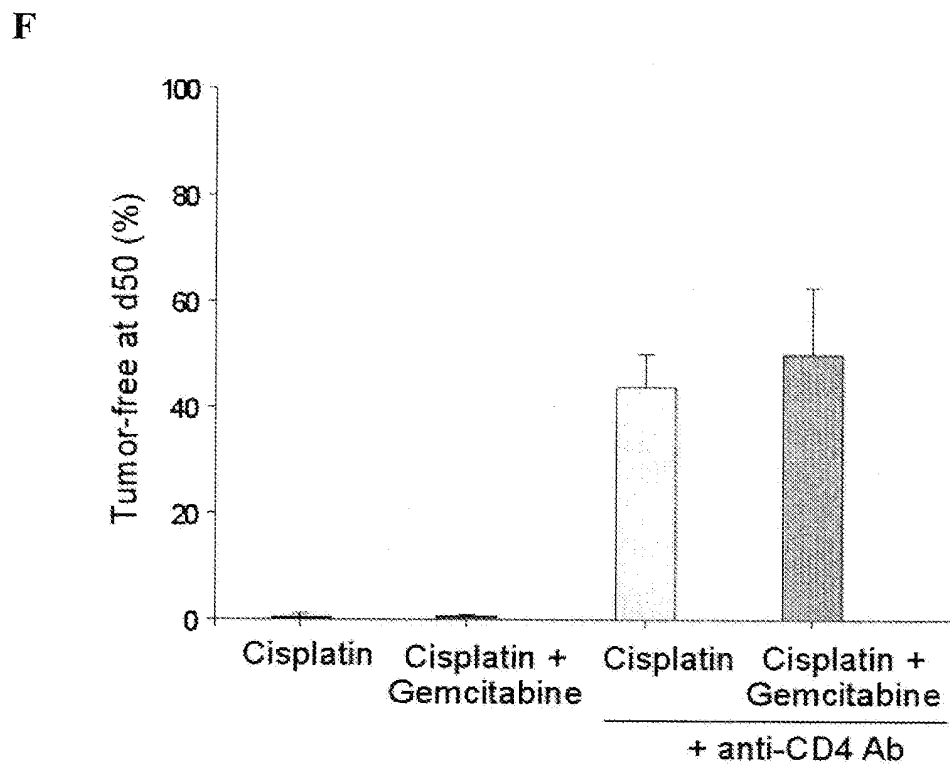


Fig. 56 E & F

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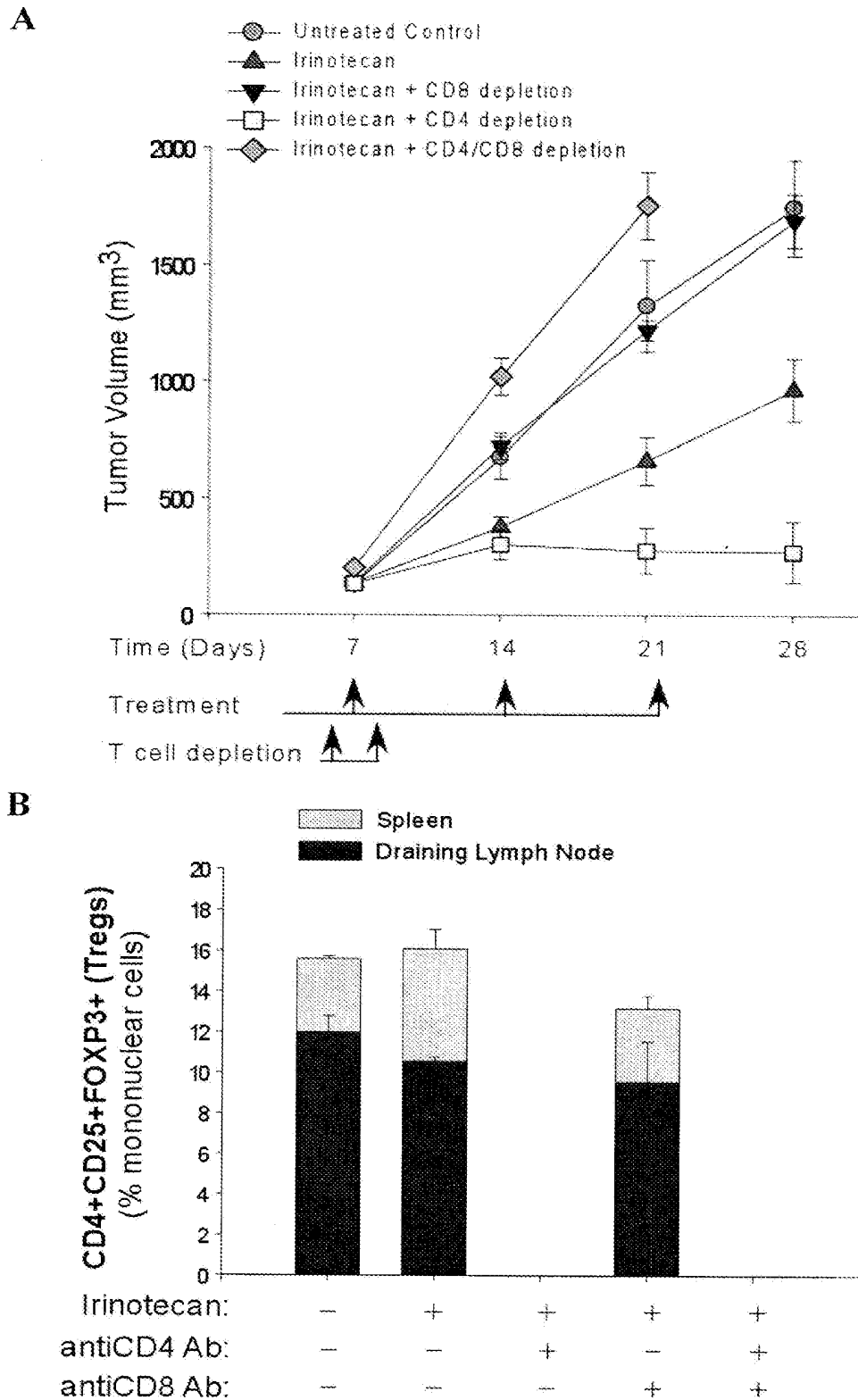


Fig. 57 A & B

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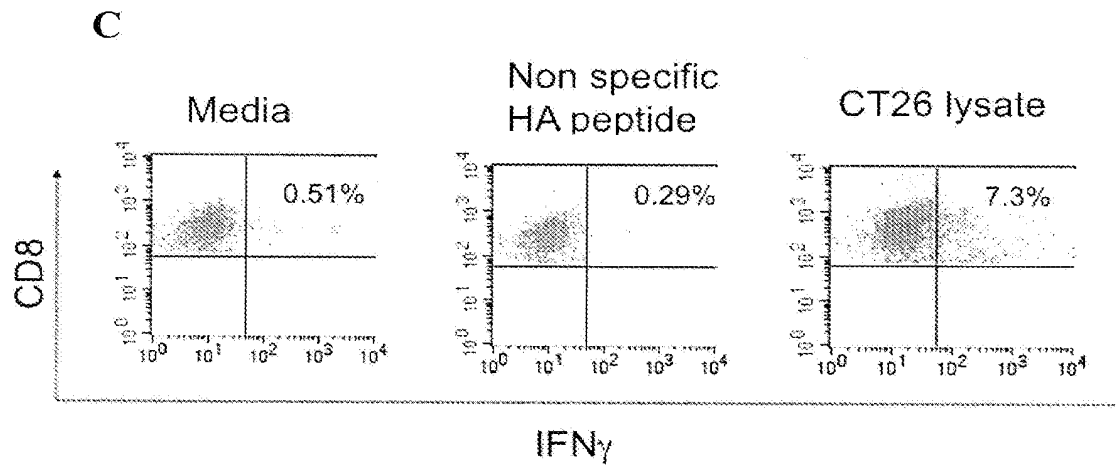
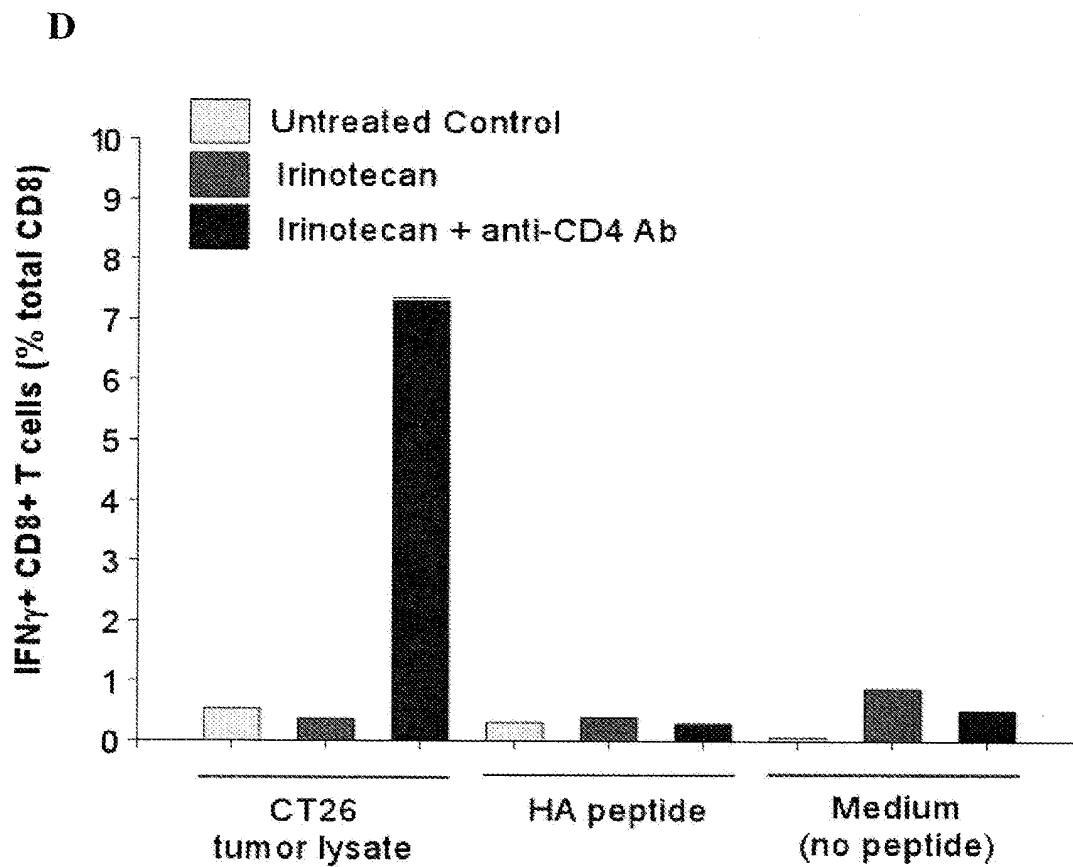


Fig. 57 C & D

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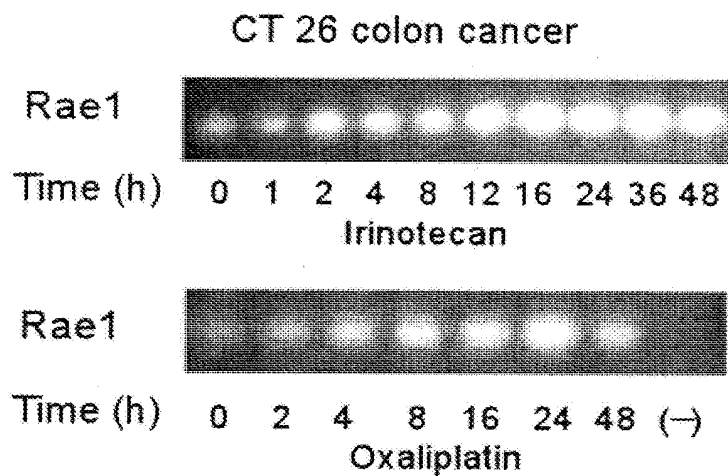
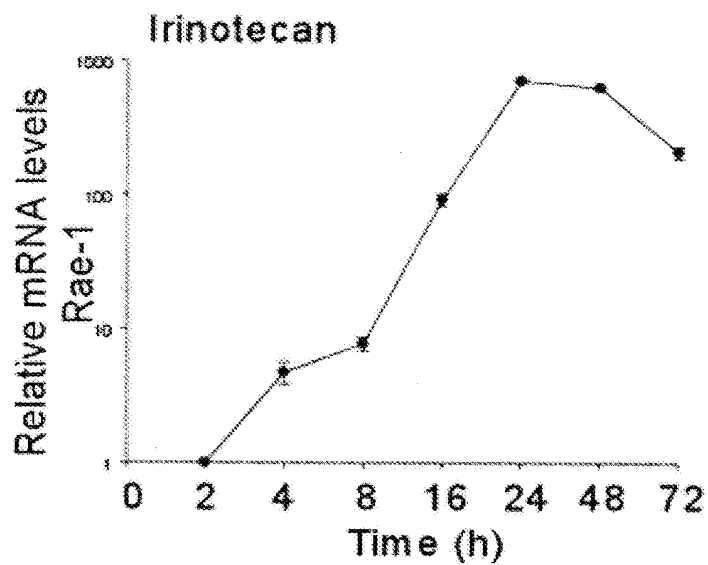
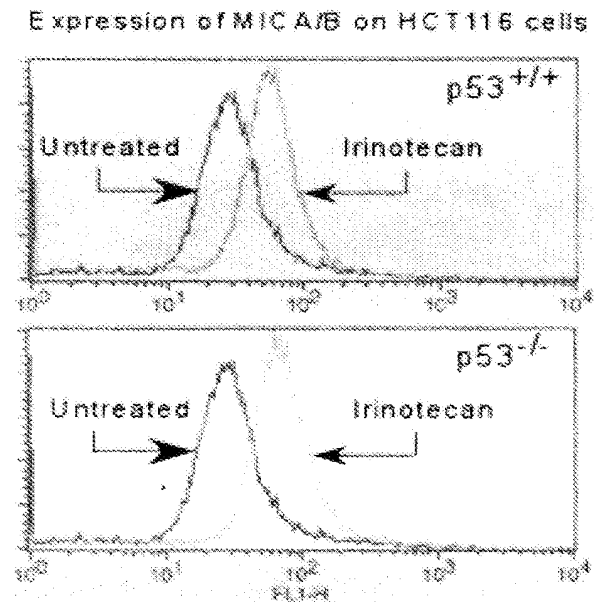
E**F**

Fig. 57 E & F

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G



H

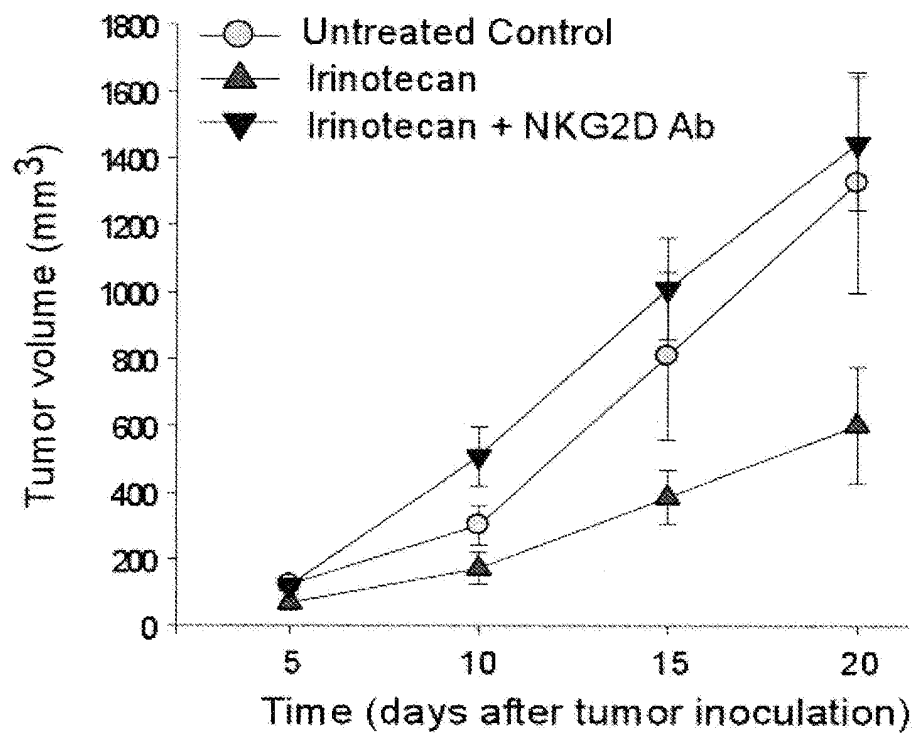


Fig. 57 G & H

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,

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

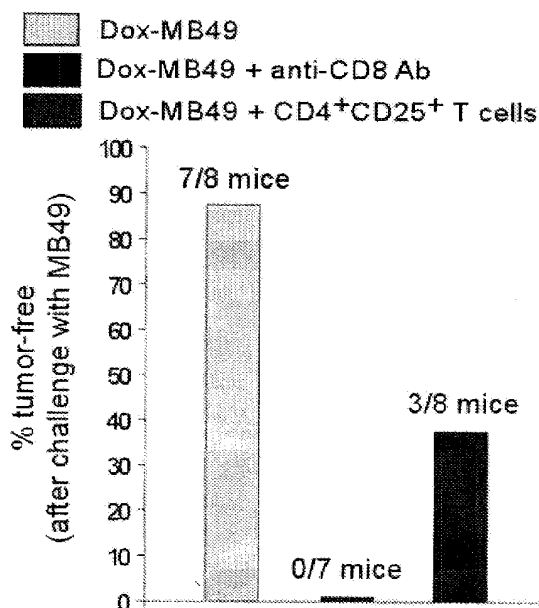


Fig. 53G

(57) Abstract: The present invention is based on the seminal discovery that targeted immunomodulatory antibodies and fusion proteins can counter act or reverse immune tolerance of cancer cells. Cancer cells are able to escape elimination by chemotherapeutic agents or tumor-targeted antibodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. Such immunosuppressive mechanisms include immunosuppressive cytokines (for example, Transforming growth factor beta (TGF- 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.



LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

— before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments (Rule 48.2(h))

— with sequence listing part of description (Rule 5.2(a))

(88) Date of publication of the international search report:

5 April 2012

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

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875					Application or Docket Number 13/582,717		Filing Date 10/17/2012		☐ To be Mailed		
APPLICATION AS FILED – PART I											
(Column 1)			(Column 2)			SMALL ENTITY ☒ OR		OTHER THAN SMALL ENTITY			
FOR	NUMBER FILED	NUMBER EXTRA	RATE (\$)	FEE (\$)			RATE (\$)	FEE (\$)			
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A				N/A				
☐ SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A	N/A				N/A				
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A				N/A				
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X \$	=	OR		X \$	=			
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X \$	=	OR		X \$	=			
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$250 (\$125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).										
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))											
* If the difference in column 1 is less than zero, enter "0" in column 2.											
APPLICATION AS AMENDED – PART II											
(Column 1)			(Column 2)			SMALL ENTITY		OR		OTHER THAN SMALL ENTITY	
AMENDMENT	09/04/2012	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)			RATE (\$)	ADDITIONAL FEE (\$)
	Total (37 CFR 1.16(i))	* 53	Minus	** 67	= 0	X \$30 =	0	OR		X \$ =	
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	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))										
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AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)			RATE (\$)	ADDITIONAL FEE (\$)
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	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))										
						TOTAL ADD'L FEE		OR		TOTAL ADD'L FEE	
<p>* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.</p> <p>** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".</p> <p>*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".</p> <p>The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.</p>											

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Legal Instrument Examiner:
/ROSS W. BROWN/

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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: Anne Corrigan

Timestamp: [year=2012; month=9; day=18; hr=11; min=10; sec=41; ms=751;]

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Asn



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U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
13/582,717	Atul BEDI	JHU3260-4

100899

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

INTERNATIONAL APPLICATION NO.	
PCT/US2011/027317	
I.A. FILING DATE	PRIORITY DATE
03/04/2011	03/05/2010

CONFIRMATION NO. 3707
371 FORMALITIES LETTER



OC000000056610879

Date Mailed: 09/19/2012

**NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371
IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)**

The following items have been submitted by the applicant or the IB to the United States Patent and Trademark Office as a Designated Office (37 CFR 1.494):

- Indication of Small Entity Status
- Priority Document
- Copy of the International Application filed on 09/04/2012
- Copy of the International Search Report filed on 09/04/2012
- Copy of IPE Report filed on 09/04/2012
- Preliminary Amendments filed on 09/04/2012
- Information Disclosure Statements filed on 09/04/2012
- Biochemical Sequence Diskette filed on 09/04/2012
- Biochemical Sequence Listing filed on 09/04/2012
- Request for Immediate Examination filed on 09/04/2012
- U.S. Basic National Fees filed on 09/04/2012
- Priority Documents filed on 09/04/2012
- Specification filed on 09/04/2012
- Claims filed on 09/04/2012
- Abstracts filed on 09/04/2012
- Drawings filed on 09/04/2012

The following items **MUST** be furnished within the period set forth below in order to complete the requirements for acceptance under 35 U.S.C. 371:

- Oath or declaration of the inventors, in compliance with 37 CFR 1.497(a) and (b), identifying the application by the International application number and international filing date.

ALL OF THE ITEMS SET FORTH ABOVE MUST BE SUBMITTED WITHIN TWO (2) MONTHS FROM THE DATE OF THIS NOTICE OR BY 32 MONTHS FROM THE PRIORITY DATE FOR THE APPLICATION, WHICHEVER IS LATER. FAILURE TO PROPERLY RESPOND WILL RESULT IN ABANDONMENT.

