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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).

Express Mail Label No. _____

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
Rajani	Ravi	Ruxtan, Maryland
Additional inventors are being named on the _____ separately numbered sheets attached hereto		
TITLE OF THE INVENTION (500 characters max):		
TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		
Direct all correspondence to: CORRESPONDENCE ADDRESS		
☒ The address corresponding to Customer Number: 28213		
OR		
☐ Firm or Individual Name		
Address		
City	State	Zip
Country	Telephone	Email
ENCLOSED APPLICATION PARTS (check all that apply)		
☒ Application Data Sheet. See 37 CFR 1.76		
☐ CD(s), Number of CDs _____		
Application Cover Sheet		
Sheets of Claims: 5		
☐ Drawing(s) Number of Sheets _____		
☒ Other (specify) <u>Appendix 1-2</u>		
☒ Specification (e.g. description of the invention) Number of Pages <u>5</u>		
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.		
<div style="border: 1px solid black; padding: 5px; display: inline-block;">110.00</div>		
☐ 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 (\$)		
☒ The Director is hereby authorized to charge the filing fee and application size fee (if applicable) or credit any overpayment to Deposit Account Number: <u>07-1896</u>		

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. 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 8 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, 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.

PROVISIONAL APPLICATION COVER SHEET
Page 2 of 2

PTO/SB/16 (12-08)

Approved for use through 06/30/2010. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.



No



Yes, the name of the U.S. Government agency and the Government contract number are: _____

WARNING:

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

SIGNATURE _____

Lisa A. Haile

Date January 24, 2011

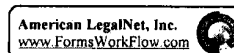
TYPED or PRINTED NAME Lisa A. Haile, J.D., Ph.D.

REGISTRATION NO. 38,347
(if appropriate)

TELEPHONE 858.677.1456

Docket Number: JHU3260-3

WEST\223022972.1
331323-000890



APPLICATION DATA SHEET

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Citizenship Country:: USA

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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.
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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 JHTT 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-adjutant 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

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 cyoreduction 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.
- Brier B and Moses HL. Tumor microenvironment: TGFβ: the molecular Jekyll and Hyde of cancer. *Nat Rev Cancer* 6:506-520, 2006.

- 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
If yes, please complete the Software Information Form which can be found at:
<http://www.jhtt.jhu.edu/For%20Hopkins%20Inventors/reporting.html>

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

13. Agreement Support Information

Were any materials, equipment or software under a special agreement, such as material transfer agreements, purchase agreements, sponsored research agreements, or the like used? Does an Inventor have a consulting agreement related to the Invention? ☐ Yes ☐ No

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

Check any prior disclosures or anticipated disclosures, either written or oral, of the Invention:

☐ Abstract(s) ☐ Publication(s) ☐ Seminar(s) ☐ Presentation(s) ☐ Other ☒ None

For each disclosure, provide the following information as appropriate in the space below:

- If **PUBLISHED**, include all journal citations and attach a reprint.
- If **NOT YET PUBLISHED**, attach a copy of the abstract or manuscript and the anticipated publication date.
- For any other written or oral disclosure, provide the names, addresses and affiliation of anyone to whom you have disclosed the Invention and the date of any disclosure.

For each disclosure please provide: The title of the disclosure; the date of the disclosure and attach electronic copy, as appropriate.

15. Was this Invention Disclosure developed within a specific JHU Center or Institute (e.g. Engineering Research Center; Brain Sciences, NanoBioTechnology, etc.) ☐ Yes ☒ No If Yes, please provide the name of the center or institution.

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

[It is not necessary to obtain all signatures before submitting this report to JHTT.]

Please provide contact information for all inventors and department chairs and JHTT will follow up for signatures]

[Please copy this page for additional Inventors as necessary]

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Are you a Howard Hughes Medical Institute employee or investigator?

☐ Yes ☒ No

Are you a Kennedy Krieger Institute employee or investigator?

☐ Yes ☒ No

Are you the Primary Contact for JHTT?

☒ Yes ☐ No

Department Director: David Sidransky M.D.; John Niparko, M.D. E-mail (if known): dsidrans@jhmi.edu ; jnipark@jhmi.edu

[The Primary Contact will be the main contact person for JHTT on all matters associated with this Invention Disclosure. It is the responsibility of the Primary Contact to keep all other JHU Inventors named on this Invention Disclosure informed of the status of such matters. Preferably, the Primary Contact is a full time JHU faculty member.]

INVENTOR INFORMATION:

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Last First Middle Degree

Title or Position: Assistant Professor of Otolaryngology-Head and Neck Surgery

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Department: Otolaryngology-Head and Neck Surgery, Division of Head and Neck Cancer Research

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Citizenship: U.S.A

Residential address: 7810 Ballston Road, Ruxton, Maryland 21204

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☐ Yes ☒ No

Are you a Kennedy Krieger Institute employee or investigator?

☐ Yes ☒ No

Are you the Primary Contact for JHTT?

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Department Director: David Sidransky M.D.; John Niparko, M.D. E-mail (if known): dsidrans@jhmi.edu ; jnipark@jhmi.edu

[Please copy this page for additional Directors or Inventors as necessary]

Section A JHU SCHOOL and DEPARTMENT DIRECTOR'S ACKNOWLEDGEMENT
I approve submission of this Invention Disclosure

JHU Department Director Signature _____ Date _____	<u>David Sidransky M.D</u> <u>Otolaryngology-Head and Neck Surgery,</u> <u>Division of Head and Neck Cancer</u> <u>Research</u> Typed or Printed Name Department and School
JHU Department Director Signature _____ Date _____	Typed or Printed Name Department and School

Section B JHU INVENTOR CERTIFICATION and ASSIGNMENT

This section is to be completed by those JHU personnel subject to The Johns Hopkins University Intellectual Property Policy. Students may or may not be subject.

- I/we, the Inventors, hereby certify that the information set forth in this Invention Disclosure is true and complete to the best of my/our knowledge.
- I/we, the Inventors, hereby certify that I/we will promptly advise JHTT of any commercial interest regarding the Invention described herein.
- I/we, the Inventor(s), subject to The Johns Hopkins University Intellectual Property Policy and not under an obligation to assign intellectual property rights to another party, hereby affirm that in consideration for The Johns Hopkins University's evaluation of commercial potential and a share of income which I/we may receive upon commercialization of my/our Invention, on the date of my/our signature(s) as indicated below do hereby assign and transfer my/our entire right, title and interest in and to the Invention described herein unto The Johns Hopkins University, its successors, legal representatives and assigns.

JHU Inventor Signature	Atul Bedi Typed or Printed Name	Date: % of Contribution <u>75</u>
JHU Inventor Signature	Rajani Ravi Typed or Printed Name	Date: % of Contribution <u>25</u>
JHU Inventor Signature	Typed or Printed Name	Date: % of Contribution
JHU Intellectual Property states that Inventor contributions are equal unless otherwise agreed upon. Inventor contribution provides for potential revenue and cost sharing. JHU Inventor percentages must TOTAL 100%.		JHU INVENTOR'S TOTAL 100 %

Section C INVENTOR CERTIFICATION without Assignment

Any Inventor(s), including HHMI or KKI inventors, who believe themselves **not** subject to assignment via The Johns Hopkins University Intellectual Property Policy for the specific Invention described herein (e.g. the Invention was made under a consulting agreement or other) must sign below and summarize the reasons for their belief. JHTT will review this summary and may, depending upon the unique facts of the case, require assignment of the Invention at a future date.

- I/we, the Inventor(s), hereby certify that the information set forth in this Invention Disclosure is true and complete to the best of my/our knowledge.
- I/we, the Inventor(s) who are HHMI or KKI Inventors at JHU, hereby certify that I/we will promptly advise JHTT of any commercial interest regarding the Invention described herein.