The time period set above may be extended by filing a petition and fee for extension of time under the provisions of 37 CFR 1.136(a).

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.

<https://portal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html>

For more information about EFS-Web please call the USPTO Electronic Business Center at **1-866-217-9197** or visit our website at <http://www.uspto.gov/ebc>.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

VONDA M WALLACE

Telephone: (571) 272-3734

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875						Application or Docket Number 13/582,717			
APPLICATION AS FILED - PART I									
(Column 1)		(Column 2)		SMALL ENTITY		OTHER THAN SMALL ENTITY			
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INDEPENDENT CLAIMS (37 CFR 1.16(h))	2	minus 3 = *	x 125 =	0.00					
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			310					
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* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL		TOTAL			
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	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					OR			
				TOTAL ADD'L FEE		TOTAL ADD'L FEE			
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	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					OR			
				TOTAL ADD'L FEE		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>									

<div> <div> MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET </div> <div> Substitute for Form PTO-1360 (For use with Form PTO/SB/06) </div> </div>							<div> Application Number 13582717 </div>		<div> Filing Date </div>					
							<div> Applicant(s) Atul BEDI </div>							
							<div> * May be used for additional claims or amendments </div>							
CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT				*		*		*	
	Indep	Depend	Indep	Depend	Indep	Depend			Indep	Depend	Indep	Depend	Indep	Depend
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Total Indep	2		2		0									
Total Depend	65	↙	51	↙	0	↙								
Total Claims	67		53		0									

51		1		1		
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53		1	---	---		
54		(1)	---	---		
55		(1)		1		
56		(1)		1		
57		(1)		1		
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59		(1)		1		
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	Not Yet Assigned
Application No.:	13/582,717	Examiner:	Not Yet Assigned
I.A. Filing Date:	March 4, 2011	Conf. No.:	3707
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

Mail Stop PCT

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

RESPONSE TO NOTIFICATION OF MISSING REQUIREMENTS UNDER 35
U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE
(DO/EO/US)

Sir:

In response to the Notification of Missing Requirements under 35 U.S.C. 371 mailed September 19, 2012, enclosed are:

1. One (1) executed Declaration for Patent Application (4 pages); and
2. One (1) Power of Attorney by Assignee (2 pages).

In re Application of:
Bedi and Ravi
Application No.: 13/582,717
I.A. Filing Date: March 4, 2011
Page 2

PATENT
Attorney Docket No.: JHU3260-4

Applicants claim **SMALL ENTITY** status in the above-identified application.

Pursuant to 37 C.F.R. § 1.27, a verified statement claiming small entity status is not required.

The filing fee has been calculated as follows.

	Number Filed		Number Extra		Rate			Fee	
					Large Entity	Small Entity		Large Entity	Small Entity
Total Claims	53-20	=	33	X		\$30	=		[\$ 990.00]
Independent Claims	2-3	=	0	X		\$125	=		[\$.00]
Multiple Dependent Claims Present: ____ Yes <u>x</u> No						\$225			N/A
Fee for Each Additional 50 Sheets in Excess of 100 Pages						\$155			[\$ 310.00]
					ELECTRONIC FILING FEE				[\$ 190.00]
					SEARCH FEE				[\$ 245.00]
					EXAM FEE				[\$ 125.00]
					SURCHARGE FEE				[\$ 65.00]
					TOTAL FEE				[\$1,925.00]

No fee is deemed necessary in connection with the filing of this paper. However, if a fee is required, 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.

Respectfully submitted,

Date: October 17, 2012


Lisa A. Haile, J.D., Ph.D.
Registration No.: 38,347
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

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

WEST\239226282.1
331323-000997

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Bedi and Ravi	Art Unit:	Not Yet Assigned
Application No.:	13/582,717	Examiner:	Not Yet Assigned
IA Filing Date:	March 4, 2011	Conf. No.:	3707
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

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

POWER OF ATTORNEY BY ASSIGNEE

As the below-named assignee of the above-identified application
("Application"), **THE JOHNS HOPKINS UNIVERSITY**, through its duly-delegated
representative, hereby appoints the attorneys of **USPTO CUSTOMER NO. 100899** at
DLA Piper LLP (US) to prosecute the Application for the assignee and transact all business
in the United States Patent and Trademark Office connected therewith.

Statement under 37 C.F.R. §3.73(b):

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

THE JOHNS HOPKINS UNIVERSITY hereby authorizes and requests
insertion of the Assignment Reel and Frame numbers when officially known.

In re Application of:
Bedi and Ravi
Application No.: 13/582,717
IA Filing Date: March 4, 2011
Page 2

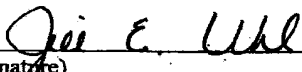
PATENT
Attorney Docket No.: JHU3260-4

Direct all telephone calls to:
LISA A. HAILE, J.D., Ph.D.
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

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

DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

THE JOHNS HOPKINS UNIVERSITY

By : 
(Signature)
Name : Jill E. Uhl
(Typed or Printed Name)
Title : Director
Date : September 7, 2012

WEST\238217409.1
331323-000997

Electronic Acknowledgement Receipt

EFS ID:	14012636
Application Number:	13582717
International Application Number:	
Confirmation Number:	3707
Title of Invention:	Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins
First Named Inventor/Applicant Name:	Atul BEDI
Customer Number:	100899
Filer:	Lisa A. Haile/Aldon Griffis
Filer Authorized By:	Lisa A. Haile
Attorney Docket Number:	JHU3260-4
Receipt Date:	17-OCT-2012
Filing Date:	
Time Stamp:	20:09:21
Application Type:	U.S. National Stage under 35 USC 371

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	Applicant Response to Pre-Exam Formalities Notice	C11123_P11123-03_20121017_OTH_DLAP_RespPreExamForm Notice.pdf	151633	no	2
			effa03a701fb362134004c64928f8edb90ee152		

Warnings:

Information:

2	Oath or Declaration filed	C11123_P11123-03_20121004_OTH_DLAP_ExecutedDeclaration.pdf	253790 d45deb1258fa278f2432327275e7157faac89fb8	no	4
Warnings:					
Information:					
3	Power of Attorney	C11123_P11123-03_20120907_OTH_DLAP_ExecutedPowerAttorney.pdf	58460 f579f195d51b6b7860b4f1a027fb3eed4fb93b69	no	2
Warnings:					
Information:					
Total Files Size (in bytes):			463883		
<p>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.</p> <p><u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p>					

DECLARATION FOR PATENT APPLICATION

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS**, the specification of which

_____ is attached hereto.

X was filed on September 4, 2012, with an International Application date of March 4, 2011 (Attorney Docket No.: JHU3260-4WO), as U.S. Application Serial No. 13/582,717 and was amended on _____ if applicable (the "Application").

I hereby authorize and request insertion of the application serial number of the Application when officially known.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability of the subject matter of the Application as defined in Title 37, Code of Federal Regulations ("C.F.R."), § 1.56.

With respect to the Application, I hereby claim the benefit under 35 U.S.C.
Section 119(e) of any United States provisional application(s) listed below:

<u>61/435,671</u> (Application Serial No.)	<u>January 24, 2011</u> (Filing Date)
<u>61/311,255</u> (Application Serial No.)	<u>March 5, 2010</u> (Filing Date)
<u> </u> (Application Serial No.)	<u> </u> (Filing Date)

With respect to the Application, I hereby claim the benefit under 35 U.S.C.
Section 120 of any United States application(s), or Section 365(c) of any PCT International
application designating the United States, listed below and, insofar as the subject matter of
each of the claims of the application is not disclosed in the prior United States or PCT
International application in the manner provided by the first paragraph of 35 U.S.C.
Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark
Office all information known to me to be material to patentability of the subject matter of the
Application as defined in Title 37, C.F.R., Section 1.56 which became available between the
filing date of the prior application and the national or PCT International filing date of the
Application:

<u>PCT/US2011/027317</u> (Application Serial No.)	<u>March 4, 2011</u> (Filing Date)	<u>pending</u> (Status) (pending, expired)
<u> </u> (Application Serial No.)	<u> </u> (Filing Date)	<u> </u> (Status) (patented, pending, abandoned)
<u> </u> (Application Serial No.)	<u> </u> (Filing Date)	<u> </u> (Status) (patented, pending, abandoned)

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

COUNTRY	APPLICATION NO.	FILING DATE	PRIORITY CLAIMED
_____	_____	_____	☐ Yes ☐ No

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first inventor: **Atul Bedi**

Inventor's signature: *Atul Bedi*

Date: October 4th, 2012

Residence: Timonium, Maryland

Country of Citizenship: US

Post Office Address: 2211 Dalewood Road
Timonium, MD 21093

ATTORNEY DOCKET NO.:
JHU3260-4

Full name of second inventor: **Rajani Ravi**

Inventor's signature: Rajani Ravi

Date: October 4th 2012

Residence: Ruxton, Maryland

Country of Citizenship: US

Post Office Address: 7810 Ballston Road
Ruxton, MD 21204



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
13/582,717	Atul Bedi	JHU3260-4

100899

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

INTERNATIONAL APPLICATION NO.	
PCT/US2011/027317	
I.A. FILING DATE	PRIORITY DATE
03/04/2011	03/05/2010

CONFIRMATION NO. 3707
371 ACCEPTANCE LETTER



Date Mailed: 11/02/2012

NOTICE OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C 371 AND 37 CFR 1.495

The applicant is hereby advised that the United States Patent and Trademark Office in its capacity as a Designated / Elected Office (37 CFR 1.495), has determined that the above identified international application has met the requirements of 35 U.S.C. 371, and is ACCEPTED for national patentability examination in the United States Patent and Trademark Office.

The United States Application Number assigned to the application is shown above and the relevant dates are:

10/17/2012
DATE OF RECEIPT OF 35 U.S.C. 371(c)(1),
(c)(2) and (c)(4) REQUIREMENTS

10/17/2012
DATE OF COMPLETION OF ALL
35 U.S.C. 371 REQUIREMENTS

A Filing Receipt (PTO-103X) will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE " FILING DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371 (c)(1), (c)(2) and (c)(4) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN ABOVE.** The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363). Once the Filing Receipt has been received, send all correspondence to the Group Art Unit designated thereon.

The following items have been received:

- Indication of Small Entity Status
- Copy of the International Application filed on 09/04/2012
- Copy of the International Search Report filed on 09/04/2012
- Copy of IPE Report filed on 09/04/2012
- Preliminary Amendments filed on 09/04/2012
- Information Disclosure Statements filed on 09/04/2012
- Biochemical Sequence Diskette filed on 09/04/2012
- Oath or Declaration filed on 10/17/2012
- Biochemical Sequence Listing filed on 09/04/2012
- Request for Immediate Examination filed on 09/04/2012
- U.S. Basic National Fees filed on 09/04/2012
- Priority Documents filed on 09/04/2012
- Power of Attorney filed on 10/17/2012
- Specification filed on 09/04/2012
- Claims filed on 09/04/2012
- Abstracts filed on 09/04/2012

page 1 of 2

- Drawings filed on 09/04/2012

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

VONDA M WALLACE

Telephone: (571) 272-3734

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875						Application or Docket Number 13/582,717			
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	195		N/A			
SEARCH FEE <small>(37 CFR 1.16(k), (l), or (m))</small>	N/A	N/A	N/A	250		N/A			
EXAMINATION FEE <small>(37 CFR 1.16(o), (p), or (q))</small>	N/A	N/A	N/A	125		N/A			
TOTAL CLAIMS <small>(37 CFR 1.16(i))</small>	53	minus 20 = * 33	x 31 =	1023	OR				
INDEPENDENT CLAIMS <small>(37 CFR 1.16(h))</small>	2	minus 3 = *	x 125 =	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).			320					
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00					
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1913		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 **	=	x =	OR	x =		
	Independent <small>(37 CFR 1.16(h))</small>	*	Minus ***	=	x =	OR	x =		
	Application Size Fee (37 CFR 1.16(s))					OR			
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					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 **	=	x =	OR	x =		
	Independent <small>(37 CFR 1.16(h))</small>	*	Minus ***	=	x =	OR	x =		
	Application Size Fee (37 CFR 1.16(s))					OR			
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					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>									



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY DOCKET NO	TOT CLAIMS	IND CLAIMS
13/582,717	10/17/2012		1925	JHU3260-4	53	2

CONFIRMATION NO. 3707

FILING RECEIPT



0000000057353159

100899

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

Date Mailed: 11/02/2012

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

Inventor(s)

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

Applicant(s)

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

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

Domestic Priority data as claimed by applicant

This application is a 371 of PCT/US2011/027317 03/04/2011
which claims benefit of 61/311,255 03/05/2010
and claims benefit of 61/435,671 01/24/2011

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see <http://www.uspto.gov> for more information.)

If Required, Foreign Filing License Granted: 10/28/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/582,717**

Projected Publication Date: 02/14/2013

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins

Preliminary Class**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>.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/582,717	10/17/2012	Atul Bedi	JHU3260-4

CONFIRMATION NO. 3707

PUBLICATION NOTICE



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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-2013-0039911-A1

Publication Date:02/14/2013

NOTICE OF PUBLICATION OF APPLICATION

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

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	Not Yet Assigned
Application No.:	13/582,717	Examiner:	Not Yet Assigned
Filed:	October 17, 2012	Conf. No.:	3707
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

Mail Stop AMENDMENT
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Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Preliminarily, please consider the following amendments and remarks prior to examination of the application.

Amendments to the Claims are reflected in the **Listing of Claims**, which begins on page **2** of this paper.

Remarks begin on page **10** of this paper.

Amendments to the Claims:

The listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

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

5. (Original) The molecule of claim 4, wherein the immunomodulatory moiety comprises an extracellular ligand-binding domain of TGF- β RII.

6. (Original) The molecule of claim 5, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, epidermal growth factor receptor (EGFR1), CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4.

7. (Original) The molecule of claim 5, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

8. (Original) The molecule of claim 5, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to Programmed Death-1 (PD-1), Programmed death-1 ligand 1 (PD-L1), or Programmed death-1 ligand 2 (PD-L2).

9. (Canceled).

10. (Previously presented) The molecule of claim 8, wherein the molecule comprises PD-1 ectodomain, immunoglobulin Fc region, and TGF β RII ectodomain.

11. (Original) The molecule of claim 10, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

12. (Original) The molecule of claim 5, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- κ B (RANK) or Receptor activator of nuclear factor- κ B ligand (RANKL).

13. (Canceled).

14. (Previously presented) The molecule of claim 12, wherein the molecule comprises RANK ectodomain, immunoglobulin Fc region, and TGF β RII ectodomain.

15. (Original) The molecule of claim 14, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

16. (Original) The molecule of claim 1, wherein the immunomodulatory moiety comprises a molecule that specifically binds to Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2).

17. (Canceled).

18. (Previously presented) The molecule of claim 16, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, Epidermal growth factor receptor (EGFR1), CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4.

19. (Original) The molecule of claim 18, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.

20. (Original) The molecule of claim 16, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor- κ B (RANK) or Receptor activator of nuclear factor- κ B ligand (RANKL).

21. (Canceled).

22. (Previously presented) The molecule of claim 20, wherein the molecule comprises RANK ectodomain, immunoglobulin Fc region, and PD-1 ectodomain.

23. (Original) The molecule of claim 22, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 25 or 26.

24. (Original) The molecule of claim 1, wherein the immunomodulatory moiety comprises a molecule that specifically binds to Receptor activator of nuclear factor- κ B ligand (RANKL).

25. (Original) The molecule of claim 24, wherein the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK).

26. (Original) The molecule of claim 25, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, Epidermal growth factor receptor (EGFR1), CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2 α receptor; IL-2 α R), or CD4.

27. (Original) The molecule of claim 26, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36.

28. (Original) The molecule of claim 1, wherein the immunomodulatory moiety comprises an antibody or ligand that specifically binds to 4-1BB, OX40, or GITR/AITR.

29. (Original) The molecule of claim 28, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, Epidermal growth factor receptor (EGFR1), CD20, vascular endothelial growth factor (VEGF), CD25, CD4, cytotoxic T-lymphocyte antigen-4 (CTLA-4), Transforming growth factor-beta (TGF- 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), glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18), 4-1BB, OX40, or Interleukin-4 receptor (IL-4R).

30. (Original) The molecule of claim 1, wherein the targeting moiety specifically binds to Tumor Necrosis Factor- α (TNF- α), and the immunomodulatory moiety comprises a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2).

31. (Original) The molecule of claim 30, wherein the targeting moiety comprises an antibody that binds TNF- α or an extracellular ligand-binding domain of tumor necrosis

factor receptor 2 (TNFR2), and the immunomodulatory moiety comprises a sequence from PD-1 ligand 1 (PD-L1 or B7-H1).

32. (Original) The molecule of claim 31, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 37, 38, or 39.

33. (Original) The molecule of claim 1, wherein the targeting moiety comprises an antibody, antibody fragment, or a peptide that specifically binds to CD20, CD25, or CD4, and the immunomodulatory moiety comprises a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2).

34. (Original) The molecule of claim 33, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 40, 41, 42, 43, or 44.

35. (Original) The molecule of claim 1, wherein the targeting moiety comprises an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety comprises a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2).

36. (Original) The molecule of claim 35, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 46 or 47.

37. (Original) The molecule of claim 1, wherein the targeting moiety comprises transforming growth factor- β (TGF- β) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety comprises a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2).

38. (Original) The molecule of claim 37, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 48 or 49.

39. (Original) The molecule of claim 1, wherein the immunomodulatory moiety comprises a sequence from transforming growth factor- β (TGF- β).

40. (Original) The molecule of claim 39, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor- α (TNF- α), CD20, CD25 (IL-2 α receptor; IL-2 α R), or CD4, and the immunomodulatory moiety comprises a sequence from transforming growth factor- β (TGF- β).

41. (Original) The molecule of claim 40, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 50, 51, 52, 53, 54, 55, 56, 57, or 58.

42. (Original) The molecule of claim 39, wherein the targeting moiety comprises an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety comprises a sequence from transforming growth factor- β (TGF- β).

43. (Original) The molecule of claim 42, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 59 or 60.

44. (Original) The molecule of claim 25, wherein the targeting moiety comprises an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor- α (TNF- α), and the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK).

45. (Original) The molecule of claim 44, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 61, 62, or 63.

46. (Original) The molecule of claim 25, wherein the targeting moiety comprises an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK).

47. (Original) The molecule of claim 46, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

48. (Original) The molecule of claim 25, wherein the targeting moiety comprises a sequence from transforming growth factor- β (TGF- β) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK).

49. (Original) The molecule of claim 48, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

50. (Original) The molecule of claim 25, wherein the targeting moiety comprises a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG C γ 1), and the immunomodulatory moiety comprises an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor- κ B (RANK).

51. (Original) The molecule of claim 50, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

52. (Original) The molecule of claim 1, wherein the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

53-54. (Canceled).

55. (Previously presented) A method of preventing or treating a neoplastic disease comprising:
administration to a subject in need thereof one or more molecule of claim 1.

56. (Original) The method of claim 55, wherein the subject is administered in combination with another anticancer therapy.

57. (Original) The method of claim 55, wherein the anticancer therapy comprises a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent, ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

58. (Original) The method of claim 55, wherein the subject is administered in combination with a vaccine.
59. (Original) The method of claim 55, wherein the subject is human.
- 60-61. (Canceled).
62. (Original) A method of treatment of a neoplastic disease comprising:
administering to a subject in need thereof an antibody or Fc-containing fusion protein that targets CD4 in combination with another therapy, wherein the neoplastic disease is not a T cell malignancy, and the therapy comprises one or more of the following:
- (i) an immunostimulatory molecule;
 - (ii) a tumor-targeted antibody; or
 - (iii) a cytotoxic anticancer agent.
- 63-67. (Canceled).

In re Application of:
Bedi and Ravi
Application No.: 13/582,717
Filed: October 17, 2012
Page 10

PATENT
Attorney Docket No.: JHU3260-4

REMARKS

Claims 1, 3-8, 10-12, 14-16, 18-20, 22-52, 55-59, and 62 are pending. Claim 4 has been amended. Support for the amendment can be found throughout the specification and claims as originally filed. No new matter has been added. Accordingly, upon entry of the present amendment, claims 1, 3-8, 10-12, 14-16, 18-20, 22-52, 55-59 be pending and under consideration in this application.

In re Application of:
Bedi and Ravi
Application No.: 13/582,717
Filed: October 17, 2012
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PATENT
Attorney Docket No.: JHU3260-4

CONCLUSION

In view of the above amendments and remarks, reconsideration and favorable action on all claims are respectfully requested. In the event any matters remain to be resolved, the Examiner is requested to contact the undersigned at the telephone number given below so that a prompt disposition of this application can be achieved.

No fee is believed due. However, if any fee is due, the Commissioner is authorized to charge any fees, or credit any overpayments, to Deposit Account No. 07-1896 referencing the above-identified docket number.

Respectfully submitted,

Date: April 25, 2013



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Electronic Acknowledgement Receipt

EFS ID:	15617619
Application Number:	13582717
International Application Number:	
Confirmation Number:	3707
Title of Invention:	Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Lisa A. Haile/Cara Grifone
Filer Authorized By:	Lisa A. Haile
Attorney Docket Number:	JHU3260-4
Receipt Date:	25-APR-2013
Filing Date:	17-OCT-2012
Time Stamp:	18:50:04
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

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File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Preliminary Amendment	JHU3260-4PreliminaryAmendment.PDF	166261	no	11
			0de4252f475d0b78516d7edae4c0142d92cad7		
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875				Application or Docket Number 13/582,717		Filing Date 10/17/2012		☐ To be Mailed		
ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO										
APPLICATION AS FILED – PART I										
(Column 1)		(Column 2)								
FOR	NUMBER FILED	NUMBER EXTRA			RATE (\$)	FEE (\$)				
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A			N/A					
☐ SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A			N/A					
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A			N/A					
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*			X \$	=				
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*			X \$	=				
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).									
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))										
* If the difference in column 1 is less than zero, enter "0" in column 2.					TOTAL					
APPLICATION AS AMENDED – PART II										
(Column 1)		(Column 2)		(Column 3)						
AMENDMENT	04/25/2013	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)			
	Total (37 CFR 1.16(i))	* 53	Minus	** 53	= 0	x \$40 =	0			
	Independent (37 CFR 1.16(h))	* 2	Minus	***3	= 0	x \$210 =	0			
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))									
						TOTAL ADD'L FEE		0		
(Column 1)		(Column 2)		(Column 3)						
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)			
	Total (37 CFR 1.16(i))	*	Minus	**	=	X \$	=			
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X \$	=			
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))									
						TOTAL ADD'L FEE				
<p>* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.</p> <p>** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".</p> <p>*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".</p> <p>The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.</p>										

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/582,717	10/17/2012	Atul Bedi	JHU3260-4	3707
100899 7590 05/07/2013 The John Hopkins University c/o DLA Piper LLP (US) 4365 Executive Drive, Suite 1100 San Diego, CA 92121-2133			EXAMINER SHAHER, SHULAMITH H	
			ART UNIT 1647	PAPER NUMBER
			MAIL DATE 05/07/2013	DELIVERY MODE PAPER

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.

Office Action Summary	Application No. 13/582,717	Applicant(s) BEDI ET AL.	
	Examiner SHULAMITH H. SHAFER	Art Unit 1647	AIA (First Inventor to File) Status No

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

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 1 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

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

Status

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

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

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

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

Disposition of Claims

5) ☒ Claim(s) 1,2,4-12,14-16,18-20,22-52,55-59 and 62 is/are pending in the application.
5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☐ Claim(s) _____ is/are rejected.

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

9) ☒ Claim(s) 1,2,4-12,14-8, 10-12,14-16,18-20,22-52,55-59 and 62 are subject to restriction and/or election requirement.

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

Application Papers

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

11) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

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

Certified copies:

a) ☐ All b) ☐ Some * c) ☐ None of the:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

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

Interim copies:

a) ☐ All b) ☐ Some c) ☐ None of the: Interim copies of the priority documents have been received.

Attachment(s)

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

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

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

4) ☐ Other: _____.

DETAILED ACTION

Applicants' amendment of 25 April 2013 is acknowledged. Claims 1, 2, 4-8, 10-12, 14-16, 18-20, 22-52, 55-59 and 62 are pending in the instant application.

REQUIREMENT FOR UNITY OF INVENTION

As provided in 37 CFR 1.475(a), an international application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept ("requirement of unity of invention"). Where a group of inventions is claimed in an international application, the requirement of unity of invention shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression "special technical features" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

The determination whether a group of inventions is so linked as to form a single general inventive concept shall be made without regard to whether the inventions are claimed in separate claims or as alternatives within a single claim. See 37 CFR 1.475(e).

When Claims Are Directed to Multiple Processes, Products, and/or Apparatuses:

Products, processes of manufacture, processes of use, and apparatuses are different categories of invention. When an application includes claims to more than one product, process, or apparatus, the first invention of the category first mentioned in the claims of the application and the first recited invention of each of the other categories related thereto will be considered as the "main invention" in the claims. In the case of non-compliance with unity of invention and where no additional fees are timely paid, the international search and/or international preliminary examination, as appropriate, will be based on the main invention in the claims. See PCT Article 17(3)(a), 37 CFR 1.475(d), 37 CFR 1.476(c) and 37 CFR 1.488(b)(3).

As provided in 37 CFR 1.475(b), an international application containing claims to different categories of invention will be considered to have unity of invention if the claims are drawn only to one of the following combinations of categories:

- (1) A product and a process specially adapted for the manufacture of said product; or
- (2) A product and process of use of said product; or
- (3) A product, a process specially adapted for the manufacture of the said product, and a use of the said product; or

Art Unit: 1647

(4) A process and an apparatus or means specifically designed for carrying out the said process; or

(5) A product, a process specially adapted for the manufacture of the said product, and an apparatus or means specifically designed for carrying out the said process.

Otherwise, unity of invention might not be present. See 37 CFR 1.475(c).

Election/Restrictions

Restriction is required under 35 U.S.C. 121 and 372.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In accordance with 37 CFR 1.499, applicant is required, in reply to this action, to elect a single invention to which the claims must be restricted.

Group I, claim(s) 1, 2, 4-8, 10-12, 14-16, 18-20, and 22-52 drawn to a molecule, comprising a targeting moiety fused with an immunomodulatory moiety.

Group II, claim(s) 55-59, a method of preventing or treating a neoplastic disease comprising administration of a targeting moiety fused with an immunomodulatory moiety.

Group III, claim(s) 62, drawn to a of treatment of a neoplastic disease comprising: administering to a subject in need thereof an antibody or Fc-containing fusion protein that targets CD4 in combination with another therapy.

The groups of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

37 CFR 1.475 states: The expression “special technical features” shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

Groups I-III lack unity of invention because even though the inventions of these groups require the technical feature of “a molecule, comprising a targeting moiety fused with an immunomodulatory moiety”, this technical feature is not a special technical feature as it does not make a contribution over the prior art in view of Adams et al. (US

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7,527,791). The first claimed product of the instant invention is “a molecule, comprising a targeting moiety fused with an immunomodulatory moiety”. Molecules comprising a targeting moiety fused with an immunomodulatory moiety are well-known in the art and taught, for example, by Adams. The reference teaches bispecific antibodies that have binding specificities for at least two different epitopes. Exemplary antibodies may combine a TGF-beta binding site with binding site(s) for HER-2, EGFR, ErbB, ErbB3, and/or ErbB4 (Column 30, line 59, bridging column 31, line 10). Therefore, since the first claimed invention is well known in the art and does not have a special technical feature, it cannot share a special technical feature with the other claimed inventions.

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

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

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

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

Further Restriction Within Groups I-III

For whatever Group is elected, further restriction within the elected Group is required. Applicants must further elect a single molecule comprising a targeting moiety fused with an immunomodulatory moiety and identify said molecule by SEQ ID NO: (SEQ ID NOs:1-69) and identify, by name, the targeting moiety and the immunomodulatory moiety. The groups of inventions listed as SEQ ID NOs:1-69 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: multiple products are claimed, each product comprising a patentably distinct invention.

Applicants are advised that this is not a species election.

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

In the event of rejoinder, the requirement for restriction between the product/apparatus claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product/apparatus are found allowable, an otherwise proper restriction requirement between product/apparatus claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product/apparatus claim will not be rejoined. See MPEP § 821.04. Additionally, in order for rejoinder to occur, applicant is advised that the process claims

should be amended during prosecution to require the limitations of the product/apparatus claims. **Failure to do so may result in no rejoinder.** Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

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

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

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

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

Notice of References Cited	Application/Control No. 13/582,717	Applicant(s)/Patent Under Reexamination BEDI ET AL.	
	Examiner SHULAMITH H. SHAFER	Art Unit 1647	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-7,527,791	05-2009	Adams et al.	424/145.1
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Bedi and Ravi	Art Unit:	1647
Application No.:	13/582,717	Examiner:	Shulamith H. Shafer
Filed:	October 17, 2012	Conf. No.:	3707
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

PRELIMINARY AMENDMENT
AND RESPONSE TO RESTRICTION REQUIREMENT

Sir:

Responsive to the Restriction Requirement mailed May 7, 2013, please consider the following amendments and remarks prior to examination of the application.

Amendments to the Claims are reflected in the **Listing of Claims**, which begins on page **2** of this paper.

Remarks begin on page **5** of this paper.

Amendments to the Claims:

Please amend the following claims: 1, 2, 4, 5, 7, 10 and 11.

Please cancel the following claims: 3, 6, 8, 9, 12-54, and 60-67.

The listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. (Currently Amended) An isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:

(a) the targeting moiety specifically binds ~~a target molecule~~; Cytotoxic T-lymphocyte associated antigen-4 (CTLA-4); and

(b) the immunomodulatory moiety ~~specifically binds to a molecule selected from:~~ comprises an amino acid sequence of the extracellular domain of

(i) ~~Transforming growth factor beta (TGF- β); Transforming growth factor-beta receptor (TGF- β R); Programmed death-1 ligand 1 (PD-L1; B7-H1) or Programmed death-1 ligand 2 (PD-L2; B7-DC); Programmed death-1 (PD-1); Receptor activator of nuclear factor- κ B ligand (RANKL); or Receptor activator of nuclear factor- κ B (RANK); and~~

(ii) ~~4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNFR4); OX40 ligand (OX40L); glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR); GITR ligand (GITRL); HVEM; LIGHT; CD27; or CD70.~~

2. (Currently Amended) The molecule of claim 1, wherein the targeting moiety comprises an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds ~~a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell~~ CTLA-4.

3. (Canceled).

4. (Currently amended) The molecule of claim 1, wherein the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor ~~TGF- β RII, TGF- β RIIb, or TGF- β RII.~~ selected from the group consisting of SEQ ID NO: 79-91

5. (Currently amended) The molecule of claim 4, wherein the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor II (TGF- β RII).

6. (Canceled).

7. (Currently amended) The molecule of claim 5, wherein the immunomodulatory moiety ~~molecule~~ comprises an amino acid sequence corresponding to SEQ ID NO: 87 ~~1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.~~

8. (Canceled).

9. (Canceled).

10. (Currently amended) The molecule of claim ~~8~~ 7, wherein the molecule comprises ~~PD-1 ectodomain, immunoglobulin Fc region, and TGF β RII ectodomain~~ an amino acid sequence corresponding to SEQ ID NO: 5.

11. (Currently amended) The molecule of claim 10, wherein the molecule comprises an amino acid sequence corresponding to SEQ ID NO: ~~11 or 12~~ 5 and an amino acid sequence corresponding to SEQ ID NO: 74.

12-54. (Canceled).

55. (Withdrawn) A method of preventing or treating a neoplastic disease comprising:

administration to a subject in need thereof one or more molecule of claim 1.

56. (Withdrawn) The method of claim 55, wherein the subject is administered in combination with another anticancer therapy.

57. (Withdrawn) The method of claim 55, wherein the anticancer therapy comprises a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent, ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

58. (Withdrawn) The method of claim 55, wherein the subject is administered in combination with a vaccine.

59. (Withdrawn) The method of claim 55, wherein the subject is human.

60-67. (Canceled).

REMARKS

Applicants elect Group I, Claims 1, 2, 4-8, 10-12, 14-16, 18-20, and 22-52, drawn to a molecule, comprising a targeting moiety fused with an immunomodulatory moiety.

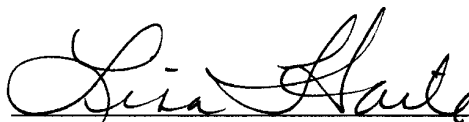
Applicants elect Group I, with traverse, and the election of a molecule wherein the targeting moiety specifically binds Cytotoxic T lymphocyte associated antigen-4 (CTLA-4) and the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor. While Applicant respectfully traverses the restriction requirement, it is believed that the election of Group I and amendments to the claims are responsive to the request.

In view of the above amendments and remarks, reconsideration and favorable action on all claims are respectfully requested. In the event any matters remain to be resolved, the Examiner is requested to contact the undersigned at the telephone number given below so that a prompt disposition of this application can be achieved.

Please charge Deposit Account No. 07-1896 in the amount of \$100.00 to cover a Petition for One Month Extension of Time fee. No additional fees are deemed necessary with the filing of this paper. However, the Commissioner is authorized to charge any fees, or credit any overpayments, to Deposit Account No. 07-1896 referencing the above-identified docket number.

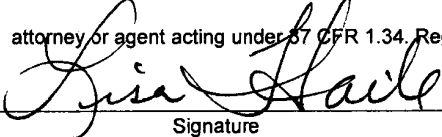
Respectfully submitted,

Date: July 3, 2013



Lisa A. Haile, J.D., Ph.D.
Reg. No. 38,347
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

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

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)		Docket Number (Optional) JHU3260-4
Application Number 13/582,717	Filed October 17, 2012	
For COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		
Art Unit 1647	Examiner Shulamith H. Shafer	
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>
☒ One month (37 CFR 1.17(a)(1))	\$200	\$100
☐ Two months (37 CFR 1.17(a)(2))	\$600	\$300
☐ Three months (37 CFR 1.17(a)(3))	\$1,400	\$700
☐ Four months (37 CFR 1.17(a)(4))	\$2,200	\$1,100
☐ Five months (37 CFR 1.17(a)(5))	\$3,000	\$1,500
Micro Entity Fee		
		\$50
		\$150
		\$350
		\$550
		\$750
☒ 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 EFS-Web.		
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. ☒ attorney or agent of record. Registration Number <u>38,347</u> . ☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.		
 _____ Signature		<u>July 3, 2013</u> _____ Date
<u>Lisa A. Haile, J.D., Ph.D.</u> _____ Typed or printed name		<u>858.677.1456</u> _____ 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>1</u> forms are submitted.		

This collection of information is required by 37 CFR 1.136(a). 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 6 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

WEST241558530.1
331323-000997

American LegalNet, Inc.
www.FormsWorkFlow.com



Electronic Patent Application Fee Transmittal				
Application Number:		13582717		
Filing Date:		17-Oct-2012		
Title of Invention:		Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins		
First Named Inventor/Applicant Name:		Atul Bedi		
Filer:		Lisa A. Haile/Cara Grifone		
Attorney Docket Number:		JHU3260-4		
Filed as Small Entity				
U.S. National Stage under 35 USC 371 Filing Fees				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 1 month with \$0 paid	2251	1	100	100

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
Total in USD (\$)				100

Electronic Acknowledgement Receipt

EFS ID:	16235964
Application Number:	13582717
International Application Number:	
Confirmation Number:	3707
Title of Invention:	Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Lisa A. Haile/Cara Grifone
Filer Authorized By:	Lisa A. Haile
Attorney Docket Number:	JHU3260-4
Receipt Date:	03-JUL-2013
Filing Date:	17-OCT-2012
Time Stamp:	18:01:06
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 100
RAM confirmation Number	7126
Deposit Account	071896
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)	

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		JHU3260-4ResponseToRestrictionAndPreliminaryAmendment.pdf	232918 84ad6199c03741ae4f2a79ea9ca072737d4f bad2	yes	6
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Response to Election / Restriction Filed		1	5	
	Extension of Time		6	6	
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30398 55376e29965c5ba1dad908684979709e843 393a0	no	2
Warnings:					
Information:					
Total Files Size (in bytes):			263316		
<p>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.</p> <p><u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p>					

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

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875				Application or Docket Number 13/582,717		Filing Date 10/17/2012		☐ To be Mailed	
ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO									
APPLICATION AS FILED – PART I									
(Column 1)		(Column 2)							
FOR	NUMBER FILED	NUMBER EXTRA			RATE (\$)	FEE (\$)			
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A			N/A				
☐ SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A			N/A				
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A			N/A				
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 = *				X \$	=			
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 = *				X \$	=			
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))			If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))									
* If the difference in column 1 is less than zero, enter "0" in column 2.					TOTAL				
APPLICATION AS AMENDED – PART II									
(Column 1)		(Column 2)		(Column 3)					
AMENDMENT	07/03/2013	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)		
	Total (37 CFR 1.16(i))	* 12	Minus	** 53	= 0	X \$40 =	0		
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X \$210 =	0		
	☐ Application Size Fee (37 CFR 1.16(s))								
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))								
						TOTAL ADD'L FEE		0	
(Column 1)		(Column 2)		(Column 3)					
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)		
	Total (37 CFR 1.16(i))	*	Minus	**	=	X \$	=		
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X \$	=		
	☐ Application Size Fee (37 CFR 1.16(s))								
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))								
						TOTAL ADD'L FEE			
<p>* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.</p> <p>** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".</p> <p>*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".</p> <p>The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.</p>									

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Substitute for form 1449/PTO

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**

(Use as many sheets as necessary)

Complete if Known

Application Number	13/582,717
Filing Date	October 17, 2012
First Named Inventor	Atul Bedi
Art Unit	1647
Examiner Name	Shulamith H. Shafer
Attorney Docket Number	JHU3260-4

Sheet

1

of

2

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)			
	AA	US-2005/054832 A1	03/10/2005	LAZAR GREGORY A.	
		US-			
		US-			
		US-			
		US-			
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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
SignatureDate
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.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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 1449B/PTO

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**

(Use as many sheets as necessary)

Complete if Known

Application Number	13/582,717
Filing Date	October 17, 2012
First Named Inventor	Atul Bedi
Art Unit	1647
Examiner Name	Shulamith H. Shafer
Attorney Docket No.	JHU3260-4

Sheet 2 of 2

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ²
	AA	Supplementary European Search Report regarding Application No.: EP 11 75 1481	

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

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ Applicant's unique citation designation number (optional). ² Applicant is to place a check mark here if English language Translation is attached.

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:	17062441
Application Number:	13582717
International Application Number:	
Confirmation Number:	3707
Title of Invention:	Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Lisa A. Haile/Tia de Langen
Filer Authorized By:	Lisa A. Haile
Attorney Docket Number:	JHU3260-4
Receipt Date:	07-OCT-2013
Filing Date:	17-OCT-2012
Time Stamp:	19:41:42
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment		no			
File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		JHU3260-4_IDS.pdf	167377	yes	4
			fff8b4318d11b1c96330dcaa18d9ca76ca5d8dd4		

Multipart Description/PDF files in .zip description								
Document Description		Start	End					
Transmittal Letter		1	2					
Information Disclosure Statement (IDS) Form (SB08)		3	4					
Warnings:								
Information:								
2	Other Reference-Patent/App/Search documents	European_Search_Report_AB.pdf	318695	no	17			
			afedf7c83876f2bb895f45cb073f8642082b958a					
Warnings:								
Information:								
Total Files Size (in bytes):			486072					
<p>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.</p> <p><u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p>								

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Bedi and Ravi	Art Unit:	1647
Application No.:	13/582,717	Examiner:	Shulamith H. Shafer
Filing Date:	October 17, 2012	Conf. No.:	3707
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

Mail Stop PCT

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

INFORMATION DISCLOSURE STATEMENT

Sir:

In accordance with 37 C.F.R. § 1.97, enclosed are references relating to the above-identified application. For the convenience of the Examiner, these 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.

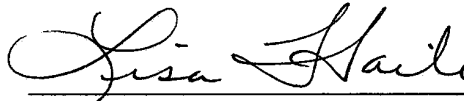
In re Application of:
Bedi and Ravi
Application No.: 13/582,717
Filing Date: October 17, 2012
Page 2

PATENT
Attorney Docket No.: JHU3260-4

No fee is deemed necessary in connection with the filing of this Information Disclosure Statement because it is being submitted within three months of the date of entry of the national stage filing. However, if any fee is required, authorization is hereby given to charge any fees associated with the filings submitted herewith, or credit any overpayment to Deposit Account No. 07-1896.

Respectfully submitted,

Date: October 7, 2013



Lisa A. Haile, J.D., Ph.D.
Registration No.: 38,347
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

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



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/582,717	10/17/2012	Atul Bedi	JHU3260-4	3707
100899	7590	10/11/2013		
The John Hopkins University c/o DLA Piper LLP (US) 4365 Executive Drive, Suite 1100 San Diego, CA 92121-2133			EXAMINER SHAHER, SHULAMITH H	
			ART UNIT 1647	PAPER NUMBER
			MAIL DATE 10/11/2013	DELIVERY MODE PAPER

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.

Office Action Summary	Application No. 13/582,717	Applicant(s) BEDI ET AL.	
	Examiner SHULAMITH H. SHAFER	Art Unit 1647	AIA (First Inventor to File) Status No

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

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 1 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

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

Status

1) ☒ Responsive to communication(s) filed on 7/3/2013.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

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

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

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

Disposition of Claims

5) ☒ Claim(s) 1,2,4,5,7,10,11 and 55-59 is/are pending in the application.
5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☐ Claim(s) _____ is/are rejected.

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

9) ☒ Claim(s) 1,2,4,5,7,10,11 and 55-59 are subject to restriction and/or election requirement.

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

Application Papers

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

11) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

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

Certified copies:

a) ☐ All b) ☐ Some * c) ☐ None of the:

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

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

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

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

Attachment(s)

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

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

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

4) ☐ Other: _____.

DETAILED ACTION

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

Applicants' response of 3 July 2013 is acknowledged. In that response, Applicants elected, with traverse, Group I, Claims 1, 2, 4-8, 10-12, 14-16, 18-20, and 22-52, drawn to a molecule, comprising a targeting moiety fused with an immunomodulatory moiety. In response of 3 July 2013, Applicants have canceled claims 3, 6, 8, 9, 12-54 and 60-67. Clarification is requested as to the elected claims.

Additionally, in light of Applicants' amendment to the claims, further Restriction/Election is required.

Species Election

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

The species of extracellular domain of TGF-beta receptor are as follows: SEQ ID NOs:79-91.

Applicant is required, in reply to this action, to elect a single species to which the claims shall be restricted if no generic claim is finally held to be allowable. The reply must also identify the claims readable on the elected species, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered non-responsive unless accompanied by an election.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which are written in dependent form or otherwise require all the limitations of an allowed generic claim. Currently, the following claim(s) are generic: Claims 1, 4 and 5.