Inventor Signature	Typed or Printed Name	Date
Exception to assignment via the JHU Intellectual Property Policy: ☐ HHMI, ☐ KKI, ☐ Non-JHU personnel, ☐ Other If Other, please provide details of the exception:		
Inventor Signature	Typed or Printed Name	Date
Exception to assignment via the JHU Intellectual Property Policy: ☐ HHMI, ☐ KKI, ☐ Non-JHU personnel, ☐ Other If Other, please provide details of the exception:		

Section D NON-JHU INVENTOR CERTIFICATION and ASSIGNMENT to JHU

This section is to be completed only by those non-JHU personnel who are not subject to The Johns Hopkins University Intellectual Property Policy or who are not subject to the Intellectual Property Policy of another institution for the Invention described herein who wish to assign such Invention to JHU.

- I/we, the Inventors, hereby certify that the information set forth in this Invention Disclosure is true and complete to the best of my/our knowledge.
- I/we, the Inventors, hereby certify that I/we will promptly advise JHTT of any commercial interest regarding the Invention described herein.
- I/we, the Inventor(s), hereby warrant that I/we am/are not subject to The Johns Hopkins University Intellectual Property Policy and am/are not subject to the Intellectual Property Policy of another institution and am/are not under an obligation to assign intellectual property rights to another party and have full rights to assign the Invention to The Johns Hopkins University, hereby affirm that in consideration for The Johns Hopkins University's evaluation of commercial potential and a share of income which I/we may receive upon commercialization of my/our Invention, on the date of my/our signature(s) as indicated below do hereby assign and transfer my/our entire right, title and interest in and to the Invention described herein unto The Johns Hopkins University, its successors, legal representatives and assigns.

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 C γ 1), 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.

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 immunocjugate 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, ARCT1, 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, 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 includes 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 product 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 13'1 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) 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 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	QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTD YNTPTSRSLINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLVT VSAASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE LLGGPSVFLFPPPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
5	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRITNGSPRLLIKYASESISGIPSRFS

	<p>GSGSGTDFTLINSVESEDIADYYCQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC</p>
6	<p>DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFS GSGSGTDFTLINSVESEDIADYYCQNNNWPTTFGAGTKLELKGGGGSGGGGSGGGG SQVQLKQSGPGLVQPSQSLSTICTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGNT DYNTPTTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGTLV TVSAGGGGS</p>
7	<p>EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYT RYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT LTVVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYITLPPSRREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK</p>
8	<p>DIQMTQSPSSLSASVGDRVITICRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVP SRFSGSRSGTDFTLTISSLQPEDFATYYCQHHYTPPTFGQGTKEIKRTVAAPSVFIFPPS DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTL TLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC</p>
9	<p>DIQMTQSPSSLSASVGDRVITICRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVP SRFSGSRSGTDFTLTISSLQPEDFATYYCQHHYTPPTFGQGTKEIKGGGGSGGGGSGG GGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTN GYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWG QGTLLTVVSSGGGGGS</p>
10	<p>QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGD TSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAG TTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK</p>
11	<p>QIVLSQSPAILSASPQEKVTMTCRASSSVSYIHWFFQQKPGSSPKPWYATSNLASGVPVR</p>

	FSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
12	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKWIYATSNLASGVPVR FSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKGGGGSGGGSGGG GSQVQLQQPGAELVKPGASVKMSCKASGYFTSYNMHWVKQTPGRGLEWIGAIYPGN GDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWG AGTTVTVSAGGGGS

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

SEQ. ID.	Sequence
13	QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSGGNT DYNTPTSTRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGT VTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCP APPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSTIPPHVQKSV NNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWR KNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNIIF SEYNTSNPD
14	QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSGGNT DYNTPTSTRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGT VTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCP APPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSPQLCKFCDVR FSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAA APKCMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPD
15	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLINSVESEDIADYYCQQNNNWPTTFGAGTKLEIKRTVAAPSVFIFPPSD

	EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLT TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSTIPPHVQKSVNNDMIVT DNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITL ETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSN PD
16	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQRTNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLT TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSPQLCKFCDVRFSTCDNQ KSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIM KEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
17	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQRTNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKGGGGSGGGGSGGG GSQVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSGG NTDYNTPTSTRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWQQG TLVTVSAGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSC MSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEK KKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
18	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQRTNGSPRLLIKYASESISGIPSRF SGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKGGGGSGGGGSGGG GSQVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSGG NTDYNTPTSTRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWQQG TLVTVSAGGGGSPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDEN ITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEY NTSNPD
19	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGY TRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQ GTLVTSSASTKQPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTISWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLSLSPGKGGGGSTIPPH VQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAV

	AVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
20	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGY TRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQ GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTSKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSPQLC KFCDFRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
21	DIQMTQSPSSLSASVGDRVITTCRASQDVNTAVAWYQKPGKAPKLLIYSASFLYSGV PSRFSGSRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSTIPPHVQKSVNNDMI VTDNNGAVKFPQLCKFCDFRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENI TLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNT SNPD
22	DIQMTQSPSSLSASVGDRVITTCRASQDVNTAVAWYQKPGKAPKLLIYSASFLYSGV PSRFSGSRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSPQLCKFCDFRFSTCD NQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKC IMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
23	DIQMTQSPSSLSASVGDRVITTCRASQDVNTAVAWYQKPGKAPKLLIYSASFLYSGV PSRFSGSRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGGGSGGGGS GGGGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIY PTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMD YWGQGTTLTVSSGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRFSTCD NQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKC IMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
24	DIQMTQSPSSLSASVGDRVITTCRASQDVNTAVAWYQKPGKAPKLLIYSASFLYSGV PSRFSGSRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGGGSGGGGS

	GGGGSEVQLVESGGGLVQPGGSLRI.SCAASGFNIKDTYIIHWVRQAPGKGLEWVARIY PTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMD YWGQGTLLTVSSGGGGSPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAV WRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECND NIIFSEYNTSNPD
25	QVQLQQPGAELVKPGASVKMSCKASGYFTSYNMHWVKQTPGRGLEWIGAIYPGNG DTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWG AGTTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSTIPP HVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEV CAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDE CNDNIIFSEYNTSNPD
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27	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVR FSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPS DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSST LTLTKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSTIPPHVQKSVNNDMIVT DNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITL ETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSN PD
28	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVR

	FSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPS DEQLKSGTASVVCLLNFPYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSST LTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSPQLCKFCDVRFSTCDN QKSCMSNCSITSICEKPQEVCAVWRKNDENTLETVCHDPKLPYHDFILEDAAASPKCI MKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
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30	QIVLSQSPAILSASPGKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVR FSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKGGGSGGGGSGG GGSQVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYP GNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFN VWGAGTTVTVSAGGGGSPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAV WRKNDENTLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECND NIIFSEEYNTSNPD

[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.