The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: The species listed as

lack unity of invention because the groups do not share the same or corresponding technical feature. Each sequence comprises an amino acid sequence that is structurally distinct from any of the other sequences. The special technical feature of SEQ ID NO:79 is distinct from the special technical feature of SEQ ID NO:91, for example, as each sequence comprises a patently distinct amino acid sequence. Each of the species requires separate, non-co-extensive searches and a search for the amino acid sequence of SEQ ID NO:79 may not result in relevant art with respect to the amino acid sequence of SEQ ID NO:91, for example.

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

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

Should applicant traverse on the ground that the inventions have unity of invention (37 CFR 1.475(a)), applicant must provide reasons in support thereof. Applicant may submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. Where such evidence or admission is provided by applicant, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

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

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

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

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

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Bedi and Ravi	Art Unit:	1647
Application No.:	13/582,717	Examiner:	Shulamith H. Shafer
Filed:	October 17, 2012	Conf. No.:	3707
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

Mail Stop AMENDMENT

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

RESPONSE TO RESTRICTION REQUIREMENT

Sir:

Responsive to the Restriction Requirement mailed October 11, 2013, Applicants elect Group I, Claims 1, 2, 4-8, 10-12, 14-16, 18-20, and 22-52, drawn to a molecule, comprising a targeting moiety fused with an immunomodulatory moiety. Applicants have canceled claims 3, 6, 8, 9, 12-54 and 60-67.

Applicants further elect the species of **SEQ ID NO: 87**, an amino acid sequence from the extracellular domain of TGF-beta receptor II (TGF-bRII).

In re Application of:
Bedi and Ravi
Application No.: 13/582,717
Filed: October 17, 2012
Page 2

PATENT
Attorney Docket No.: JHU3260-4

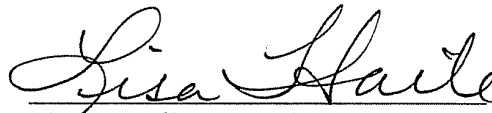
REMARKS

In the event any matters remain to be resolved, the Examiner is requested to contact the undersigned at the telephone number given below so that a prompt disposition of this application can be achieved.

No fee is deemed necessary with the filing of this paper. However, the Commissioner is authorized to charge any fees, or credit any overpayments, to Deposit Account No. 07-1896 referencing the above-identified docket number.

Respectfully submitted,

Date: November 11, 2013



Lisa A. Haile, J.D., Ph.D.

Reg. No. 38,347

Telephone: (858) 677-1456

Facsimile: (858) 677-1465

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

WEST\245195955.1
303110-000036

Electronic Acknowledgement Receipt

EFS ID:	17368597
Application Number:	13582717
International Application Number:	
Confirmation Number:	3707
Title of Invention:	Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Lisa A. Haile/Cara Grifone
Filer Authorized By:	Lisa A. Haile
Attorney Docket Number:	JHU3260-4
Receipt Date:	11-NOV-2013
Filing Date:	17-OCT-2012
Time Stamp:	17:02:01
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no
------------------------	----

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Response to Election / Restriction Filed	JHU3260-4ResponseToRestriction.PDF	21261 28ee89feaa491a8be37dbfccc4416d7e4ab317c2	no	2

Warnings:

Information:

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/582,717	10/17/2012	Atul Bedi	JHU3260-4	3707
100899 7590 02/26/2014 The John Hopkins University c/o DLA Piper LLP (US) 4365 Executive Drive, Suite 1100 San Diego, CA 92121-2133			EXAMINER SHAHER, SHULAMITH H	
			ART UNIT 1647	PAPER NUMBER
			MAIL DATE 02/26/2014	DELIVERY MODE PAPER

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.

Office Action Summary	Application No. 13/582,717	Applicant(s) BEDI ET AL.	
	Examiner SHULAMITH H. SHAFER	Art Unit 1647	AIA (First Inventor to File) Status No

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

Period for Reply

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

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

Status

1) ☒ Responsive to communication(s) filed on 7/3/2013, 11/11/2013.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

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

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

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

Disposition of Claims*

5) ☒ Claim(s) 1,2,4,5,7,10,11 and 55-59 is/are pending in the application.
5a) Of the above claim(s) 55-59 is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☒ Claim(s) 1,2,4,5,7,10,11 is/are rejected.

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

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

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

Application Papers

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

11) ☒ The drawing(s) filed on 9/4/2012 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

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

Certified copies:

a) ☐ All b) ☐ Some** c) ☐ None of the:

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

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

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

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

Attachment(s)

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

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)
Paper No(s)/Mail Date 9/4/2012, 10/7/2013.

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

4) ☐ Other: _____.

Detailed Action

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

Status of Application, Amendments, And/Or Claims:

Applicants' amendment of 3 July 2013 is acknowledged. Claims 1, 2, 4, 5, 7, 10, 11 and 55-59 are pending in the instant application.

Restriction Requirement:

In response of 3 July 2013, Applicants' elected, with traverse, Group I, claims 1, 2, 4-8, 10-12, 14-16, 18-20 and 22-52, drawn to a molecule, comprising a targeting moiety fused with an immunomodulatory moiety. In response to requirement for species election, applicants elect a molecule wherein the targeting moiety specifically binds CTLA-4 and the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of TGF- β receptor.

Because Applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

In response of 11 November 2013, applicants elect the species of SEQ ID NO:87, an amino acid sequence from the extracellular domain of TGF- β receptor II.

Claims 1, 2, 4, 5, 7, 10, 11 and 55-59 are pending in the instant application. Claims 55-59 have been withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Claims 1, 2, 4, 5, 7, 10 and 11 are under consideration to the extent they read on the elected invention, an isolated molecule comprising a targeting moiety wherein the targeting moiety specifically binds CTLA-4 and an immunomodulatory moiety wherein the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of TGF- β receptor II, wherein the amino acid sequence is SEQ ID NO:87.

Information Disclosure Statement:

The Information Disclosure statements (IDS) submitted on the 14 September 2012 and 7 October 2013 have been considered. The signed copies are attached.

Objections

Abstract:

The abstract of the disclosure is objected to because it contains misspellings ("antibodies"). Correction is required. See MPEP § 608.01(b).

Applicant is reminded of the proper language and format for an abstract of the disclosure. The abstract should be in narrative form and generally limited to a single paragraph on a separate sheet within the range of 50 to 150 words. The form and legal phraseology often used in patent claims, such as "means" and "said," should be avoided. The abstract should describe the disclosure sufficiently to assist readers in deciding whether there is a need for consulting the full patent text for details. The language should be clear and concise and should not repeat information given in the title. It should avoid using phrases which can be implied, such as, "The disclosure concerns," "The disclosure defined by this invention," "The disclosure describes," etc.

Rejections

35 U.S.C. § 112:

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

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

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

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

Claims 7, 10 and 11 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The cited claims are vague and indefinite in reciting “an amino acid sequence corresponding to SEQ ID NO:87” (Claim 7), “an amino acid sequence corresponding to SEQ ID NO:5” (Claim 10) and “an amino acid sequence corresponding to SEQ ID NO:74” (Claim 11).

These phrases encompass proteins that comprises the full length sequences of SEQ ID NOs:87, 5 and 74 or any portion of SEQ ID NOs: 87, 5 and 74. These claims would be anticipated by polypeptides comprising a fragment of SEQ ID NOs: 87, 5 and 74 of two or more amino acid residues, if it were applicants’ intent to encompass such breadth. If applicant intends a polypeptide sequence encoding the full length molecule, it is suggested that claim be amended to read for example, “the amino acid sequence corresponding to”

35 U.S.C. § 103:

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1, 2, 4, 5 and 7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lazar et al. (US 8,399,618, priority claimed to SN 12/020443, now US 8,101,720, filed 25 January 2008) and Sun et al. (US 7,795,389, filed 28 September 2005).

The cited references establish the following fact pattern:

Lazar et al. teach Fc variants which comprise antibody Fc regions, Fc fusions (Fc-containing polypeptides), or scFvs (column 22, line 36, bridging column 23, line 20); the reference specifically teaches antibodies which target CTLA-4 (SEQ ID NOs:9 and 10, Figures 19e and f). Lazar teaches a sequence, SEQ ID NO:13, which is 100% identical to SEQ ID NO:74 of the instantly claimed invention (see results in SCORE and alignment below).

```
Query Match          100.0%;  Score 1119;  DB 5;  Length 215;
  Best Local Similarity 100.0%;
  Matches 215;  Conservative 0;  Mismatches 0;  Indels 0;  Gaps
0;

Qy      1  EIVLTQSPGTLSPGERATLSCRASQSVGSSYLAWYQQKPGQAPRLLIYGAFSRATGIP 60
      ||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1  EIVLTQSPGTLSPGERATLSCRASQSVGSSYLAWYQQKPGQAPRLLIYGAFSRATGIP 60

Qy      61  DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIKRTVAAPSVFIFP
120      ||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      61  DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIKRTVAAPSVFIFP
120      ||||||||||||||||||||||||||||||||||||||||||||||||||||

Qy      121 PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTL
180      ||||||||||||||||||||||||||||||||||||||||||||||||||||
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Db          121 PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
180
Qy          181 TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 215
          |||
Db          181 TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 215

```

The sequence is identified as a anti-CTLA-4 light chain.

The disclosed antibodies may be part of a fusion protein or Fc conjugate, where one or more polypeptides are linked to Fc; these polypeptides may comprise the antibody with the target-binding region of a receptor (column 23, lines 35-49). The reference teaches that antibodies that target CTLA-4 block inhibition of T-cell activation and are effective at promoting anti-tumor immune responses (column 70, lines 37-42).

Lazar et al do not teach an isolated molecule comprising a targeting moiety that specifically binds to CTLA-4 and the extracellular domain of TGF β R, wherein the extracellular domain of TGF β R comprises an amino acid sequence of the extracellular domain of TGF β -RII, wherein the amino acid sequence is SEQ ID NO:87.

Sun et al. teach TGF-beta antagonists comprising soluble cell surface type II TGF-beta receptors and fusion proteins comprising said soluble cell surface type II TGF-beta receptors (column 3, lines 26-28, 31-33). These soluble type II receptors comprise the extracellular domain of the TGF β -RII (see claim 1, for example). Said antagonists may be used to treat cancers (abstract). The reference teaches a sequence, SEQ ID NO:8, which comprises an amino acid sequence which is 100% identical to SEQ ID NO:87 of the instantly claimed invention (see results in SCORE and alignment below).

```

Query Match          100.0%;  Score 764;  DB 3;  Length 538;
  Best Local Similarity 100.0%;
  Matches 137;  Conservative 0;  Mismatches 0;  Indels 0;  Gaps
0;

Qy          1  TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE 60
          |||
Db          23 TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE 82

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Query Match 73.3%; Score 2382; DB 5; Length 448;
 Best Local Similarity 99.3%;
 Matches 445; Conservative 1; Mismatches 2; Indels 0; Gaps 0;

```

Qy      1 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYTMHWVRQAPGKGLEWVTFISYDGNNKYY 60
      |||
Db      1 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYTMHWVRQAPGKGLEWVTFISYDGNNKYY 60

Qy      61 ADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAIYYCARTGWLGPFDYWGQGT LVT VSSAS
120      |||
Db      61 ADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAIYYCARTGWLGPFDYWGQGT LVT VSSAS
120      |||

Qy      121 TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGL
180      |||
Db      121 TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGL
180      |||

Qy      181 YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPELLGGPS
240      |||: |||
Db      181 YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPELLGRGPS
240      |||

Qy      241 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
300      |||
Db      241 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
300      |||

Qy      301 YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT
360      |||
Db      301 YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT
360      |||

Qy      361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
420      |||
Db      361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
420      |||