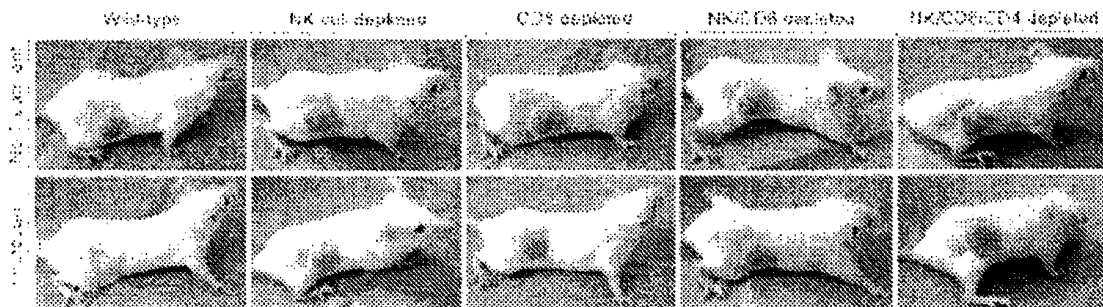
TUMOR TARGETING COMPOSITIONS

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

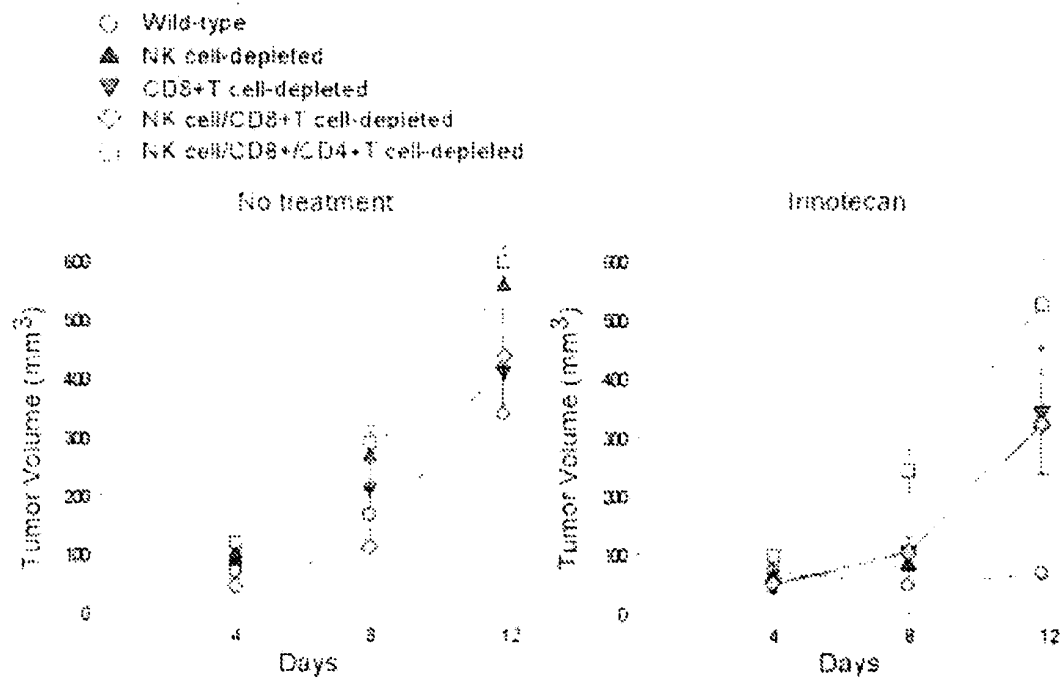
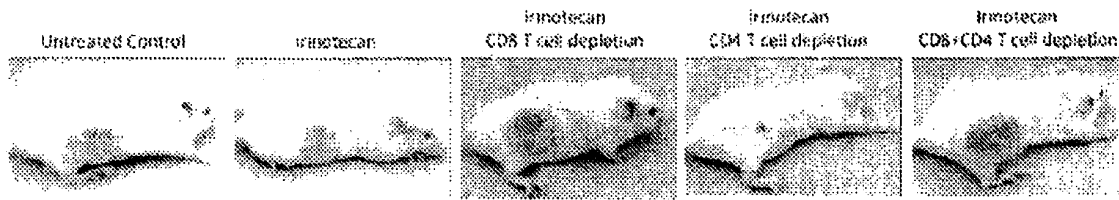


FIGURE 2

A



B

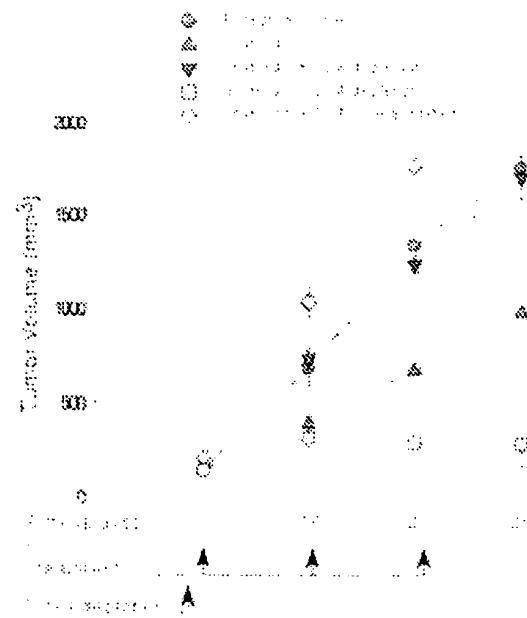


FIGURE 2

C.

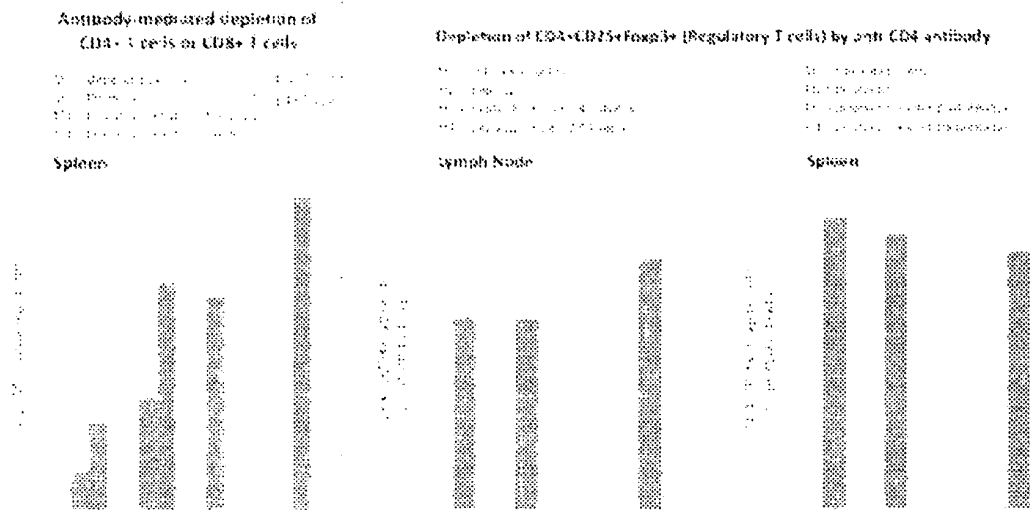


FIGURE 3

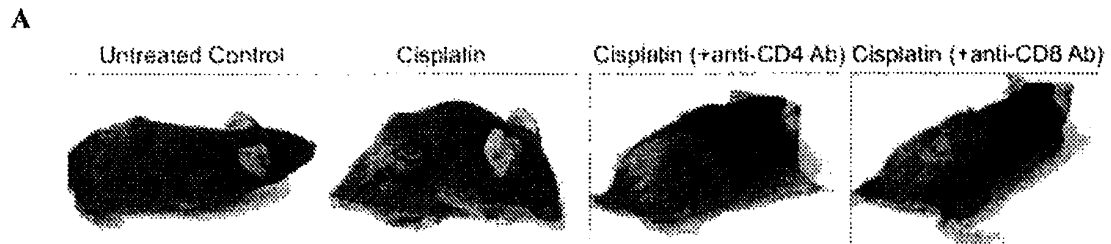
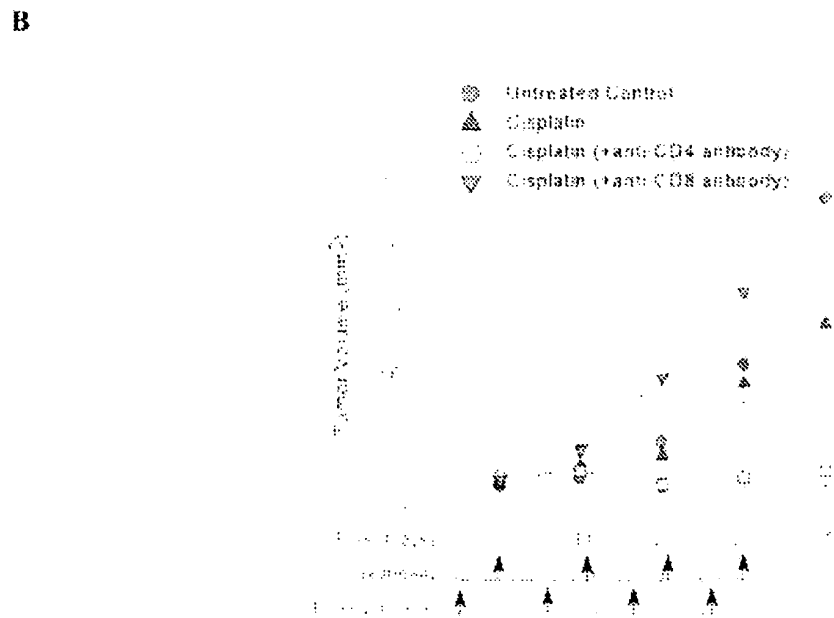


FIGURE 3

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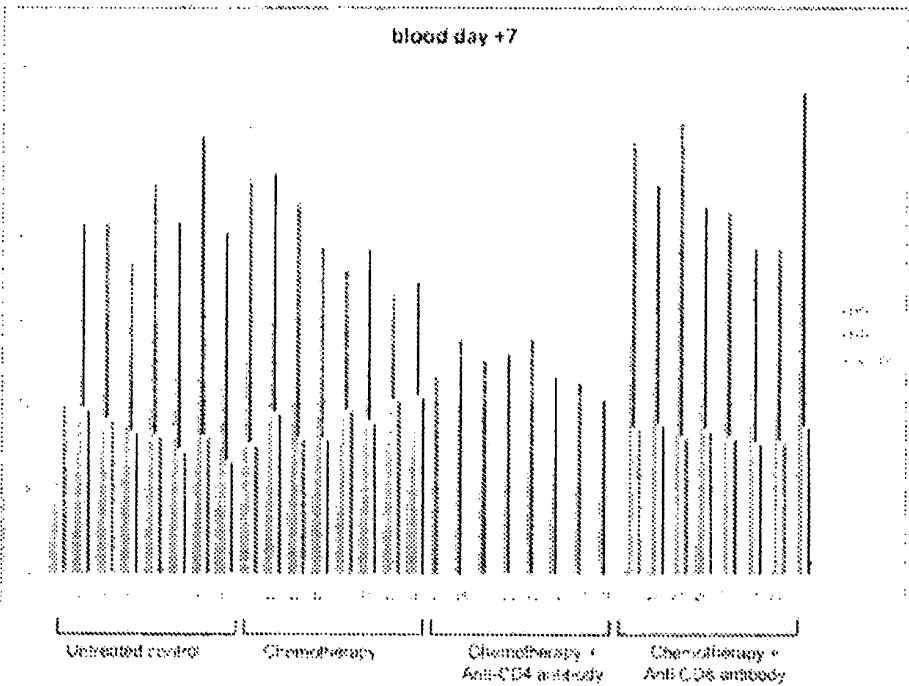
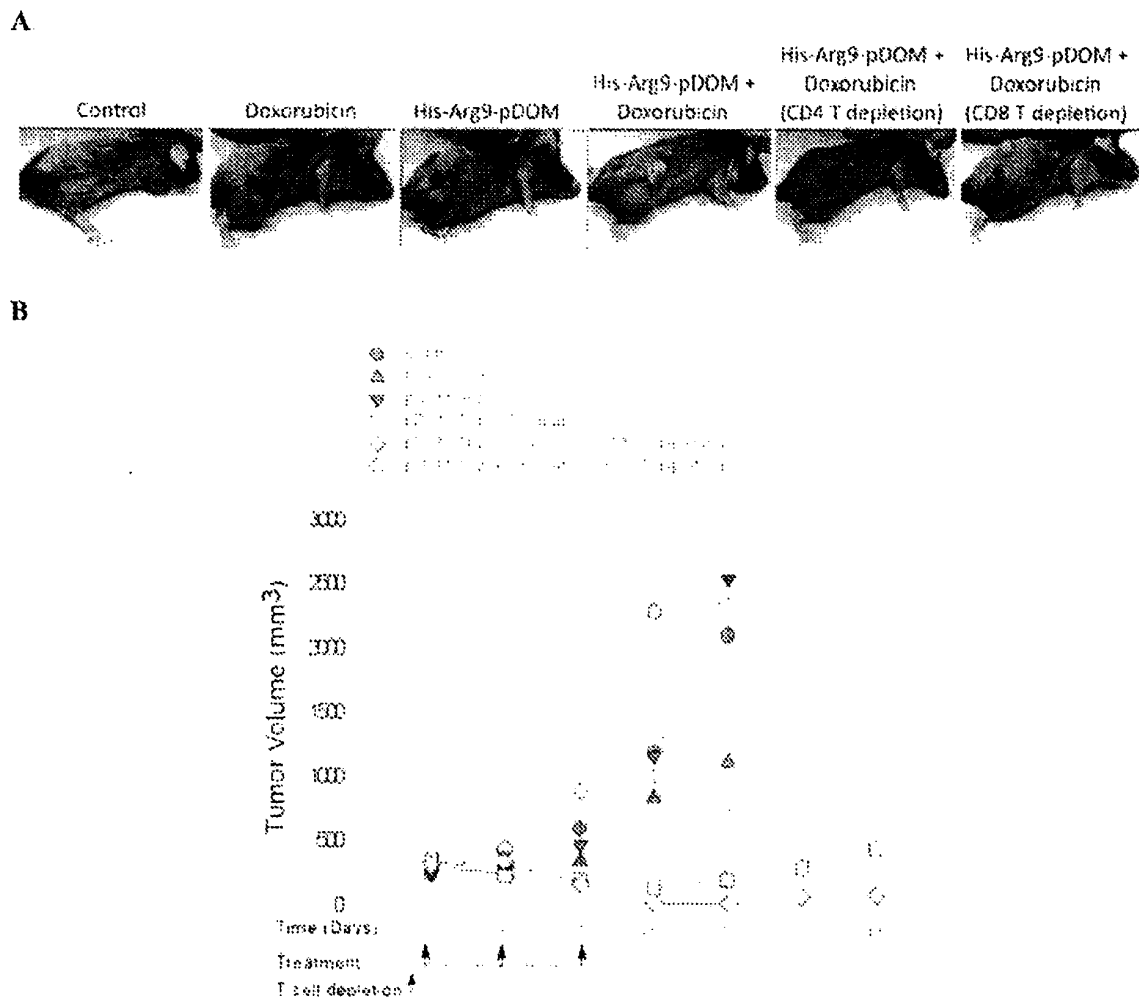
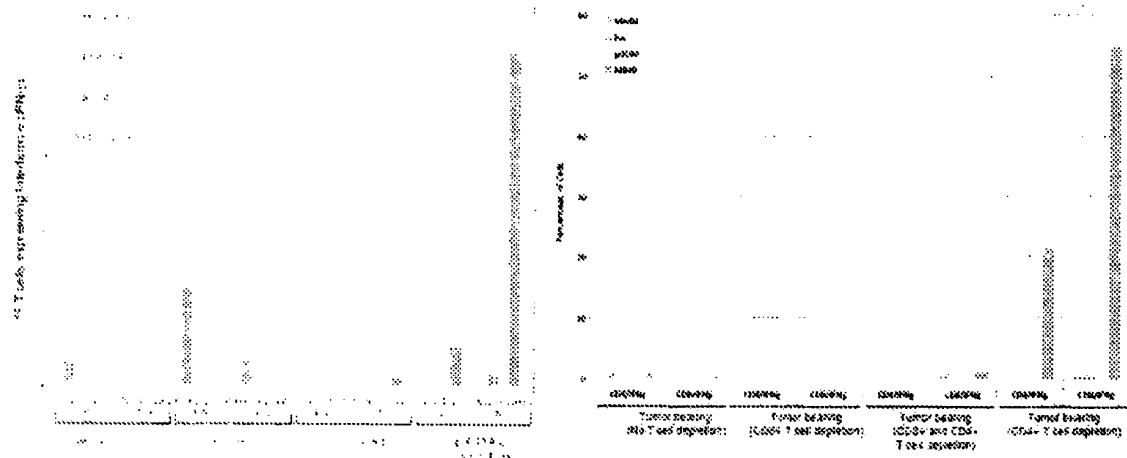