Qy      421 GNVFSCSVMHEALHNHYTQKSLSLSPGK 448
      |||
Db      421 GNVFSCSVMHEALHNHYTQKSLSLSPGK 448

```

As discussed above, the claim recites “**an** amino acid sequence corresponding to SEQ ID NO:5”. This phrase encompasses proteins that comprises the full length

sequence of SEQ ID NO:5 or any portion of SEQ ID NO:5. Thus, the claim is be anticipated by the polypeptide disclosed by Lazar et al. which comprises a 214-amino acid residue which is 100% identical to a fragment of SEQ ID NO:5.

The reference also teaches a polypeptide comprising a sequence, SEQ ID NO:13 which is 100% identical to the instantly claimed SEQ ID NO:74 (see results in SCORE and alignment below).

The examiner is unable to determine whether the prior art disclosures actually possesses the characteristic of an isolated molecule comprising a targeting moiety fused to an immunomodulatory moiety wherein the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of TGF-beta receptor. Under such circumstances, where the product seems to be identical, then the burden shifts to applicant to provide evidence that the prior art would neither anticipate nor render obvious the claimed invention. Note the case law of *In re Best* 195 USPQ 430, 433 (CCPA 1977).

Note: this rejection could be overcome by amending the claims to recite, for example “**the** amino acid sequence corresponding....”

Conclusion:

No claims are allowed.

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

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.

Art Unit: 1647

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

SHULAMITH H. SHAFER
Primary Examiner
Art Unit 1647

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

Notice of References Cited	Application/Control No. 13/582,717		Applicant(s)/Patent Under Reexamination BEDI ET AL.	
	Examiner SHULAMITH H. SHAFER		Art Unit 1647	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-8,399,618	03-2013	Lazar et al.	530/350
*	B	US-7,795,389	09-2010	Sun et al.	530/350
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

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(FILE 'HOME' ENTERED AT 11:08:44 ON 12 FEB 2014)

FILE 'MEDLINE, AGRICOLA, BIOSIS, CAPLUS, EMBASE, SCISEARCH' ENTERED AT
11:09:04 ON 12 FEB 2014

- L1 134 SEA ABB=ON PLU=ON BEDI ATUL/AU OR RAVI RAJANI/AU
- L2 78 SEA ABB=ON PLU=ON L1 AND PROTEIN
- L3 9 SEA ABB=ON PLU=ON L2 AND ANTIBODY
- L4 7 DUP REM L3 (2 DUPLICATES REMOVED)

DIS L4 1-7 TI SO AU
- L5 23276 SEA ABB=ON PLU=ON CTLA-4 OR "CYTOTOXIC T-LYMPOCYTE ANTIGEN
4" OR CD152 OR "CLUSTER OF DIFFERENTIATION 152"
- L6 78 SEA ABB=ON PLU=ON L5 AND ("TRANSFORMING GROWTH FACTOR BETA
RECEPTOR" OR TGF-BETAR OR "TGF-BETA RECEPTOR")
- L7 51 DUP REM L6 (27 DUPLICATES REMOVED)

DIS L7 1-51 TI SO AU
- L8 649 SEA ABB=ON PLU=ON L5 (P) ((IMMUNE OR IMMUNO) (S) (SUPPRESS
OR SUPPRESSION))
- L9 167 SEA ABB=ON PLU=ON L5 (S) ((IMMUNE OR IMMUNO) (S) (SUPPRESS
OR SUPPRESSION))
- L10 92 SEA ABB=ON PLU=ON L9 AND (CANCER OR TUMOR OR METASTASIS)
- L11 76 SEA ABB=ON PLU=ON L9 (P) (CANCER OR TUMOR OR METASTASIS)
- L12 26 DUP REM L11 (50 DUPLICATES REMOVED)

DIS L12 1-26 TI SO AU

L13 29249 SEA ABB=ON PLU=ON ("TRANSFORMING GROWTH FACTOR BETA RECEPTOR"
OR TGF-BETAR OR "TGF-BETA RECEPTOR")

L14 105 SEA ABB=ON PLU=ON L13 (P) ((IMMUNE OR IMMUNO) (S) (SUPPRESS
OR SUPPRESSION))

L15 10 SEA ABB=ON PLU=ON L13 (S) ((IMMUNE OR IMMUNO) (S) (SUPPRESS
OR SUPPRESSION))

L16 38 DUP REM L10 (54 DUPLICATES REMOVED)

L17 7 DUP REM L15 (3 DUPLICATES REMOVED)

DIS L17 TI SO AU

DIS L17 1-7 TI SO AU

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**

(Use as many sheets as necessary)

Complete if Known

Application Number	
Filing Date	
First Named Inventor	Atul Bedi
Art Unit	
Examiner Name	
Attorney Docket Number	JHU3260-4

Sheet

1

of

1

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)			
	AA	US-7,803,378 B2	09-28-2010	Tranchand-Bunel	
	AB	US-2006/0135459 A1	06-22-2006	Epstein et al.	
	AC	US-2006/0263368 A1	11-23-2006	Rosenblum et al.	
	AD	US-2007/0212337 A1	09-13-2007	Bedi et al.	
	AE	US-2008/0075717 A1	03-27-2008	Tranchand-Bunel	
	AF	US-2009/0226435 A1	09-10-2009	Khare	
		US-			
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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

/Shulamith Shafer/

Date
Considered

10/09/2013

*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.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	2	("20130039911").PN.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2013/10/09 11:42
S2	5	bedi-atul.in. or ravi-rajani.in.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2013/10/09 11:45
S3	2	("8372952").PN.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2014/02/11 13:21
S4	2	("20130039911").PN.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2014/02/11 13:28
S5	2	("7527791").PN.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2014/02/11 13:33
S6	1	("8647622").PN.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2014/02/11 15:02
S7	5	bedi-atul.in. or ravi-rajani.in.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:08
S8	6603	"cytotoxic T-lymphocyte Antigen 4" or CTLA-4 or CD152 or "cluster of differentiation 152"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:10
S9	4539	S8 same (bind or target or antibody or antibodies or Fc)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:11
S10	3182	S8 with (bind or target or antibody or antibodies or Fc)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:11
S11	955	S10 same (fusion or conjugate or chimera or chimeric)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:11
S12	628	S10 with (fusion or conjugate or chimera or chimeric)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:11
S13	2617	"transforming growth factor beta receptor" or TGF-betaR or "TGF-beta receptor"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:12
S14	100	S8 and S13	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:12
S15	27	S8 same S13	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:13
S16	76	(S8 and S13) and (fusion or chimera or chimeric or hybrid or	US-PGPUB; USPAT; USOCR; EPO; JPO;	OR	ON	2014/02/11 16:15

		conjugate)	DERWENT			
S17	1	("5885776").PN.	USPAT; USOCR	OR	OFF	2014/02/11 16:19
S18	3930	S8 same (antibody or antibodies or Fc)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:22
S19	2664	S8 with (antibody or antibodies or Fc)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:22
S20	1130	S8 with (antibody or antibodies or Fc) same (cancer or tumor or metastasis)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:22
S21	780	S8 with (antibody or antibodies or Fc) with (cancer or tumor or metastasis)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:23
S22	137	S13 with (extracellular)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:24
S23	18	S13 with (extracellular) same (fusion or conjugate or hybrid or chimera or chimeric)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:24
S24	18	(S13 with (extracellular)) same (fusion or conjugate or hybrid or chimera or chimeric)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:25
S25	264	"transforming growth factor beta receptor II" or TGF-betaRII or "TGF-beta receptorII"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:27
S26	15	S25 and S8	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:28
S27	2452	S8 with (antibody or antibodies)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:31
S28	548	S27 with (immune)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:31
S29	219	(S27 with (immune)) same (cancer or tumor or metastasis or metastatic)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:32
S30	219	(S27 with (immune)) same (cancer or tumor or metastasis or metastatic)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:32
S31	176	(S27 with (immune)) with (cancer or tumor or metastasis or metastatic)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:32
S32	6	((S27 with (immune)) with (cancer or tumor or metastasis or metastatic)) and S13	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2014/02/11 16:33

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Search Notes 	Application/Control No. 13582717	Applicant(s)/Patent Under Reexamination BEDI ET AL.
	Examiner SHULAMITH H SHAFER	Art Unit 1647

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Inventor name search, PALM, EAST and STN, search history included	2/12/14	SHS
EAST search, search history included	2/12/14	SHS
STN search, search history included	2/12/14	SHS
SEQ search, see results in SCORE	10/9/2013	SHS

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

	/SHULAMITH H SHAFER/ Primary Examiner.Art Unit 1647
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CONFIRMATION NO. 3707

SERIAL NUMBER 13/582,717	FILING or 371(c) DATE 10/17/2012 RULE	CLASS 424	GROUP ART UNIT 1647	ATTORNEY DOCKET NO. JHU3260-4		
APPLICANTS Atul Bedi, Timonium, MD; Rajani Ravi, Ruxton, MD; ** CONTINUING DATA ***** This application is a 371 of PCT/US2011/027317 03/04/2011 which claims benefit of 61/311,255 03/05/2010 and claims benefit of 61/435,671 01/24/2011 ** FOREIGN APPLICATIONS ***** ** IF REQUIRED, FOREIGN FILING LICENSE GRANTED *** SMALL ENTITY ** 10/28/2012						
Foreign Priority claimed ☐ Yes ☒ No 35 USC 119(a-d) conditions met ☐ Yes ☐ No Verified and /SHULAMITH H Acknowledged SHAFER/ Examiner's Signature		☐ Met after Allowance Initials	STATE OR COUNTRY MD	SHEETS DRAWINGS 6	TOTAL CLAIMS 53	INDEPENDENT CLAIMS 2
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 1925	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:		☐ All Fees			
			☐ 1.16 Fees (Filing)			
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☐ Credit						

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**

(Use as many sheets as necessary)

Complete if Known

Application Number	13/582,717
Filing Date	October 17, 2012
First Named Inventor	Atul Bedi
Art Unit	1647
Examiner Name	Shulamith H. Shafer
Attorney Docket Number	JHU3260-4

Sheet

1

of

2

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)			
	AA	US-2005/054832 A1	03/10/2005	LAZAR GREGORY A.	
		US-			
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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)				

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Signature

/Shulamith Shafer/

Date
Considered

10/09/2013

*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.

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**

(Use as many sheets as necessary)

Complete if Known

Application Number	13/582,717
Filing Date	October 17, 2012
First Named Inventor	Atul Bedi
Art Unit	1647
Examiner Name	Shulamith H. Shafer
Attorney Docket No.	JHU3260-4

Sheet 2 of 2

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ²
	AA	Supplementary European Search Report regarding Application No.: EP 11 75 1481	

Examiner Signature	/Shulamith Shafer/	Date Considered	02/20/2014
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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:

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Bedi and Ravi	Art Unit:	1647
Application No.:	13/582,717	Examiner:	Shulamith H. Shafer
Filed:	October 17, 2012	Conf. No.:	3707
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

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

RESPONSE TO OFFICE ACTION

Sir:

Responsive to the non-final Office Action mailed February 26, 2014, please consider the following amendments and remarks prior to examination of the application.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the **Listing of Claims**, which begins on page 3 of this paper.

Remarks begin on page 6 of this paper.

Amendments to the Specification

Please replace the abstract with the following amended abstract:

The present invention is based on the seminal discovery that targeted immunomodulatory anti-[[o]]bodies 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 anti-[[o]]bodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. Such immune[[-]]suppressive 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.

Amendments to the Claims:

Please amend claims 1, 7, 10 and 11 as shown in the listing of claims.

Please add new claims 68-70 as shown in the listing of claims.

The listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. (Currently Amended) An isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:

(a) the targeting moiety specifically binds Cytotoxic T-lymphocyte associated antigen-4 (CTLA-4); and

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

2. (Previously Presented) The molecule of claim 1, wherein the targeting moiety comprises an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds CTLA-4.

3. (Canceled).

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

5. (Previously Presented) The molecule of claim 4, wherein the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor II (TGF- β RII).

6. (Canceled).

7. (Currently amended) The molecule of claim 5, wherein the immunomodulatory moiety comprises [[an]] the amino acid sequence corresponding to SEQ ID NO: 87 or a binding fragment thereof.

8. (Canceled).

9. (Canceled).

10. (Currently amended) The molecule of claim 7, wherein the molecule comprises [[an]] the amino acid sequence corresponding to SEQ ID NO: 5 or a binding fragment thereof.

11. (Currently amended) The molecule of claim 10, wherein the molecule comprises [[an]] the amino acid sequence corresponding to SEQ ID NO: 5 or a binding fragment thereof and [[an]] the amino acid sequence corresponding to SEQ ID NO: 74 or a binding fragment thereof.

12-54. (Canceled).

55. (Withdrawn) A method of preventing or treating a neoplastic disease comprising: administration to a subject in need thereof one or more molecule of claim 1.

56. (Withdrawn) The method of claim 55, wherein the subject is administered in combination with another anticancer therapy.

57. (Withdrawn) The method of claim 55, wherein the anticancer therapy comprises a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent, ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

58. (Withdrawn) The method of claim 55, wherein the subject is administered in combination with a vaccine.

59. (Withdrawn) The method of claim 55, wherein the subject is human.
- 60-67. (Canceled).
68. (New) The isolated molecule of claim 1, further comprising a linker at the C_H3 region of the Fc.
69. (New) The isolated molecule of claim 68, wherein the linker is (GGGGS)_n, and wherein n is 1, 2, 3, 4, 5, 6, 7 or 8.
70. (New) The isolated molecule of claim 68, wherein the linker comprises the amino acid sequence GGGSGGGSGGGGS.

REMARKS

Claim amendments

Prior to this Response, claims 1, 2, 4, 5, 7, 10, 11 and 55-59 were pending and under consideration. With the present communication, claims 55-59 have been withdrawn, claims 1, 7, 10 and 11 have been amended and new claims 68-70 have been added. Support for the claim amendments and new claims may be found generally throughout the application and claims as originally filed and specifically at paragraphs [0013], [0039], [0040], [0078] and [0182] and Figure 1. No new matter is added. After entry of the amendments, claims 1, 2, 4, 5, 7, 10, 11 and 68-70 will be under consideration.

Objections to the Specification

The Examiner has objected to the Specification stating that the abstract contains misspellings.

Applicants have amended the abstract to correct the misspellings. Applicants submit that these amendments render the objection moot and respectfully request withdrawal of the objection.

Rejections under 35 USC § 112:

Claims 7, 10 and 11 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner states that the cited claims recite the phrases “an amino acid sequence corresponding to SEQ ID NO:87” (Claim 7); “an amino acid sequence corresponding to SEQ ID NO:5” (Claim 10); and “an amino acid sequence corresponding to SEQ ID NO:74” (Claim 11) which are vague and indefinite. Specifically, the Examiner states that these phrases encompass proteins that comprises the full length sequences of SEQ ID NOs:87, 5 and 74 or any portion of SEQ ID NOs: 87, 5 and 74 and that these claims would be anticipated by polypeptides

comprising a fragment of SEQ ID NOs: 87, 5 and 74 of two or more amino acid residues.

Applicants respectfully disagree, but solely to expedite prosecution, Applicants have amended claims 7, 10 and 11 to recite “ the amino sequence corresponding to SEQ ID NO: XX or a binding fragment thereof”. Applicants submit that these amendments render the objection moot and respectfully request withdrawal of the rejection.

Rejections under 35 U.S.C. § 103:

Claims 1, 2, 4, 5 and 7 stand rejected under 35 USC § 103(a) as allegedly being unpatentable over Lazar et al. (U.S. Patent No. 8,399,618; hereafter “Lazar”) and Sun et al. (U.S. Patent No. 7,795,389; hereafter “Sun”). Applicants respectfully traverse this objection.

The U.S. Supreme Court decision in KSR International v. Teleflex Inc. modified the standard for establishing a prima facie case of obviousness. KSR, 550 US 398, 82 USPQ 2d 1385 (2007). Under the KSR rule, three basic criteria are considered. First, some suggestion or motivation to modify a reference or to combine the teachings of multiple references still has to be shown. Second, the combination has to suggest a reasonable expectation of success. Third, the prior art reference or combination has to teach or suggest all of the recited claim limitations. Factors such as the general state of the art and common sense may be considered when determining the feasibility of modifying and/or combining references.

The 2010 KSR Guidelines Update provides additional guidance in view of decisions by the United States Court of Appeals for the Federal Circuit (Federal Circuit) since KSR. However, familiar lines of argument still apply, including teaching away from the claimed invention by the prior art, lack of a reasonable expectation of success, and unexpected results. Indeed, such arguments may have even taken on added importance in view of the recognition in KSR of a variety of possible rationales. See Federal Register, Vol. 75, No. 169, September 1, 2010, page 53645. Applicants respectfully submit that the Office has not met the burden of establishing a prima facie case of obviousness for the reasons discussed below.

The Examiner states that Lazar discloses Fc variants which comprise antibody Fc regions, Fc fusions (Fc-containing polypeptides), or scFvs; antibodies which target CTLA-4; a sequence, SEQ ID NO:13, which is 100% identical to SEQ ID NO:74 of the instantly claimed invention; that the disclosed antibodies may be part of a fusion protein or Fc conjugate, where one or more polypeptides are linked to Fc; these polypeptides may comprise the antibody with the target-binding region of a receptor; and antibodies that target CTLA-4 block inhibition of T-cell activation and are effective at promoting anti-tumor immune responses. However, Lazar does not disclose an isolated molecule comprising a targeting moiety that specifically binds to CTLA-4 and the extracellular domain of TGF β R, wherein the extracellular domain of TGF β R comprises an amino acid sequence of the extracellular domain of TGF β -RII, wherein the amino acid sequence is SEQ ID NO:87. Sun discloses TGF-beta antagonists comprising soluble cell surface type II TGF-beta receptors and fusion proteins comprising said soluble cell surface type II TGF-beta receptors; soluble type II receptors comprise the extracellular domain of the TGF β -RII; the antagonists may be used to treat cancers; and a sequence, SEQ ID NO:8, which comprises an amino acid sequence which is 100% identical to SEQ ID NO:87 of the instantly claimed invention. The Examiner concludes that it would have been obvious to combine the prior art teachings of Lazar and Sun to construct the isolated molecule comprising a targeting moiety which specifically binds to CTLA-4 (as disclosed by Lazar) and an immunomodulatory moiety comprising an amino acid sequence of the extracellular domain of TGF β -RII of SEQ ID NO:87, as disclosed by Sun.

Applicants respectfully disagree. Lazar discloses an Fc variant of a parent Fc polypeptide, wherein the Fc variant comprises amino acid modifications which may be amino acid insertions, deletions or substitutions particularly in the Fc region of the Fc polypeptide (see Lazar column 2 lines 46-51). Lazar further discloses that the Fc variants may include a variety of structures including Fc regions, Fc fusions or scFvs (see Lazar column 22, line 46 to column 23, line 20). However, Lazar does not disclose or suggest an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein: the targeting moiety specifically binds Cytotoxic T-lymphocyte associated antigen-4 (CTLA-4); and the

immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor (TGF- β R) as is presently claimed.