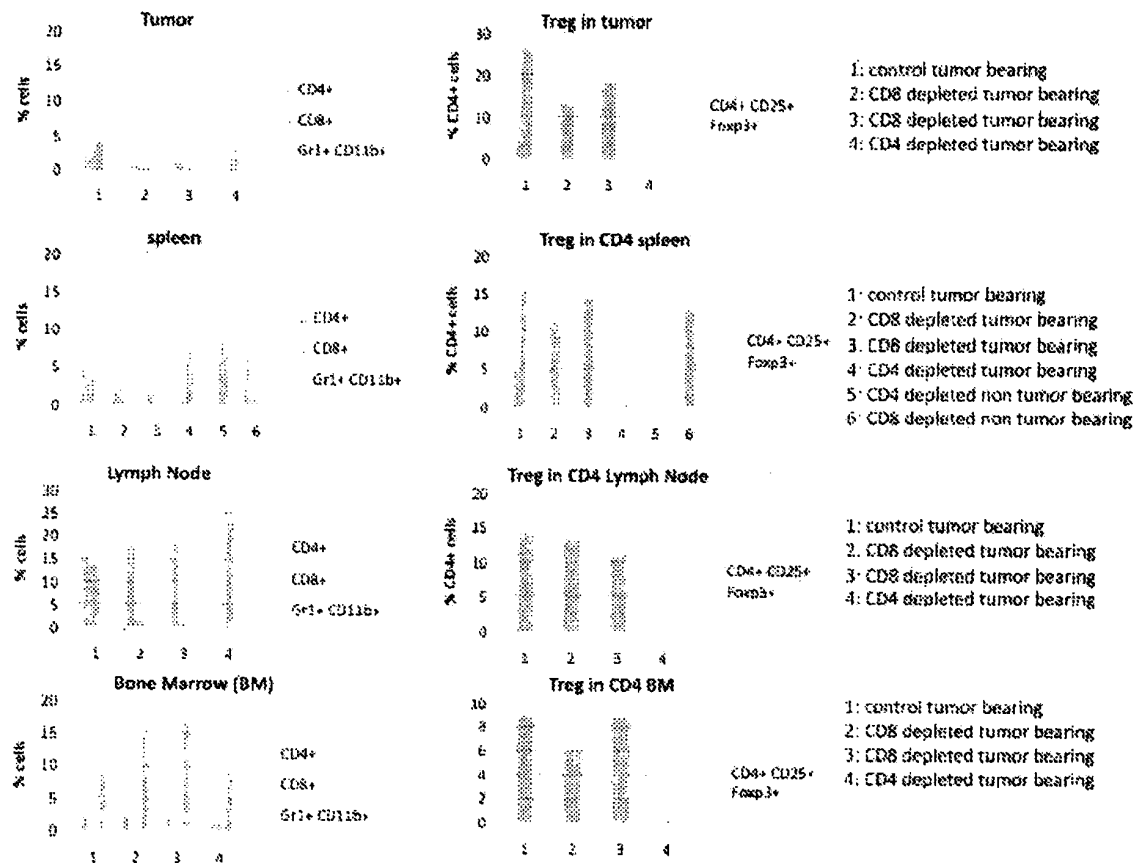
FIGURE 4



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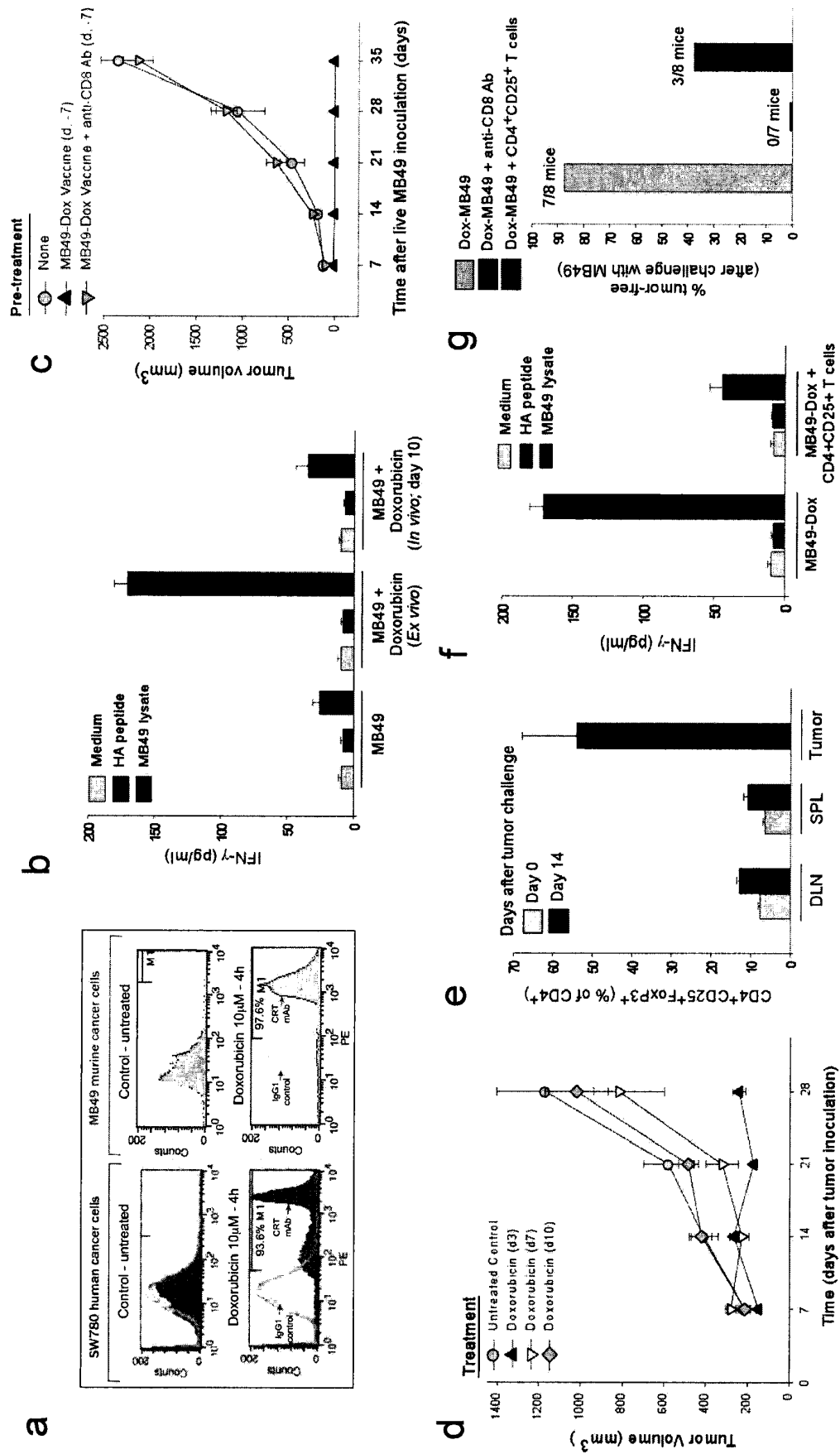


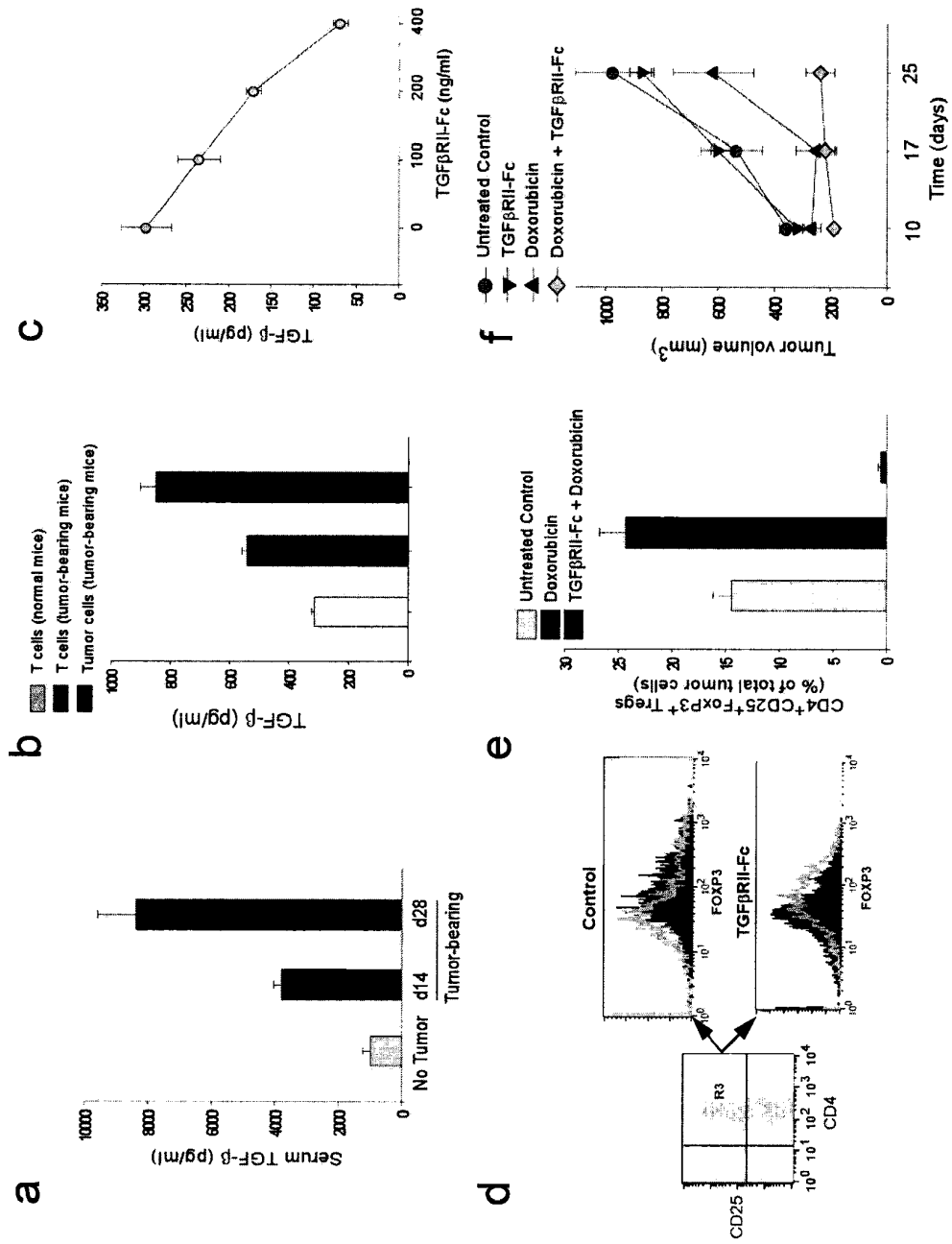
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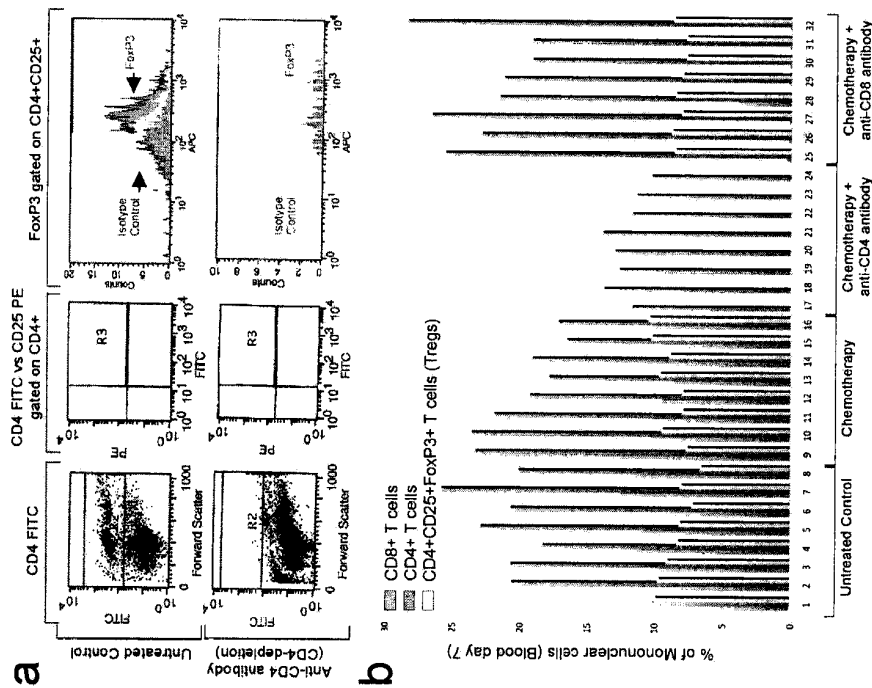
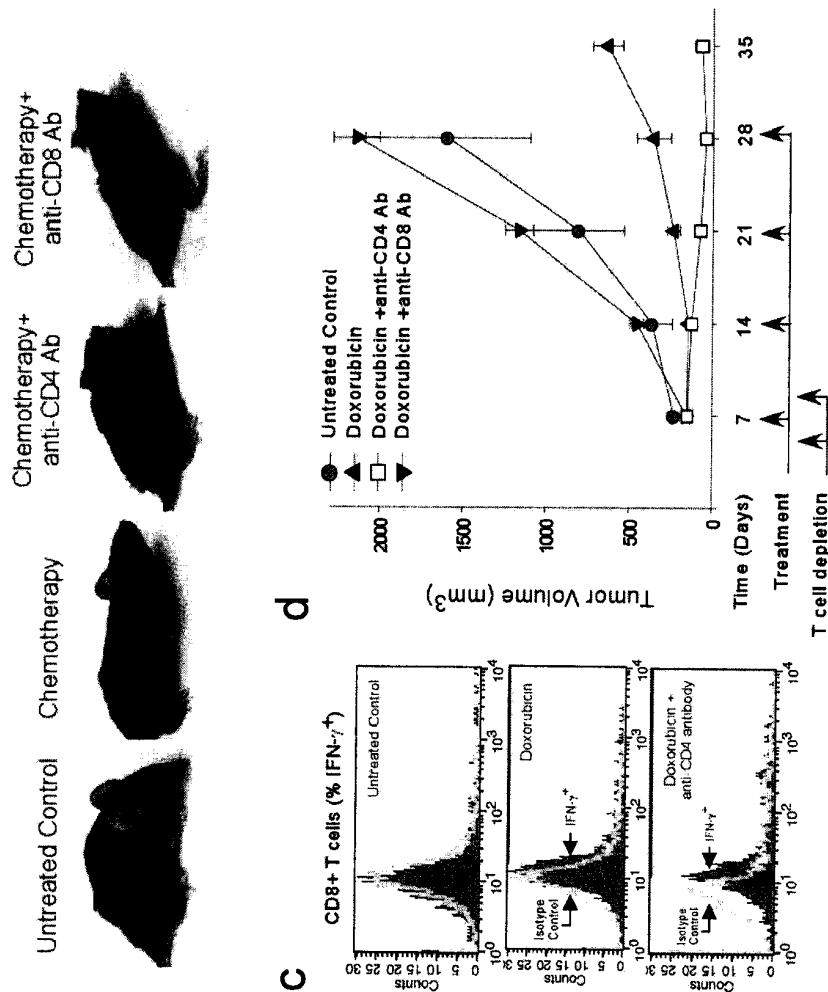