As provided in the declaration by Dr. Atul Bedi submitted herewith, targeted immunostimulatory antibodies target a disease modifying molecule on a tumor cell or tumor infiltrating T cell and simultaneously trap and disable specific ligands that mediate tumor progression and tumor-induced immune dysfunction within the localized microenvironment of the targeted tumor cell or immune cell. The ability to sequester and disable a specific ligand in the immediate microenvironment of the targeted tumor cell or T cell enables interruption of ligand driven autocrine/paracrine communication that promotes tumor induced immune tolerance, tumor metastases and treatment resistance. Specifically, tumors stimulate the development and function of regulatory T cells (Treg) which attenuate the activation and function of immune effector cells. Tregs express FoxP3 which induces the expression of CTLA-4, a molecule that restrains the costimulation of T cells. Data provided in the declaration show that the autonomous expression of TGF β by tumor cells and/or Tregs cooperates with CTLA-4 blockade to promote the development of FoxP3-expressing Tregs. Therefore, autocrine and paracrine TGF β signaling in the immediate microenvironment of tumor-infiltrating T cells promotes the development of Tregs and attenuates the activation and cytotoxic function of immune effector cells, thereby limiting the anti-tumor efficacy of CTLA-4 antagonists.

The present invention provides a molecule which both functions to have anti-tumor activity as well as counteracting tumor induced immune tolerance by targeting CTLA-4 and simultaneously binding and disabling TGF β within the localized microenvironment of tumor-infiltrating Tregs. The presently claimed invention is directed to a molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein: the targeting moiety specifically bind CTLA-4; and the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of TGF- β R. The claimed molecule is as an illustrative example, a bifunctional antibody (α -CTLA-4-TGF β R II) that targets and antagonizes CTLA-4 and simultaneously inactivates TGF- β by an extracellular domain sequence of TGF- β Receptor

II (TGF- β RII) (SEQ ID NO: 87) fused to the C-terminus of the heavy chain of an α -CTLA-4 antibody via a flexible linker sequence, (GGGGS)₃ (see Appendix Figure 1a).

α -CTLA-4-TGF β RII has several novel features and key advantages over α -CTLA-4, and/or α -TGF β , administered either alone or in combination. α -CTLA-4-TGF β RII not only maintains *bivalent* binding to the constitutively expressed CTLA-4 on Tregs, but also decorates the surface of the targeted cells with a decoy TGF β RII ectodomain that traps and disables TGF- β in their immediate microenvironment. The CTLA-4 targeting anchoring moiety of the bifunctional antibody directs the decoy TGF β RII ectodomain to the Treg cell membrane and sustains its microlocalized retention and bioavailability by tethering it to the surface of the target cell. Unlike small molecules or antibodies that target TGF- β or free TGF β RII-Fc, α -CTLA-4-TGF β RII exploits the constitutive FoxP3-mediated expression of CTLA-4 on Tregs to render them incapable of responding to autocrine or paracrine TGF- β signals in their immediate milieu. Since α -CTLA-4-TGF β RII can simultaneously disable the CTLA-4 immune checkpoint and counteract TGF- β in the localized microenvironment of the targeted Treg, it has the unique ability to inhibit the development and function of Tregs as a single maneuver that enables the activation of cytotoxic immune effector cells within the tumor microenvironment. As such, α -CTLA-4-TGF β RII is more effective in activating tumor-reactive cytotoxic CD8⁺ T cells and demonstrates superior antitumor efficacy compared to either an α -CTLA-4 antibody or any TGF- β antagonist in the prior art (including TGF- β antibody or free TGF β RII-Fc).

A unique and key feature of α -CTLA-4-TGF β RII is its ability to counteract the immunosuppressive function of Tregs and simultaneously convert Tregs into immunostimulatory TH1 helper cells. This distinctive function of α -CTLA-4-TGF β RII is derived from its ability to bind and antagonize CTLA-4 on Treg cells while concurrently localizing the dimeric decoy TGF β RII ectodomain to the Treg cell membrane. This enables α -CTLA-4-TGF β RII to not only counteract the immunosuppressive function of the targeted Tregs (via blockade of both CTLA-4 and TGF- β), but also sequester and disable TGF- β in their immediate microenvironment. Since α -CTLA-4-TGF β RII executes targeted blockade of autocrine and paracrine TGF- β at the Treg

cell surface, it inhibits TGF- β -driven expression of FoxP3 and promotes expression of IFN- γ . This unique ability to contraconvert Tregs to TH1 cells distinguishes α -CTLA-4-TGF β RII from either α -CTLA-4 antibody or any TGF- β antagonist known in the art. It is especially noteworthy that treatment with α -CTLA-4-TGF β RII is significantly more effective in activating tumor-reactive IFN- γ expressing CD8⁺ T cells and inhibiting tumor progression *in vivo* compared to combinatorial administration of both α -CTLA-4 antibody and a TGF- β antagonist (e.g. α -TGF- β antibody or TGF β RII-Fc). By disabling CTLA-4 and TGF- β in the localized microenvironment of the targeted Treg, the bifunctional immunostimulatory antibody enables activation of adaptive immune responses to cross-presented tumor antigens during the course of therapy (as an *in situ* tumor vaccine) that can sustain antitumor activity after cessation of therapy. The ability of this improved class of next-generation molecule to unleash potent innate and adaptive immune responses against tumor cells by counteracting tumor-induced immune tolerance offers a promising new targeted immunotherapeutic strategy for many types of advanced treatment-refractory cancers.

Applicants submit herewith evidence of surprising and unexpected activity of the claimed molecule. Figure 2 shows that α -CTLA-4-TGF β RII was significantly more effective in inhibiting the development and suppressive function of regulatory T cells compared to α -CTLA-4 alone. Whereas α -CTLA-4 antibody promoted TGF- β -induced development of Tregs *in vitro*, α -CTLA-4-TGF β RII was able to significantly reduce the production of Tregs by sequestering and disabling TGF- β (See Appendix Figure 2a). Whereas α -CTLA-4 antibody failed to counteract Treg-mediated suppression, α -CTLA-4-TGF β RII antibody restored activation of tumor antigen-specific Interferon- γ (IFN- γ)-expressing T cells in the presence of Tregs (See Appendix Figure 2b).

Figure 3 demonstrates that α -CTLA-4-TGF β RII significantly reduces Tregs and exhibits superior antitumor efficacy compared with α -CTLA-4 antibody in human tumor-bearing NOG mice reconstituted with human CD34⁺ cells. Treatment with α -CTLA-4-TGF β RII resulted in significantly smaller tumors compared to α -CTLA-4 antibody ($P < 0.001$) or untreated controls ($p < 0.0001$) (see Appendix Figure 3a). Tumors isolated from mice treated with α -CTLA-4-

TGF β RII exhibited significantly reduced tumor-infiltrating Tregs compared with mice treated with α -CTLA-4 mAb or untreated controls (see Appendix Figure 3b).

Figure 4 demonstrates that α -CTLA-4-TGF β RII is more effective in activating tumor-reactive IFN γ -expressing T cells and inhibiting tumor growth compared to α -CTLA-4 alone, α -TGF β alone, and the combination of α -CTLA-4 and α -TGF β administered as separate molecules (see Appendix Figures 4a-b). Treatment with α -CTLA-4-TGF β RII resulted in significantly smaller tumors compared to either α -CTLA-4 ($p=0.001$) or the combination of α -CTLA-4 and α -TGF β antibody ($p=0.03$) (see Appendix Figure 4a). Treatment with α -CTLA-4-TGF β RII resulted in a greater elevation in tumor-reactive IFN- γ -expressing CD8 $^{+}$ cells (percentage of CD3 $^{+}$ CD8 $^{+}$ cells) (37.08%) compared to treatment with either α -CTLA-4 (8.89%) or the combination of α -CTLA-4 and α -TGF- β (14.16%) (see Appendix Figure 4b).

Figure 5 demonstrates that α -CTLA-4-TGF β RII is more effective than either α -CTLA-4 alone and the combination of α -CTLA-4 and TGF β RII-Fc (administered as separate molecules) in reducing Tregs, activating tumor-reactive IFN γ -expressing T cells, and inhibiting growth of *patient-derived primary melanoma tumor* xenografts in NOG mice reconstituted with human CD34 $^{+}$ cells.

This data demonstrates that the presently claimed molecule has unexpected and surprising properties and demonstrates that the presence of both a CTLA-4 antagonist and the ECD of TGF β on the **same molecule** is critical for anti-tumor efficacy.

Applicants submit that prior to the present invention one of skill in the art would have no motivation to combine a CTLA-4 antagonist and the ECD of TGF β RII in a fusion protein due to the inability of the ECD of TGF β RII to increase the anti-tumor efficacy of α -CTLA-4 when administered in combination as separate molecules. Further, Applicants submit that one of skill in the art would not have predicted the surprising and unexpected properties of the presently claimed molecule based on the disclosure in Lazar. Accordingly, applicants submit that Lazar does not disclose or suggest the presently claimed invention

The Examiner relies on Sun to cure the deficiencies of Lazar. Sun discloses TGF β antagonists comprising TGF β receptors and fusion proteins comprising sRII TGF β R (see column 3, lines 26-28, 31-33). The TGF β antagonists may include a therapeutically effective amount of a mixture or combination of therapeutically active portion of sRII, E domain of sRIII or U domain of sRIII (see Sun column 3, lines 47-51). Further, the TGF β antagonists may be used for the treatment of cancer (see Sun abstract). Sun does not disclose or suggest an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein: the targeting moiety specifically binds Cytotoxic T-lymphocyte associated antigen-4 (CTLA-4); and the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor (TGF- β R) as is presently claimed. As such, Sun fails to cure the deficiencies of Lazar.

Applicants submit that neither Lazar nor Sun disclose or suggest the present invention and that one of skill in the art would not arrive at the instant invention based on the disclosure of Lazar or Sun, either alone or in combination. Therefore, Applicants respectfully request reconsideration and withdrawal of the rejection.

Rejections under 35 U.S.C. § 102/35 U.S.C. § 103:

Claims 10 and 11 stand rejected under 35 U.S.C. 102(e) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Lazar.

“[A]nticipation requires that the four corners of a single, prior art document describe every element of the claimed invention, either expressly or inherently, such that a person of ordinary skill in the art could practice the invention without undue experimentation.” *Advanced Display Systems, Inc. v. Kent State University*, 212 F.3d 1272, 1282 (Fed. Cir. 2000). “Anticipation requires identity of invention. The claimed invention, as described in appropriately construed claims, must be the same as that of the reference in order to anticipate.” *Glaverbel Societe Anonyme v. Northlake Marketing & Supply Inc.*, 45 F.3d 1550, 1554 (Fed. Cir. 1995).

The Examiner states that Lazar discloses a polypeptide, SEQ ID NO:14, which comprises a 448-amino acid sequence that is 73.3% identical to SEQ ID NO:5 of the instant invention. Thus, the claim is anticipated by the polypeptide disclosed by Lazar et al. which comprises a 214-amino acid residue which is 100% identical to a fragment of SEQ ID NO:5. Lazar also discloses a polypeptide comprising a sequence, SEQ ID NO:13 which is 100% identical to the instantly claimed SEQ ID NO:74. The Examiner states that this rejection could be overcome by amending the claims to recite, for example “the amino acid sequence corresponding....”.

Applicants respectfully disagree. As stated above Lazar does not disclose or suggest an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein: the targeting moiety specifically binds Cytotoxic T-lymphocyte associated antigen-4 (CTLA-4); and the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor (TGF- β R) as is presently claimed. Further, Applicants have amended claims 10 and 11 to recite “the amino sequence corresponding to SEQ ID NO: XX or a binding fragment thereof”.

Applicants submit that Lazar does not disclose or suggest the presently claimed invention and respectfully request reconsideration and withdrawal of the rejection.

In re Application of:
Bedi and Ravi
Application No.: 13/582,717
Filed: October 17, 2012
Page 15

PATENT
Attorney Docket No.: JHU3260-4

CONCLUSION

In the event any matters remain to be resolved, the Examiner is requested to contact the undersigned at the telephone number given below so that a prompt disposition of this application can be achieved.

Please charge Deposit Account 07-1896 in the amount of \$700.00 to cover a Petition for Three Month Extension of Time fee. No additional fee is deemed necessary with the filing of this paper. However, the Commissioner is authorized to charge any fees, or credit any overpayments, to Deposit Account No. 07-1896 referencing the above-identified docket number.

Respectfully submitted,

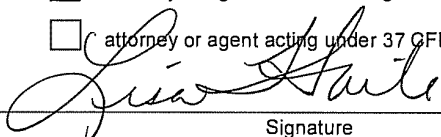
Date: August 25, 2014



Lisa A. Haile, J.D., Ph.D.
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San Diego, California 92121-2133

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

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)		Docket Number (Optional) JHU3260-4
Application Number 13/582,717	Filed October 17, 2012	
For COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		
Art Unit 1647	Examiner Shulamith H. Shafer	
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))	\$200	\$100 \$50 \$ _____
☐ Two months (37 CFR 1.17(a)(2))	\$600	\$300 \$150 \$ _____
☒ Three months (37 CFR 1.17(a)(3))	\$1,400	\$700 \$350 \$ <u>700.00</u>
☐ Four months (37 CFR 1.17(a)(4))	\$2,200	\$1,100 \$550 \$ _____
☐ Five months (37 CFR 1.17(a)(5))	\$3,000	\$1,500 \$750 \$ _____
☒ 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 EFS-Web.		
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. ☒ attorney or agent of record. Registration Number <u>38,347</u> . ☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.		
 _____ Signature		August 25, 2014 _____ Date
Lisa A. Haile, J.D., Ph.D. _____ Typed or printed name		858.677.1456 _____ 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>1</u> forms are submitted.		

This collection of information is required by 37 CFR 1.136(a). 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 6 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Bedi and Ravi	Art Unit:	1647
Application No.:	13/582,717	Examiner:	Shafer, Shulamith H.
Filed:	October 17, 2012	Conf. No.:	3707
Title:	Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins		

MAIL STOP AMENDMENT

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

DECLARATION UNDER 37 C.F.R. § 1.132

Dear Sir:

I, Dr. Atul Bedi, declare and state that:

1. I am a named inventor of the subject matter described and claimed in U.S. Patent Application Serial No. 13/582,717, filed on September 4, 2012 entitled, "Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins." I am a physician-scientist and medical oncologist, and an Associate Professor of Otolaryngology/Head & Neck Surgery and Oncology at the Johns Hopkins University School of Medicine.

2. I understand that claims 1, 2, 4, 5 and 7 of the patent application have been rejected as being obvious given the disclosures of Lazar et al. (U.S. Patent No. 8,399,618; hereafter "Lazar") and Sun et al. (U.S. Patent No. 7,795,389; hereafter "Sun") and claims 10 and 11 have been rejected as being obvious given the disclosures of Lazar.

3. I disagree with the Examiner's assertion that Lazar and Sun render the present invention obvious. Lazar discloses an Fc variant of a parent Fc polypeptide, wherein the Fc variant comprises amino acid modifications which may be amino acid insertions, deletions or

substitutions particularly in the Fc region of the Fc polypeptide. Lazar further discloses that the Fc variants may include a variety of structures including Fc regions, Fc fusions or scFvs.

4. Sun discloses TGF β antagonists comprising TGF β receptors and fusion proteins comprising sRII TGF β R. The TGF β antagonists may include a therapeutically effective amount of a mixture or combination of therapeutically active portion of sRII, E domain of sRIII or U domain of sRIII. Further, the TGF β antagonists may be used for the treatment of cancer.

5. My invention provides a molecule which counteracts tumor-induced immune tolerance and enhances antitumor immune responses by simultaneously targeting CTLA-4 and binding and disabling TGF β within the localized microenvironment of tumor-infiltrating regulatory T cells (Tregs). My invention is directed to a molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein: the targeting moiety specifically binds CTLA-4; and the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of TGF- β R. The α -CTLA-4-TGF β RII molecule is, as an illustrative example, a bifunctional antibody that targets and antagonizes CTLA-4 and simultaneously inactivates TGF- β by an extracellular domain sequence of TGF- β Receptor II (TGF- β RII) fused to the C-terminus of the heavy chain of an α -CTLA-4 antibody via a flexible linker sequence, (GGGGS)₃ (see Appendix Figure 1a). Figure 1b is an SDS-PAGE comparison of α -CTLA-4 antibody and the α -CTLA-4-TGF β RII molecule under reducing conditions showing the expected higher molecular weight of the heavy chain of α -CTLA-4-TGF β RII. In contrast to α -CTLA-4 antibody, the α -CTLA-4-TGF β RII antibody exhibited simultaneous binding to CTLA-4 and TGF- β 1 (Figure 1c).

6. I have shown that α -CTLA-4-TGF β RII was significantly more effective in inhibiting the development and suppressive function of regulatory T cells (Treg). In one experiment, we demonstrated that α -CTLA-4-TGF β RII was significantly more effective than α -CTLA-4 in inhibiting the development of Tregs. Human peripheral blood mononuclear cells

(PBMC) were cultured with rhIL-2 (100 IU/ml) and anti-CD3-/anti-CD28-coated Dynabeads (T cell: bead, 1:3 in plates coated with huCTLA-4-Fc (0.3 µg/ml) or left uncoated), in the presence or absence of 2.5 ng/ml rhTGFβ1 with or without either α-CTLA-4-TGFβRII or α-CTLA-4 mAb (5 µg/ml). On day 5, anti-CD3/anti-CD28 beads were magnetically removed and the number of Tregs (CD4⁺/CD25^{high}/CD127^{low}/FOXP3⁺) were enumerated by flow cytometry. Whereas α-CTLA-4 mAb promoted TGF-β-induced development of Tregs, α-CTLA-4-TGFβRII was able to significantly reduce the production of Tregs by sequestering and disabling TGF-β (see Appendix Figure 2a).

In another experiment, myeloma patient bone marrow (BM) was stained with α-CD3 and Glycophorin A to enumerate T cells. Following activation for 7 days with rhIL-2 and α-CD3/α-CD28 beads, the cells were magnetically separated and stained with α-CD3. Concurrently, autologous Tregs were isolated from the same patient's peripheral blood lymphocytes using α-CD4/α-CD25 beads. The activated T cells were CFSE labeled and added to autologous BM that had been pulsed for 30 min in medium with or without either SW780 cell lysate (non-specific antigen) or U266/H929 myeloma cell lysate (tumor-specific antigen) and plated in the presence or absence of the selected autologous Tregs. Following culture for 3 days with or without either α-CTLA-4-TGFβRII or α-CTLA-4 mAb (5 µg/ml), the cells were stained with α-CD3 and α-IFNγ. Tumor antigen-specific T cells were considered as CD3⁺/CFSE^{low}/IFNγ⁺. Exposure of α-CD3/CD28 activated marrow infiltrating lymphocytes (aMILs) to autologous Tregs suppressed the activation of tumor antigen-specific T cells. Whereas α-CTLA-4 mAb failed to counteract Treg-mediated suppression, α-CTLA-4-TGFβRII antibody restored activation of tumor antigen-specific T cells in the presence of Tregs (see Appendix Figure. 2b).

7. I have also demonstrated that α-CTLA-4-TGFβRII significantly reduces Tregs and exhibits superior antitumor efficacy compared with α-CTLA-4 antibody in human tumor-bearing NOG mice reconstituted with human CD34⁺ cells. The comparative antitumor efficacy of α-CTLA-4 antibody or α-CTLA-4-TGFβRII mAb was evaluated in A-375 human tumor-bearing NOG mice (NOD/Shi-*scid* *IL-2rg*^{null}) reconstituted with matched HLA A2⁺ human BM

CD34⁺ cells. NOG mice were irradiated (200 cGy), followed by adoptive transfer of human CD34⁺ cells (7×10^4). Mice demonstrating engraftment of human CD3⁺ cells at 7 weeks were inoculated with A375 tumor cells (2×10^6 cells). At 10d following tumor cell inoculation, mice were treated with either α -CTLA-4 mAb or α -CTLA-4-TGF β RII (5mg/kg i.p. weekly). Tumor size was evaluated weekly and tumor volume calculated [length $\times$ width $\times$ height]. Treatment with α -CTLA-4-TGF β RII resulted in significantly smaller tumors compared to α -CTLA-4 mAb ($P < 0.001$) or untreated controls ($p < 0.0001$) (see Appendix Fig. 3a). Tumors were isolated from tumor-bearing mice in each group for immunophenotype analysis of T cells. Tumor samples were subjected to collagenase digestion for 20 min at 37⁰ C followed by RBC lysis. Tumor cells were resuspended in RPMI, and counted with trypan blue to enumerate live cells. Tregs in tumor-infiltrating T cells from mice in each group were measured by immunophenotype analysis of CD4⁺CD25⁺CD127^{low}FoxP3⁺ cells. Cells were stained extracellularly with α -human CD4-PE, α -human CD25-PE-CYTM5, and α -human CD127-FITC. The cells were then permeabilized and stained intracellularly with α -human FOXP3-APC or the corresponding isotype control mouse IgG1-APC. The stained cells were washed twice with FACS buffer, run on the Gallios Flow Cytometer, and analyzed utilizing Kaluza Software. Tumor-infiltrating cells from mice treated with α -CTLA-4-TGF β RII exhibited significantly reduced Tregs compared with mice treated with α -CTLA-4 mAb or untreated controls (see Appendix Figure 3b).