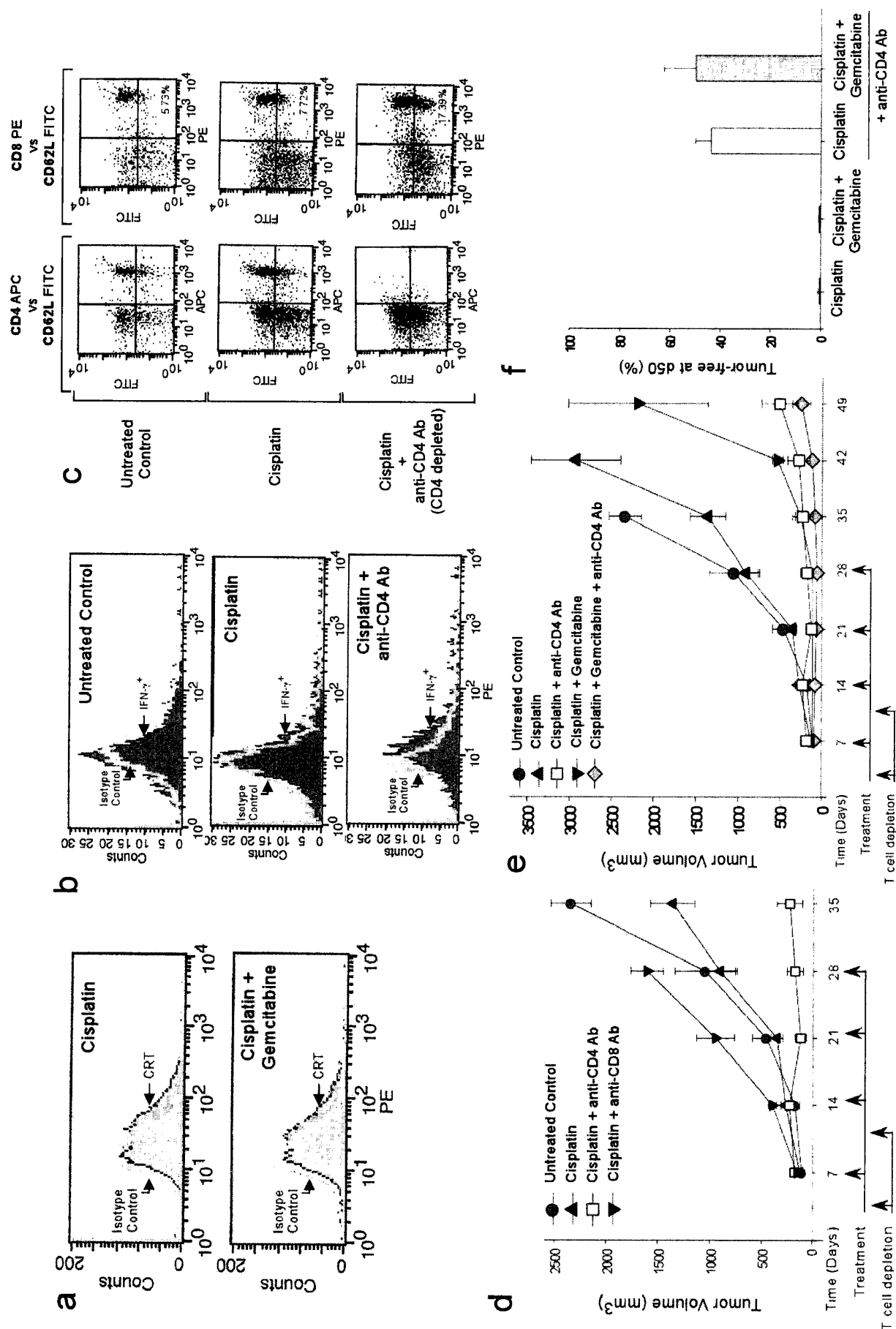
- Chemotherapy is a cornerstone of systemic treatment of patients with advanced cancers.
- The vast majority of human cancers harbor genetic alterations and signaling mechanisms that impair the direct death signaling pathways entrained by chemotherapeutic agents.
- Chemotherapeutic agents and tumor-targeted antibodies (mAbs) have immuno-adjutant 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.

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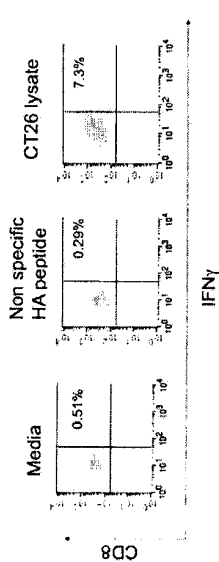