8. Additionally, I have demonstrated that α -CTLA-4-TGF β RII is more effective in activating tumor-reactive IFN γ -expressing T cells and inhibiting tumor growth compared to α -CTLA-4 alone, α -TGF β alone, and the combination of α -CTLA-4 and α -TGF β administered as separate molecules. The comparative antitumor efficacy of *in vivo* treatment with α -CTLA-4-TGF β RII, versus the combination of α -CTLA-4 antibody and α -TGF β antibody (α -TGF β Ab) was evaluated in A-375 human tumor-bearing NOG mice (NOD/Shi-*scid* IL-2 rg^{null}) reconstituted with matched HLA A2⁺ human BM CD34⁺ cells. NOG mice were irradiated (200 cGy), followed by adoptive transfer of human CD34⁺ cells (7×10^4). Mice demonstrating engraftment of human CD3⁺ cells at 7 weeks were inoculated with A375 tumor cells (2×10^6 cells). At 10d following

tumor cell inoculation, mice were randomized into groups and treated (i.p. weekly) with the following: (i) α -CTLA-4-TGF β RII (5mg/kg); (ii) α -CTLA-4 mAb (5 mg/kg); (iii) α -TGF- β Ab (ID11) (5 mg/kg); (iv) combination of α -CTLA-4 mAb (5 mg/kg) and α -TGF- β Ab (5 mg/kg); (v) Vehicle (Control). Tumor size was evaluated weekly and tumor volume calculated [length $\times$ width $\times$ height]. Treatment with α -CTLA-4-TGF β RII resulted in significantly smaller tumors compared to either α -CTLA-4 mAb ($p=0.001$) or the combination of α -CTLA-4 mAb and α -TGF- β antibody ($p=0.03$) (see Appendix Figure 4a). To evaluate tumor-specific IFN- γ expression in CD3 $^{+}$ /CD8 $^{+}$ T cells, BM cells were plated in 96 well plates (2×10^5 cells/ well) in the presence of tumor cell lysate (A-375 cells), non-specific control peptide, or medium alone. Cells were cultured for 72 h followed by incubation with Golgi stop for 4 h. Cells were stained extracellularly with anti-CD3-FITC and anti-CD8-APC, permeabilized, stained intracellularly with anti-IFN- γ -PE or its corresponding isotype control, and then analyzed by flow cytometry. In accordance with its superior ability to deplete and counteract Tregs (Figures 2b and 3b), treatment with α -CTLA-4-TGF β RII resulted in a greater elevation in tumor-reactive IFN- γ -expressing CD8 $^{+}$ cells (percentage of CD3 $^{+}$ CD8 $^{+}$ cells) (37.08%) compared to treatment with either α -CTLA-4 mAb (8.89%) or the combination of α -CTLA-4 mAb and α -TGF- β Ab (14.16%) (see Appendix Figure 4b).

9. In another experiment, I demonstrated that α -CTLA-4-TGF β RII is more effective than either α -CTLA-4 alone and the combination of α -CTLA-4 and TGF β RII-Fc (administered as separate molecules) in reducing Tregs, activating tumor-reactive IFN γ -expressing T cells, and inhibiting growth of patient-derived primary melanoma tumor xenografts in NOG mice reconstituted with human CD34 $^{+}$ cells. The comparative antitumor efficacy of *in vivo* treatment with α -CTLA-4-TGF β RII, versus the combination of α -CTLA-4 antibody and TGF β RII-Fc was evaluated in NOG mice (NOD/Shi-*scid* IL-2 rg^{null}) reconstituted with matched HLA A2 $^{+}$ human BM CD34 $^{+}$ cells and implanted with patient-derived primary melanoma tumor grafts. NOG mice were irradiated (200 cGy), followed by adoptive transfer of human CD34 $^{+}$ cells (7×10^4). Mice demonstrating engraftment of human CD3 $^{+}$ cells at 7 weeks were implanted with primary tumor

xenografts (2 x 2 mm) and randomized into groups treated (i.p. weekly x 3 wks) with the following: (i) α -CTLA-4-TGF β RII (5mg/kg); (ii) α -CTLA-4 mAb (5 mg/kg); (iii) TGF- β RII-Fc (5 mg/kg); (iv) combination of α -CTLA-4 mAb (5 mg/kg) and TGF- β RII-Fc (5 mg/kg); (v) Vehicle (Control). Tumors were isolated from tumor-bearing mice in each group for immunophenotype analysis of T cells. Tumor samples were subjected to collagenase digestion for 20 min at 37⁰ C followed by RBC lysis. Tumor cells were resuspended in RPMI, and counted with trypan blue to enumerate live cells. Tregs in tumor-infiltrating T cells isolated from mice in each group were measured by immunophenotype analysis of CD4⁺CD25⁺CD127^{low}FoxP3⁺ cells, as in Figure 3b. Tumor-infiltrating cells from mice treated with α -CTLA-4-TGF β RII exhibited significantly reduced Tregs compared with mice treated with either α -CTLA-4 mAb or the combination of α -CTLA-4 mAb and TGF- β RII-Fc (see Appendix Figure. 5a). Tumor-reactive CD3⁺ and CD8⁺ T cells in BM were measured by immunophenotype analysis of their tumor-specific expression of interferon gamma (IFN- γ). To evaluate tumor-specific IFN- γ expression in CD3⁺/CD8⁺ T cells, BM cells were plated in 96 well plates (2x10⁵ cells/ well) in the presence of tumor cell lysate (patient-derived tumor), non-specific control peptide, or medium alone. Cells were cultured for 72 h followed by incubation with Golgi stop for 4 h. Cells were stained extracellularly with α -CD3-FITC and α -CD8-APC, permeabilized, stained intracellularly with α -IFN- γ -PE or it corresponding isotype control, and then analyzed by flow cytometry. Treatment with α -CTLA-4-TGF β RII resulted in a greater elevation in tumor-reactive IFN- γ -expressing CD8⁺ cells (percentage of CD3⁺CD8⁺ cells) compared to treatment with either α -CTLA-4 mAb or the combination of α -CTLA-4 mAb and TGF- β RII-Fc (see Appendix Figure 5b). Tumor size was evaluated weekly and tumor volume calculated [length x width x height]. Evaluation of tumor volume at 21 days following treatment initiation showed that α -CTLA-4-TGF β RII induced tumor regression and resulted in significantly smaller tumors compared to either α -CTLA-4 mAb (p=0.0018) or the combination of α -CTLA-4 mAb and TGF- β RII-Fc (p=0.024)(Fig. 5c).

In re Application of:
Bedi and Ravi
Application No.: 13/582,717
Filed: October 17, 2012
Page 7

PATENT
Attorney Docket No.: JHU3260-4

10. This data demonstrates that the α -CTLA-4-TGF β RII molecule has unexpected and surprising properties and demonstrates that the presence of both a CTLA-4 antagonist and the ECD of TGF β on the same molecule is critical for anti-tumor efficacy.

11. In my opinion, one of skill in the art would have no motivation to combine a CTLA-4 antagonist and the ECD of TGF β RII in a fusion protein due to the inability of the ECD of TGF β RII to increase the anti-tumor efficacy of α -CTLA-4 when administered in combination as separate molecules. Further, one of skill in the art would not have predicted the surprising and unexpected properties of the presently claimed molecule based on the disclosures in Lazar and Sun.

12. I declare that all statements made herein are of my own knowledge and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

August 25, 2014
Date

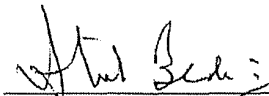
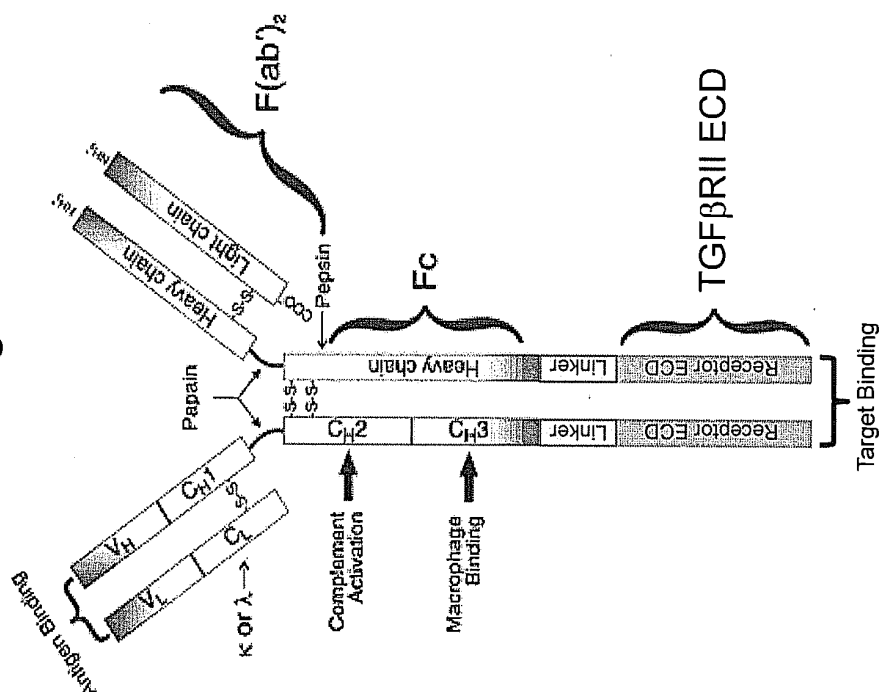

Dr. Atul Bedi

FIGURE 1

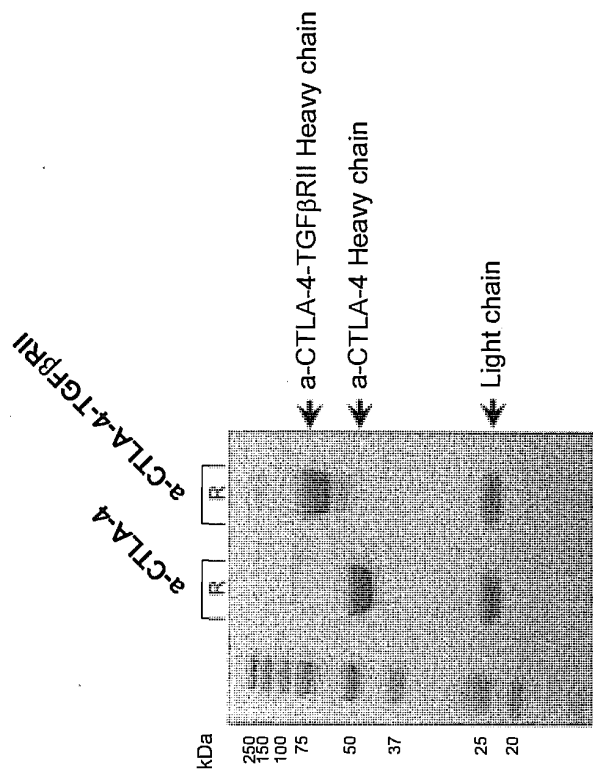
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Bivalent binding to CTLA-4



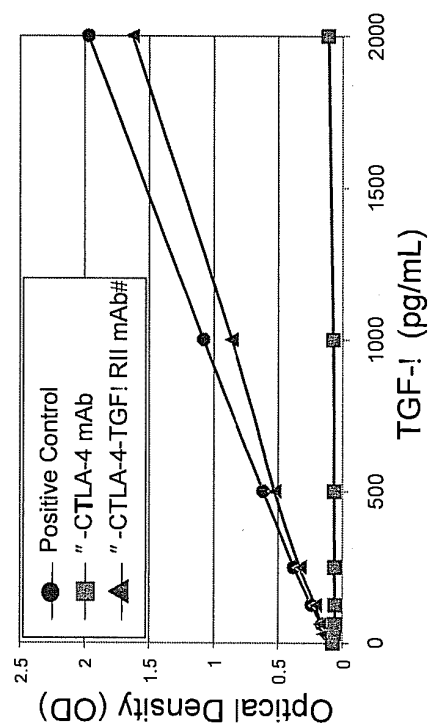
TGFβRII ECD dimer binds TGF-β

2



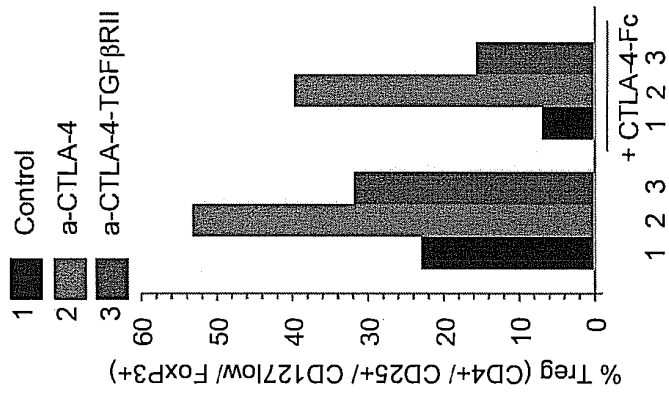
9

α-CTLA-4-TGFβRII simultaneously binds CTLA-4 and TGF-β



a

FIGURE 2



b

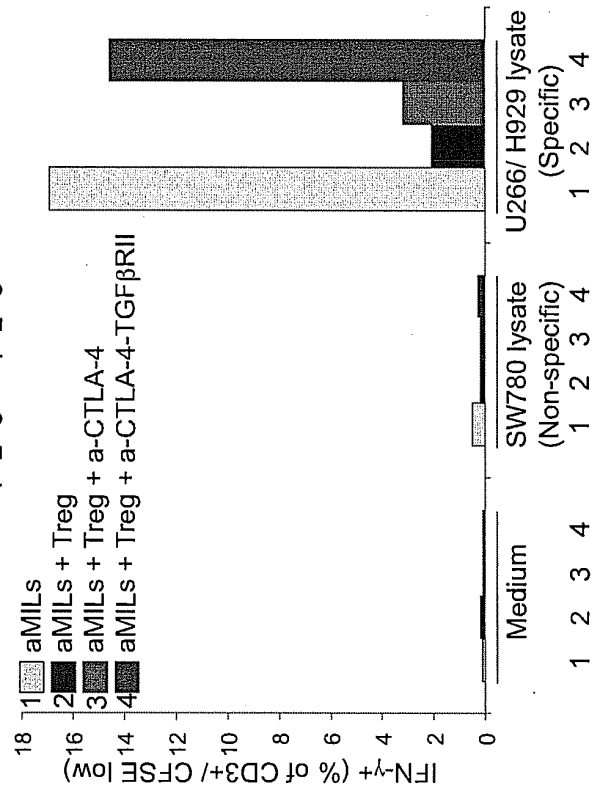
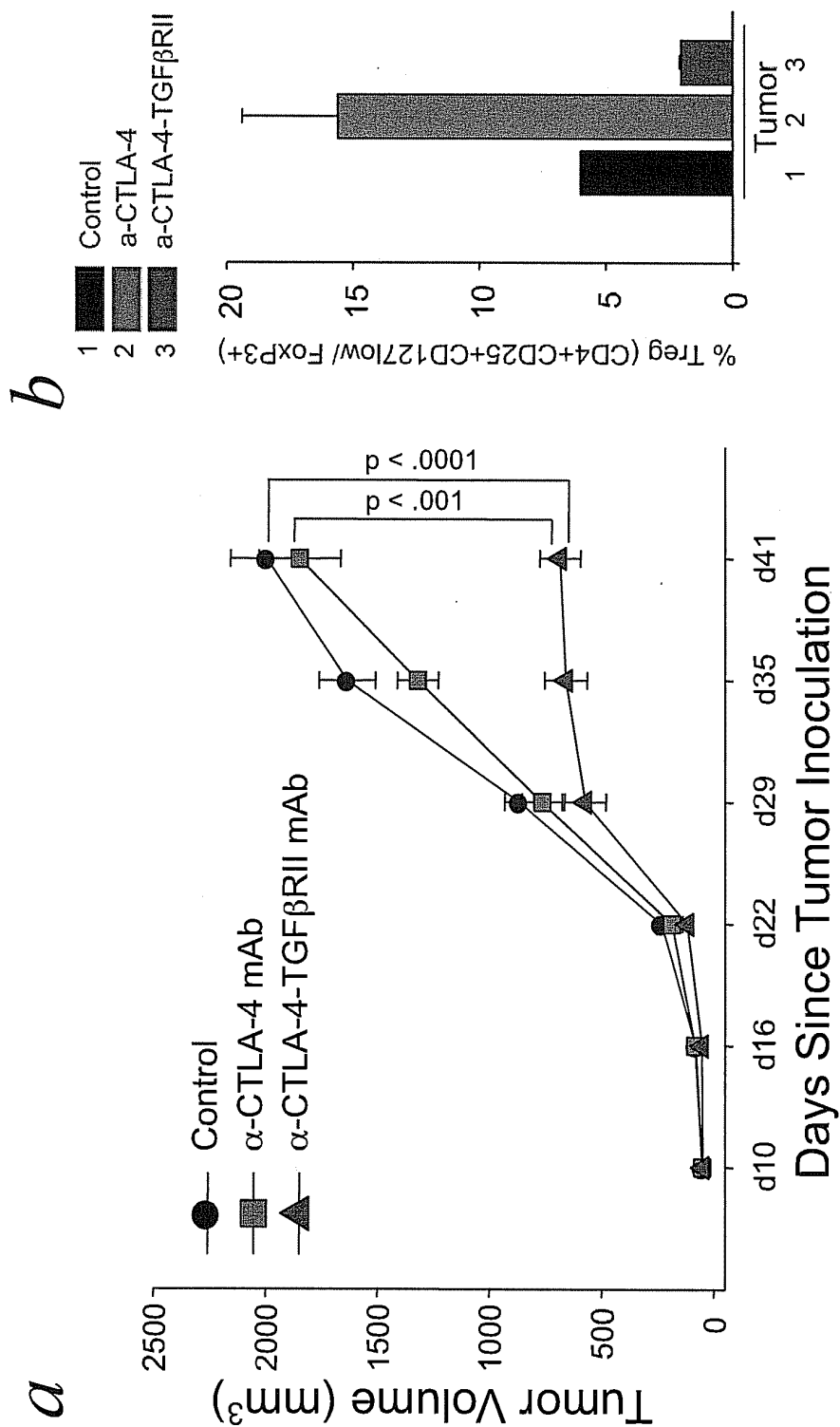


FIGURE 3

Treatment of hu A375 tumor-bearing NOG mice reconstituted with huCD34+ BM cells:



Treatment of hu A375 tumor-bearing NOG mice reconstituted with huCD34+ BM cells:

FIGURE 4

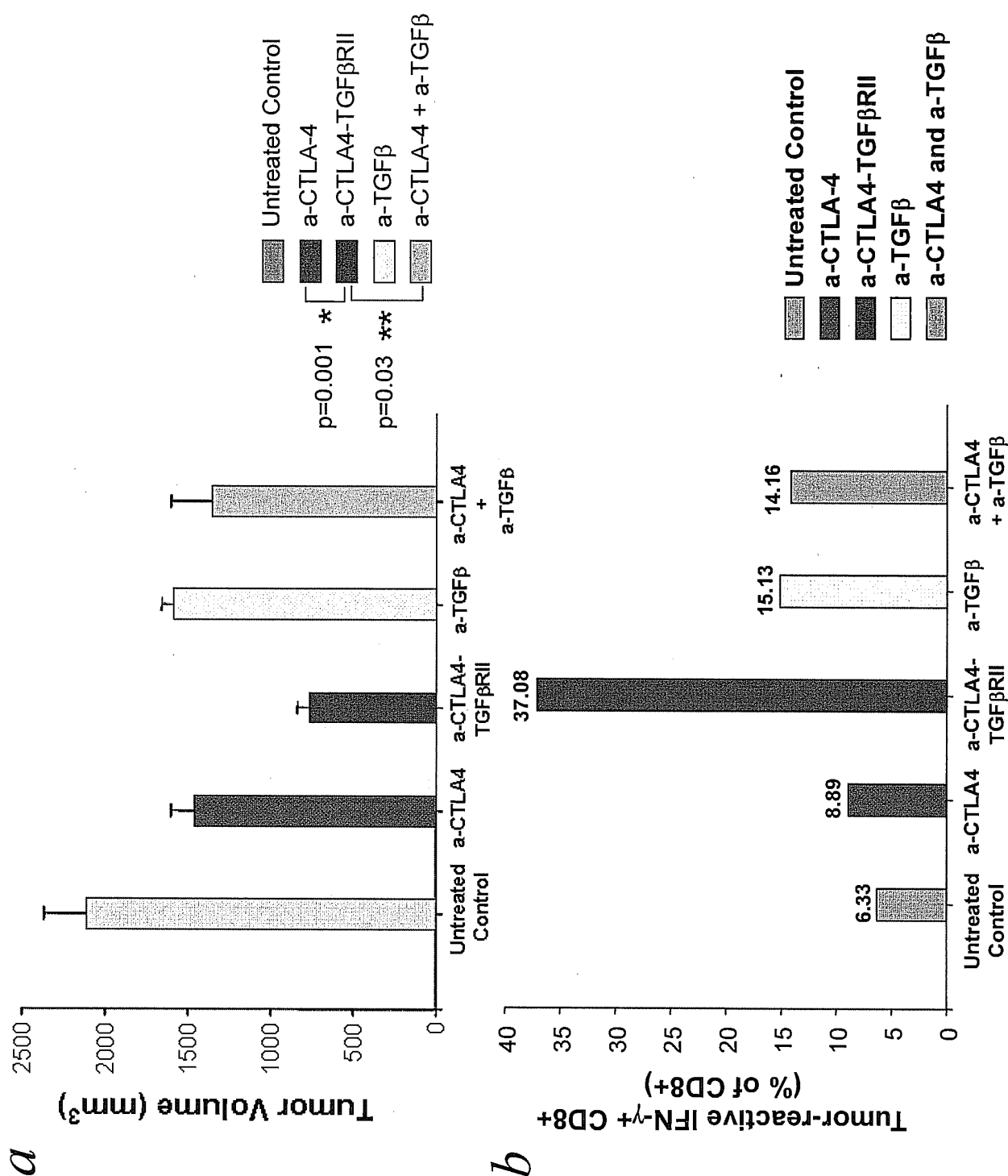
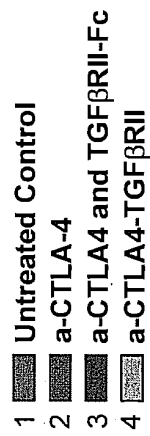


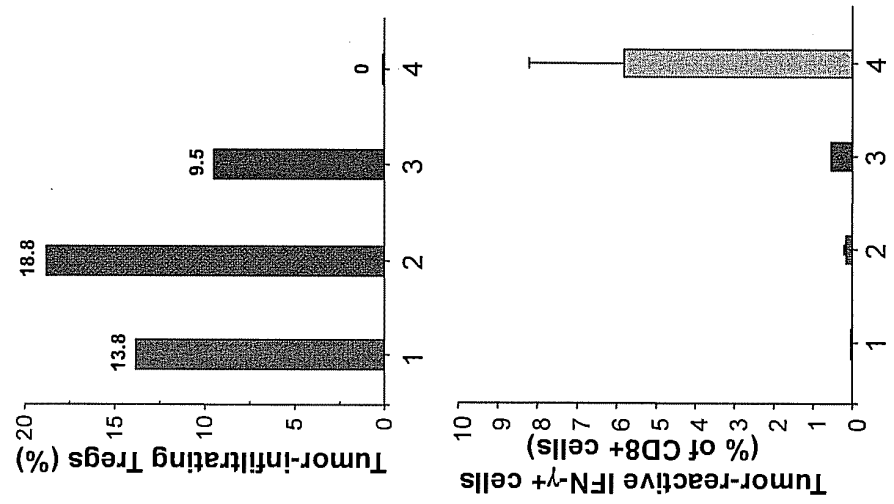
FIGURE 5

Patient-derived primary melanoma tumor xenograft in huCD34⁺ reconstituted NOG mice

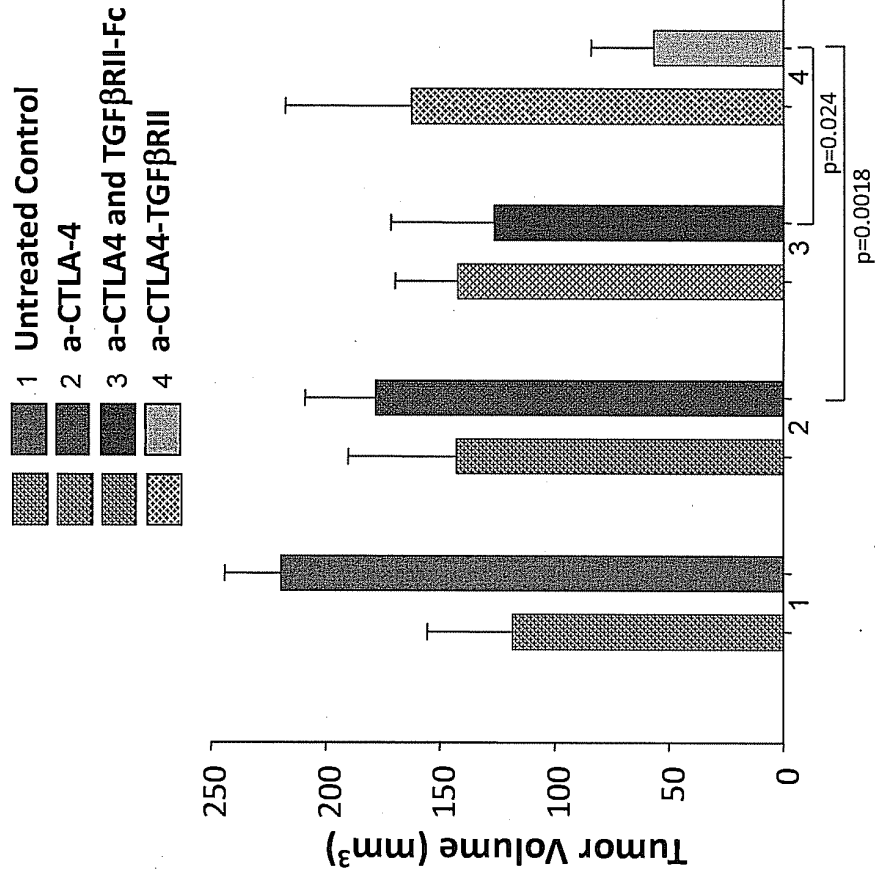
a



b



c Treatment Day: 1 21



Electronic Patent Application Fee Transmittal				
Application Number:		13582717		
Filing Date:		17-Oct-2012		
Title of Invention:		Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins		
First Named Inventor/Applicant Name:		Atul Bedi		
Filer:		Lisa A. Haile/Cara Grifone		
Attorney Docket Number:		JHU3260-4		
Filed as Small Entity				
U.S. National Stage under 35 USC 371 Filing Fees				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 3 months with \$0 paid	2253	1	700	700

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
Total in USD (\$)				700

Electronic Acknowledgement Receipt

EFS ID:	19960919
Application Number:	13582717
International Application Number:	
Confirmation Number:	3707
Title of Invention:	Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Lisa A. Haile/Cara Grifone
Filer Authorized By:	Lisa A. Haile
Attorney Docket Number:	JHU3260-4
Receipt Date:	25-AUG-2014
Filing Date:	17-OCT-2012
Time Stamp:	19:52:55
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$700
RAM confirmation Number	6283
Deposit Account	071896
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)	

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		JHU3260-4Amendment.PDF	496046 83d67993d008b7de60210628ea8c1a3eacf6c3e0	yes	28
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Amendment/Req. Reconsideration-After Non-Final Reject		1	15	
	Extension of Time		16	16	
	Affidavit-traversing rejectns or objectns rule 132		17	28	
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30401 abcf9f0e3579468a7241f59e016603f372186bd1	no	2
Warnings:					
Information:					
Total Files Size (in bytes):			526447		
<p>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.</p> <p><u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p>					

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

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875				Application or Docket Number 13/582,717		Filing Date 10/17/2012		☐ To be Mailed	
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ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

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

APPLICATION AS AMENDED – PART II

AMENDMENT	(Column 1)	(Column 2)	(Column 3)	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)
	08/25/2014	CLAIMS REMAINING AFTER AMENDMENT					
	Total (37 CFR 1.16(i))	* 15	Minus	** 53	= 0	x \$40 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	x \$210 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
TOTAL ADD'L FEE						0	

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

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

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/582,717	10/17/2012	Atul Bedi	JHU3260-4	3707
100899 7590 08/29/2014 The John Hopkins University c/o DLA Piper LLP (US) 4365 Executive Drive, Suite 1100 San Diego, CA 92121-2133			EXAMINER SHAHER, SHULAMITH H	
			ART UNIT 1647	PAPER NUMBER
			MAIL DATE 08/29/2014	DELIVERY MODE PAPER

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.

COMMUNICATION

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

The amendment to the claims submitted on 25 August 2014 is not in compliance with USPTO practice. The status of Claim 1 is indicated as "currently amended"; however the changes in the claim are not identified.

MPEP 714 states

(c) Claims. Amendments to a claim must be made by rewriting the entire claim with all changes (e.g., additions and deletions) as indicated in this subsection, except when the claim is being canceled •

(2) When claim text with markings is required. All claims being currently amended in an amendment paper shall be presented in the claim listing, indicate a status of "currently amended," and be submitted with markings to indicate the changes that have been made relative to the immediate prior version of the claims. The text of any added subject matter must be shown by underlining the added text. The text of any deleted matter must be shown by strike-through except that double brackets placed before and after the deleted characters may be used to show deletion of five or fewer consecutive characters. The text of any deleted subject matter must be shown by being placed within double brackets if strike-through cannot be easily perceived. Only claims having the status of "currently amended," or "withdrawn" if also being amended, shall include markings. If a withdrawn claim is currently amended, its status in the claim listing may be identified as "withdrawn— currently amended."

Correction is required.

Since the above-mentioned reply appears to be *bona fide*, applicant is given **ONE (1) MONTH or THIRTY (30) DAYS** from the mailing date of this notice, whichever is longer, within which to supply the omission or correction in order to avoid abandonment. EXTENSIONS OF THIS TIME PERIOD MAY BE GRANTED UNDER 37 CFR 1.136(a).

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

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

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

SHULAMITH H. SHAFER
Primary Examiner
Art Unit 1647

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

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Bedi and Ravi	Art Unit:	1647
Application No.:	13/582,717	Examiner:	Shulamith H. Shafer
Filed:	October 17, 2012	Conf. No.:	3707
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

Mail Stop AMENDMENT

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

RESPONSE TO NOTICE OF NON-COMPLIANCE

Sir:

Responsive to the Notice of Non-Compliance mailed August 29, 2014, please consider the following amendments and remarks prior to examination of the application.

REMARKS

Objections to the Claims

The Examiner has objected to the claims stating that the claim amendments submitted on August 25, 2014 are not in compliance with USPTO practice. Specifically, the Examiner states that claim 1 is indicated as “Currently Amended” however that changes in the claim are not identified and correction is required.

Applicants respectfully disagree. As recited in the Listing of Claims in the Response to Office Action filed on August 25, 2014 claim 1 recites:

1. (Currently Amended) An isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:
 - (a) the targeting moiety specifically binds Cytotoxic T-lymphocyte associated antigen-4 (CTLA-4); and
 - (b) the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor (TGF- β R). (emphasis added)

Applicants have amended the word “bind” in the first line of step (a) to add an “s”. As shown above and in the Listing of Claims in the Response to Office Action filed on August 25, 2014, the amended claim recites “binds” and the “s” is underlined as required by MPEP 714(c)(2). Applicants submit that the claim amendments are compliant with USPTO practice and respectfully withdraw of the objection.

In re Application of:
Bedi and Ravi
Application No.: 13/582,717
Filed: October 17, 2012
Page 3

PATENT
Attorney Docket No.: JHU3260-4

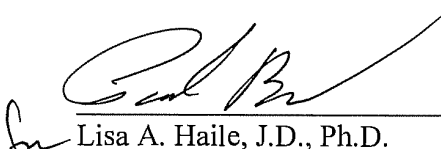
CONCLUSION

In the event any matters remain to be resolved, the Examiner is requested to contact the undersigned at the telephone number given below so that a prompt disposition of this application can be achieved.

No fee is believed due in connection with this submission. The Commissioner is hereby authorized to charge any additional fees required by this submission, or make any credits or overpayments, to Deposit Account No. 07-1896 referencing the above-identified attorney docket number.

Respectfully submitted,

Date: September 17, 2014


for Lisa A. Haile, J.D., Ph.D.
Reg. No. 38,347
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

Res #54984
Pamela Bechtel

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

Electronic Acknowledgement Receipt	
EFS ID:	20169779
Application Number:	13582717
International Application Number:	
Confirmation Number:	3707
Title of Invention:	Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Lisa A. Haile/Cara Grifone
Filer Authorized By:	Lisa A. Haile
Attorney Docket Number:	JHU3260-4
Receipt Date:	17-SEP-2014
Filing Date:	17-OCT-2012
Time Stamp:	18:13:25
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment		no			
File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Amendment/Req. Reconsideration-After Non-Final Reject	JHU3260-4Response.PDF	26922	no	3
			2619d4d7a8e38de820c50ff6757693c2399268c2		
Warnings:					
Information:					

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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



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NOTICE OF ALLOWANCE AND FEE(S) DUE

100899 7590 11/24/2014
The John Hopkins University
c/o DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

EXAMINER

SHAHER, SHULAMITH H

ART UNIT

PAPER NUMBER

1647

DATE MAILED: 11/24/2014

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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13/582,717

10/17/2012

Atul Bedi

JHU3260-4

3707

TITLE OF INVENTION: Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$480	\$0	\$0	\$480	02/24/2015

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: **Mail** **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

100899 7590 11/24/2014
The John Hopkins University
c/o DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/582,717	10/17/2012	Atul Bedi	JHU3260-4	3707

TITLE OF INVENTION: Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$480	\$0	\$0	\$480	02/24/2015

EXAMINER	ART UNIT	CLASS-SUBCLASS
SHAFER, SHULAMITH H	1647	514-019200

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

- (1) The names of up to 3 registered patent attorneys or agents OR, alternatively, 1 _____
- (2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. 2 _____
- 3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:

- ☐ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): (Please first reapply any previously paid issue fee shown above)

- ☐ A check is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☐ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. Change in Entity Status (from status indicated above)

- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
- ☐ Applicant asserting small entity status. See 37 CFR 1.27
- ☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____

Date _____

Typed or printed name _____

Registration No. _____



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/582,717	10/17/2012	Atul Bedi	JHU3260-4	3707
100899	7590	11/24/2014	EXAMINER	
The John Hopkins University c/o DLA Piper LLP (US) 4365 Executive Drive, Suite 1100 San Diego, CA 92121-2133			SHAHER, SHULAMITH H	
			ART UNIT	PAPER NUMBER
			1647	

DATE MAILED: 11/24/2014

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)

(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

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

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

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 13/582,717	Applicant(s) BEDI ET AL.	
	Examiner SHULAMITH H. SHAFER	Art Unit 1647	AI/A (First Inventor to File) Status No

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

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to 8/25/14, 9/17/14, telephonic interview 11/17/14.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

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

3. ☒ The allowed claim(s) is/are 1,2,4,5,7,10,11,55-59 and 68-70. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

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

Certified copies:
a) ☐ All b) ☐ Some *c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: _____.

Applicant has **THREE MONTHS FROM THE "MAILING DATE"** of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in **ABANDONMENT** of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☒ Notice of References Cited (PTO-892) 2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____ 3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material 4. ☒ Interview Summary (PTO-413), Paper No./Mail Date <u>11/17/2014</u> .	5. ☒ Examiner's Amendment/Comment 6. ☐ Examiner's Statement of Reasons for Allowance 7. ☐ Other _____.
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/SHULAMITH H. SHAFER/ Primary Examiner, Art Unit 1647	
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Detailed Action

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

Status of Application, Amendments, And/Or Claims:

Applicants' response of 25 August 2014 and 17 September 2014 is acknowledged.

Claims 1, 7, 10 and 11 have been amended and the amendment made of record. Claims 68-70 are newly presented and entered into the record.

Additionally, applicants have submitted a declaration under 37 CFR 1.132 by Dr. Atul Bedi, which will be discussed below.

Claims 1, 2, 4, 5, 7, 10, 11, 55-59 and 68-70 are pending in the instant application. Claims 55-59 stand withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim.

Claims 1, 2, 4, 5, 7, 10, 11 and 68-70 are under consideration to the extent they read on the elected invention, an isolated molecule comprising a targeting moiety wherein the targeting moiety specifically binds CTLA-4 and an immunomodulatory moiety wherein the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of TGF- β receptor II.

Withdrawn Objections/Rejections

The objection to the abstract of the disclosure is withdrawn in light of Applicants' amendment to the specification by submission of an amended abstract.

Applicants' amendment to the claims has overcome the following rejections of record:

The rejection of Claims 7, 10 and 11 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention

The rejection of Claims 10 and 11 under 35 U.S.C. 102(e) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Lazar et al.

The Declaration under 37 CFR 1.132 filed 25 August 2014 by Dr. Atul Bedi is sufficient to overcome the rejection of claims 1, 2, 4, 5 and 7 under 35 U.S.C. 103(a) as being unpatentable over Lazar et al. (US 8,399,618, priority claimed to SN 12/020443, now US 8,101,720, filed 25 January 2008) and Sun et al. (US 7,795,389, filed 28 September 2005)

Rejoinder of Claims

Claims 1, 2, 4, 5, 7, 10 and 68-70 are directed to an allowable product. Pursuant to the procedures set forth in MPEP § 821.04(b), claims 55-59, directed to the process of making or using the allowable product, previously withdrawn from consideration as a result of a restriction requirement, are hereby rejoined and fully examined for patentability under 37 CFR 1.104.

Because a claimed invention previously withdrawn from consideration under 37 CFR 1.142 has been rejoined, **the restriction requirement between groups I and II as set forth in the Office action mailed on 7 May 2013 is hereby withdrawn.** In view of the withdrawal of the restriction requirement as to the rejoined inventions, applicant(s) are advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application. Once the restriction requirement is withdrawn, the provisions of 35 U.S.C. 121 are no longer applicable. See *In re Ziegler*, 443 F.2d 1211, 1215, 170 USPQ 129, 131-32 (CCPA 1971). See also MPEP § 804.01.

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided

Art Unit: 1647

by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Dr. L. A. Haile on 17 November 2014.

Please amend claim 55 so that the claim reads as follows:

55. A method of treating a neoplastic disease comprising: administration to a subject in need thereof one or more molecules of claim 1, wherein the neoplastic disease is bladder cancer or melanoma.

Please amend claim 69 so that the claim reads as follows:

69. The isolated molecule of claim 68, wherein the linker is (GGGGS)_n (SEQ ID NO:104), and wherein n is 1, 2, 3, 4, 5, 6, 7 or 8.

70. The isolated molecule of claim 68, wherein the linker comprises the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO:104).

Art made of record:

The following art is made of record and not relied upon is considered pertinent to applicant's disclosure. Govindappa, et al. (US 8,815,247) disclose chimeric polypeptides containing at least one targeting moiety to target a cancer cell and at least one immunomodulatory molecule (column 1, lines 58-60). The immunomodulatory molecule may bind a TGF-beta receptor (column 2, lines 23-29); the targeting moiety may be an antibody that binds to CTLA4 (column 3, lines 19-20). However, the '247 patent is not available as prior art, as the earliest priority date is 13 March 2013.

Conclusion:

Claims 1, 2, 4, 5, 7, 10, 11, 55-59 and 68-70 are allowable with the aforementioned amendment.

Art Unit: 1647

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

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

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

SHULAMITH H. SHAFER
Primary Examiner
Art Unit 1647

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

Application/Control Number: 13/582,717
Art Unit: 1647

Page 6

<i>Examiner-Initiated Interview Summary</i>	Application No. 13/582,717	Applicant(s) BEDI ET AL.	
	Examiner SHULAMITH H. SHAFER	Art Unit 1647	

All participants (applicant, applicant's representative, PTO personnel):

(1) SHULAMITH H. SHAFER. (3)_____.

(2) Dr. L. A. Haile. (4)_____.

Date of Interview: 17 November 2014.

Type: ☒ Telephonic ☐ Video Conference
 ☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
 If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☒Others
 (For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: 55,69 and 70.

Identification of prior art discussed: N/A.

Substance of Interview
 (For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

The Examiner suggested amending rejoined claim 55 to delete reference to "preventing" and limiting neoplastic disease to bladder cancer and melanoma to put claims 55-59 in condition for allowance. Claims 69 and 70 require the amendment to recite SEQ ID NO:. Dr. Haile authorized amendment to claim 55 and stated that claims 69 and 70 should be amended to include recitation of SEQ ID NO:104.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/SHULAMITH H. SHAFER/ Primary Examiner, Art Unit 1647	
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Notice of References Cited	Application/Control No. 13/582,717		Applicant(s)/Patent Under Reexamination BEDI ET AL.	
	Examiner SHULAMITH H. SHAFER		Art Unit 1647	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-8,815,247	08-2014	Govindappa et al.	424/179.1
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes 	Application/Control No. 13582717	Applicant(s)/Patent Under Reexamination BEDI ET AL.
	Examiner SHULAMITH H SHAFER	Art Unit 1647

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Inventor name search, PALM, EAST and STN, search history included	2/12/14	SHS
EAST search, search history included	2/12/14	SHS
STN search, search history included	2/12/14	SHS
SEQ search, see results in SCORE	10/9/2013	SHS
Inventor name search update, PALM and EAST, search history included	11/18/2014	SHS
EAST search update, search history included	11/18/2014	SHS
SEQ search update, SEQ ID NOs:79-91, see results in SCORE	11/4/2014	SHS

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
	EAST search of claims, search history included	11/18/2014	SHS
	SEQ search, patent and pending databases	11/4/2014	SHS

	/SHULAMITH H SHAFER/ Primary Examiner.Art Unit 1647
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Issue Classification 	Application/Control No. 13582717	Applicant(s)/Patent Under Reexamination BEDI ET AL.	
	Examiner SHULAMITH H SHAFER	Art Unit 1647	

CPC					
Symbol				Type	Version
C07K	14	/	495	F	2013-01-01
C07K	14	/	70532	I	2013-01-01
C07K	14	/	70575	I	2013-01-01
C07K	14	/	70578	I	2013-01-01
C07K	14	/	70596	I	2013-01-01
C07K	14	/	71	I	2013-01-01
C07K	16	/	28	I	2013-01-01
C07K	16	/	2812	I	2013-01-01
C07K	16	/	2815	I	2013-01-01
C07K	16	/	2851	I	2013-01-01
C07K	2319	/	00	A	2013-01-01
A61K	2039	/	505	A	2013-01-01
C07K	2317	/	73	A	2013-01-01
C07K	2317	/	76	A	2013-01-01
		/			

CPC Combination Sets				
Symbol	Type	Set	Ranking	Version

NONE (Assistant Examiner) _____ (Date) _____		Total Claims Allowed: 15	
/SHULAMITH H SHAFER/ Primary Examiner. Art Unit 1647 (Primary Examiner) _____ (Date) 11/18/2014		O.G. Print Claim(s) 1	O.G. Print Figure none

Issue Classification 	Application/Control No. 13582717	Applicant(s)/Patent Under Reexamination BEDI ET AL.
	Examiner SHULAMITH H SHAFER	Art Unit 1647

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47															
Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	1														
2	2														
3	4														
4	5														
5	7														
6	10														
7	11														
8	55														
9	56														
10	57														
11	58														
12	59														
13	68														
14	69														
15	70														

NONE	Total Claims Allowed: 15	
(Assistant Examiner)	(Date)	
/SHULAMITH H SHAFER/ Primary Examiner.Art Unit 1647	11/18/2014	O.G. Print Claim(s) 1
(Primary Examiner)	(Date)	O.G. Print Figure none

EAST Search History**EAST Search History (Prior Art)**

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	27	bedi-atul.in. or ravi-rajani.in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2014/11/18 08:26
L2	8054	"cytotoxic T-lymphocyte Antigen 4" or CTLA-4 or CD152 or "cluster of differentiation 152"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2014/11/18 08:29
L3	5389	I2 same (antibody or antibodies or bind or target or Fc pr scFv)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2014/11/18 08:29
L4	3874	I2 with (antibody or antibodies or bind or target or Fc pr scFv)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2014/11/18 08:30
L5	32	"transforming growth factor-beta receptor II" or TGF-betaRII	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2014/11/18 08:31
L6	959759	"transforming growth factor beta receptor" or TGF-betaR pr "TGF-beta receptor"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2014/11/18 08:31
L7	3073	"transforming growth factor beta receptor" or TGF-betaR or "TGF-beta receptor"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2014/11/18 08:32
L8	40	I4 and I7	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT	OR	ON	2014/11/18 08:32

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L9	518	"cytotoxic T-lymphocyte Antigen 4".clm. or CTLA-4.clm. or CD152.clm. or "cluster of differentiation 152".clm"	US-PGPUB; USPAT; UPAD	OR	ON	2014/11/18 08:38
L10	264	I9 with (bind.clm. or antibody.clm. or antibodies.clm. or Fc.clm. or scFv.clm.)	US-PGPUB; USPAT; UPAD	OR	ON	2014/11/18 08:39
L11	2	I10 and ("transforming growth factor beta receptor".clm. or TGF-betaR.clm. or "TGF-beta receptor".clm.)	US-PGPUB; USPAT; UPAD	OR	ON	2014/11/18 08:40

11/ 18/ 2014 8:41:42 AM**C:\Users\sshafer\Documents\EAST\Workspaces\13582717.wsp**

Substitute for form 1449/PTO

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**

(Use as many sheets as necessary)

Complete if Known

Application Number	13/582,717
Filing Date	October 17, 2012
First Named Inventor	Atul Bedi
Art Unit	1647
Examiner Name	Shulamith H. Shafer
Attorney Docket Number	JHU3260-4

Sheet

1

of

2

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)			
	AA	US-2005/054832 A1	03/10/2005	LAZAR GREGORY A.	
		US-			
		US-			
		US-			
		US-			
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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

/Shulamith Shafer/

Date
Considered

10/09/2013

*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.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

/S.H.S./

PTO/SB/08b (07-09)

Approved for use through 07/31/2012. OMB 0651-0031

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction 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 1449B/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>		Complete if Known			
		Application Number	13/582,717		
		Filing Date	October 17, 2012		
		First Named Inventor	Atul Bedi		
		Art Unit	1647		
		Examiner Name	Shulamith H. Shafer		
Sheet	2	of	2	Attorney Docket No.	JHU3260-4

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ²
	AA	Supplementary European Search Report regarding Application No.: EP 11 75 1481	

Examiner Signature	/Shulamith Shafer/	Date Considered	02/20/2014
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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.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: **Mail** Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

100899 7590 11/24/2014
 The John Hopkins University
 c/o DLA Piper LLP (US)
 4365 Executive Drive, Suite 1100
 San Diego, CA 92121-2133

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

Aldon Griffis	(Depositor's name)
<i>Aldon Griffis</i>	(Signature)
February 20, 2015	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/582,717	10/17/2012	Atul Bedi	JHU3260-4	3707

TITLE OF INVENTION: Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$480	\$0	\$0	\$480	02/24/2015

EXAMINER	ART UNIT	CLASS-SUBCLASS
SHAFFER, SHULAMITH H	1647	514-019200

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. Use of a Customer Number is required.

2. For printing on the patent front page, list

- (1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
 (2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1. DLA Piper LLP (US)

2.

3.

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

The Johns Hopkins University

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Baltimore, Maryland

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:

- ☒ Issue Fee
☐ Publication Fee (No small entity discount permitted)
☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): (Please first reapply any previously paid issue fee shown above)

- ☐ A check is enclosed.
☐ Payment by credit card. Form PTO-2038 is attached.
☒ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number 07-1896 (enclose an extra copy of this form).

5. Change in Entity Status (from status indicated above)

- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
☐ Applicant asserting small entity status. See 37 CFR 1.27
☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Lisa A. Haile/

Date February 20, 2015

Typed or printed name Lisa A. Haile, J.D., Ph.D.

Registration No. 38,347

Electronic Patent Application Fee Transmittal				
Application Number:		13582717		
Filing Date:		17-Oct-2012		
Title of Invention:		Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins		
First Named Inventor/Applicant Name:		Atul Bedi		
Filer:		Lisa A. Haile/Aldon Griffis		
Attorney Docket Number:		JHU3260-4		
Filed as Small Entity				
Filing Fees for U.S. National Stage under 35 USC 371				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Utility Appl Issue Fee	2501	1	480	480

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				480

Electronic Acknowledgement Receipt

EFS ID:	21556163
Application Number:	13582717
International Application Number:	
Confirmation Number:	3707
Title of Invention:	Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Lisa A. Haile/Aldon Griffis
Filer Authorized By:	Lisa A. Haile
Attorney Docket Number:	JHU3260-4
Receipt Date:	20-FEB-2015
Filing Date:	17-OCT-2012
Time Stamp:	14:45:16
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$480
RAM confirmation Number	1122
Deposit Account	071896
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. 1.492 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		C11123_P11123-03_20121017_OTH_DLAP_IssueFeeEFS.PDF	47863 467029dbee9bf4e2e002fa013bbcd9232b149b9a	yes	3

Multipart Description/PDF files in .zip description

Document Description	Start	End
Transmittal Letter	1	1
Miscellaneous Incoming Letter	2	2
Issue Fee Payment (PTO-85B)	3	3

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	30547 1f93dadae12d03512102759714e426f571849f9d	no	2
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Warnings:

Information:

Total Files Size (in bytes):	78410
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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

Inventors: Bedi and Ravi
Application No.: 13/582,717
Filed: October 17, 2012
Title: COMPOSITIONS AND METHODS FOR TARGETED
IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Art Unit: 1647
Examiner: S.H. Shafer
Conf. No.: 3707

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

TRANSMITTAL SHEET

Sir:

Transmitted herewith for the above-identified application, please find:

1. Response to Notice of Allowance mailed November 24, 2014 (1 page);
and
2. Form PTOL-85B Issue Fee Transmittal (1 page).

The fees for this filing total \$480.00, which consist of \$480.00 for the Issue Fee pursuant to 37 CFR § 1.18(a). The Commissioner is hereby authorized to charge this fee and any other fees associated with the filing submitted herewith or credit any overpayment to Deposit Account No. 07-1896.

Respectfully submitted,

Date: February 20, 2015

Res # 54984


Lisa A. Haile, J.D., Ph.D.
Registration No. 38,347
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

Pamela Becluli

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

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	1647
Application No.:	13/582,717	Examiner:	S.H. Shafer
Filed:	October 17, 2012	Conf. No.:	3707
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

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

RESPONSE TO NOTICE OF ALLOWANCE

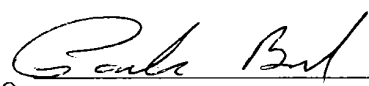
Sir:

In response to the Notice of Allowance mailed November 24, 2014, attached is the completed Issue Fee Transmittal Form PTOL-85B.

The Commissioner is hereby authorized to charge \$480.00 to Deposit Account No. 07-1896 for the required Issue Fees. The Commissioner is also authorized to charge any other fees associated with the filing submitted herewith or credit any overpayment to the aforementioned Deposit Account.

Respectfully submitted,

Date: February 20, 2015

 *Rs # 54984*
Pamela Bickel

Lisa A. Haile, J.D., Ph.D.
Registration No. 38,347
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

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

WEST255330956.1
331323-000997



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/582,717	03/31/2015	8993524	JHU3260-4	3707

100899 7590 03/11/2015
The John Hopkins University
c/o DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional applicants):

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

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): Bedi, et al. Assignee: The Johns Hopkins University
Patent No.: 8,993,524 Appl. No.: 13/582,717 Conf. No. 3707
Issued: March 31, 2015 Filed:
Title: COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY
ANTIBODIES AND FUSION PROTEINS

ATTN: CERTIFICATE OF CORRECTION BRANCH
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 C.F.R. §1.323

Sir:

The undersigned requests the issuance of a Certificate of Correction in the above-identified patent to correct the government support clause. Accompanying this Request is a completed form PTO/SB/44.

Applicants submit that the Request for Certificate of Correction does not raise issues of new matter.

In accordance with 37 C.F.R. §1.20(a), the amount of \$150.00 in payment of the certificate of correction fee due is hereby paid electronically via USPTO EFS-Web.

Respectfully submitted,

Date: February 1, 2018

/Joseph Contrera/
Joseph Contrera
Reg. No. 44628

Customer No. 54110
Johns Hopkins Technology Transfer
1812 Ashland Avenue, Suite 110
Baltimore, MD 21205
Tel: 410-614-0300
Fax: 410-223-1730

UNITED STATES PATENT AND TRADEMARK OFFICE CERTIFICATE OF CORRECTION

Page 1 of 1

PATENT NO. : 8,993,524
APPLICATION NO.: 15/582,717
ISSUE DATE : March 31, 2015
INVENTOR(S) : Atul Bedi, Rajani Ravi

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On page 1, immediately after the title, please add the following paragraph:

STATEMENT OF GOVERNMENTAL INTEREST

This invention was made with government support under grant number CA123277, awarded by the National Institutes of Health. The government has certain rights in the invention.

MAILING ADDRESS OF SENDER (Please do not use customer number below):

Johns Hopkins Technology Ventures
1812 Ashland Avenue, Suite 110
Baltimore, MD 21205

This collection of information is required by 37 CFR 1.322, 1.323, and 1.324. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1.0 hour to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Attention Certificate of Corrections Branch, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

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

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

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal				
Application Number:		13582717		
Filing Date:		17-Oct-2012		
Title of Invention:		Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins		
First Named Inventor/Applicant Name:		Atul Bedi		
Filer:		Joseph Contrera/Cheryl Oliver		
Attorney Docket Number:		JHU3260-4		
Filed as Large Entity				
Filing Fees for U.S. National Stage under 35 USC 371				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Certificate of correction	1811	1	150	150

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				150

Electronic Acknowledgement Receipt

EFS ID:	31672057
Application Number:	13582717
International Application Number:	
Confirmation Number:	3707
Title of Invention:	Compositions and Methods for Targeted Immunomodulatory Antibodies and Fusion Proteins
First Named Inventor/Applicant Name:	Atul Bedi
Customer Number:	100899
Filer:	Joseph Contrera/Cheryl Oliver
Filer Authorized By:	Joseph Contrera
Attorney Docket Number:	JHU3260-4
Receipt Date:	01-FEB-2018
Filing Date:	17-OCT-2012
Time Stamp:	12:57:54
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	\$ 150
RAM confirmation Number	020118INTEFSW12581500
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Certificate of Correction	P11123-03_COC.pdf	207848	no	3
			5fd3fb4a3fb2958ceb7357b581ee993cd55a4ac0		

Warnings:**Information:**

2	Fee Worksheet (SB06)	fee-info.pdf	30180	no	2
			49d30aa2a752bac6c51f7d975cf78893ea83d6de		

Warnings:**Information:**

Total Files Size (in bytes):			238028
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This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 8,993,524 B2
APPLICATION NO. : 13/582717
DATED : March 31, 2015
INVENTOR(S) : Atul Bedi and Rajani Ravi

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

Column 1, immediately after the title, please add the following paragraph:

STATEMENT OF GOVERNMENTAL INTEREST

This invention was made with government support under grant number CA123277, awarded by the National Institutes of Health. The government has certain rights in the invention.

Signed and Sealed this
Tenth Day of April, 2018



Andrei Iancu
Director of the United States Patent and Trademark Office



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY.DOCKET NO./TITLE	REQUEST ID
13/582,717	10/17/2012	Atul Bedi	JHU3260-4	103566

Acknowledgement of Loss of Entitlement to Entity Status Discount

The entity status change request below filed through Private PAIR on 01/17/2020 has been accepted.

CERTIFICATIONS:

Change of Entity Status:

☒ Applicant changing to regular undiscounted fee status.

NOTE: Checking this box will be taken to be notification of loss of entitlement to small or micro entity status, as applicable.

This portion must be completed by the signatory or signatories making the entity status change in accordance with 37 CFR 1.4(d)(4).

Signature:	/Aldon Griffis/
Name:	Aldon Griffis
Registration Number:	55949