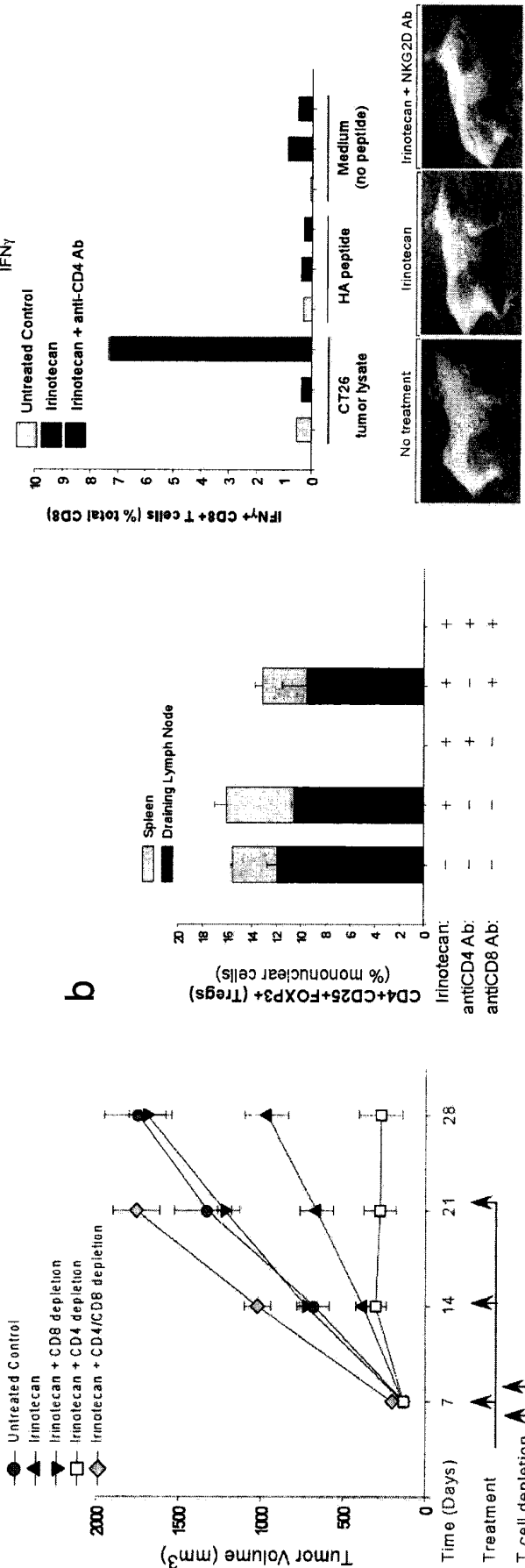
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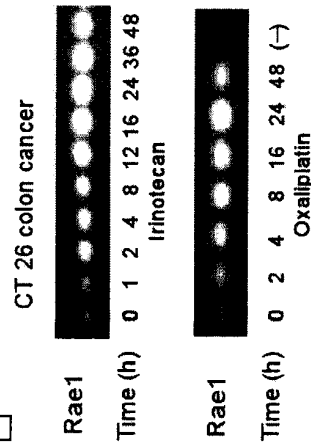
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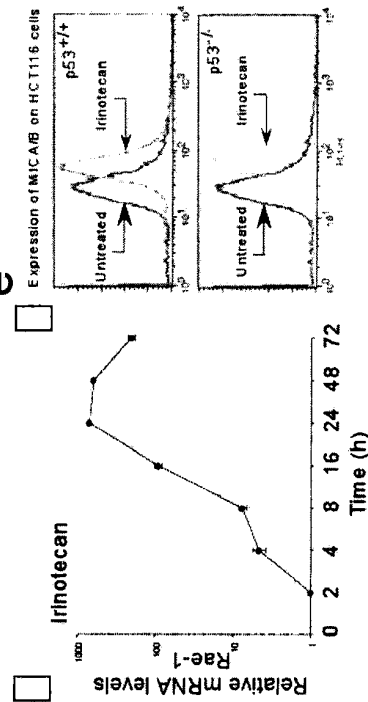
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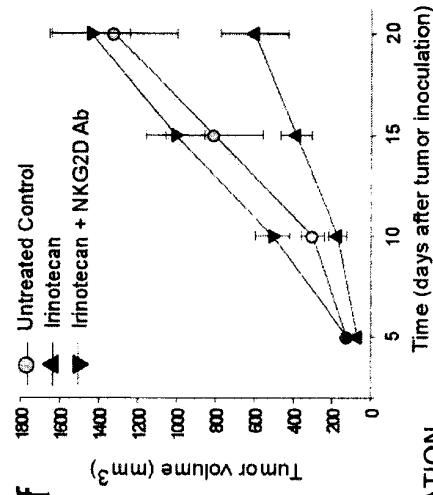
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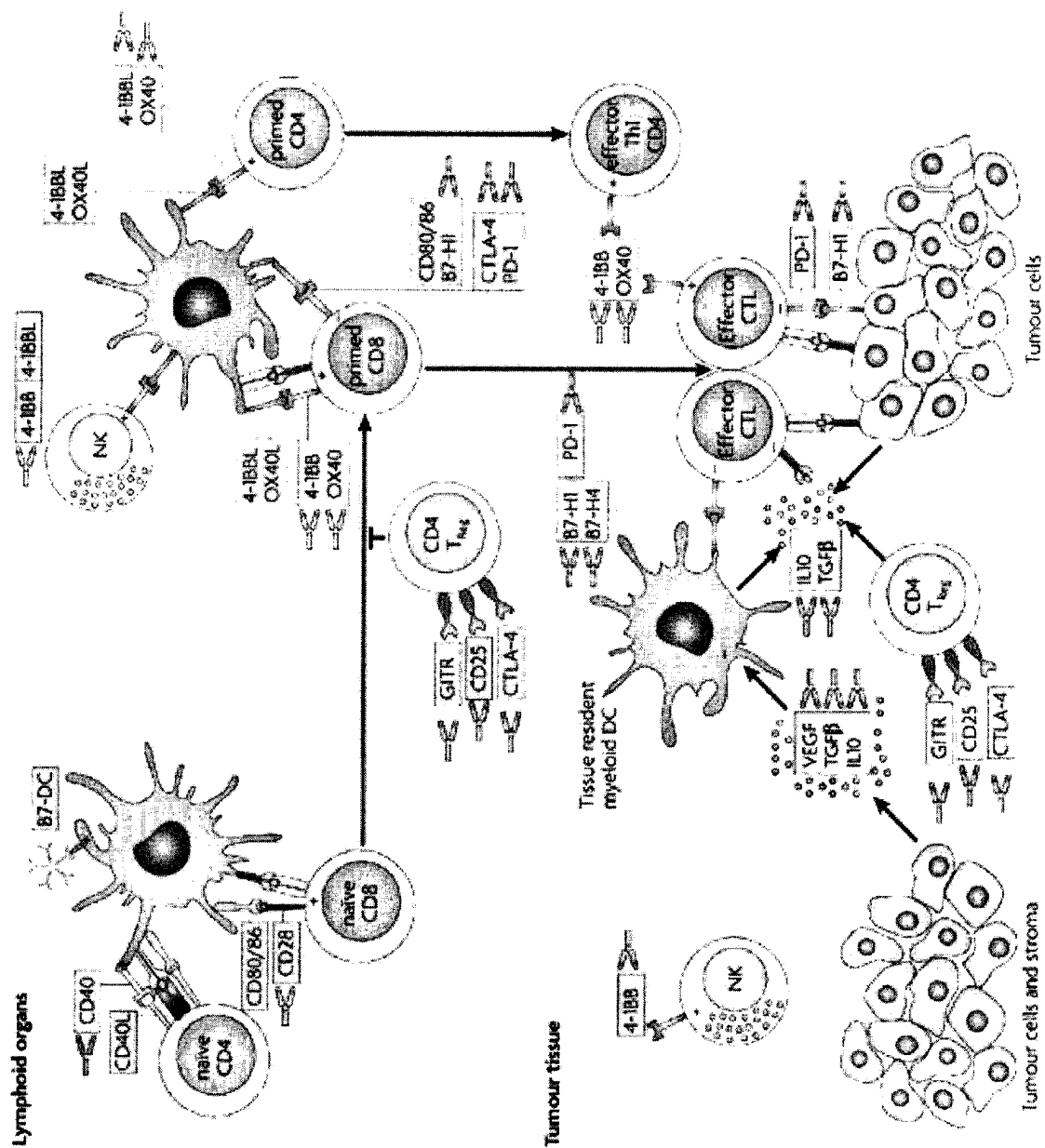
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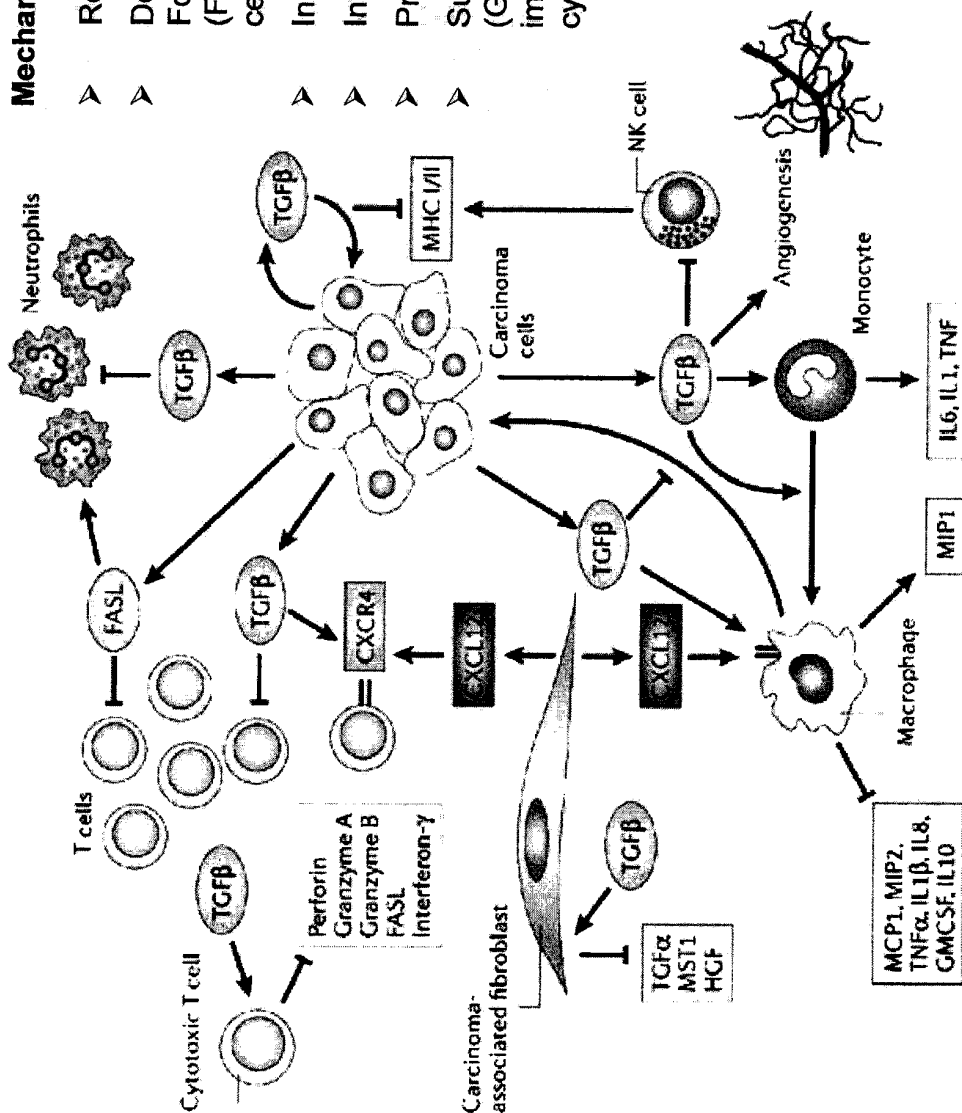


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Mechanisms of TGF- β -induced immune tolerance:

- 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-adjuvant 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- 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)

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

I. Targeted Immunostimulatory Antibodies and Fusion Proteins:

- 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)
- 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 (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:
- (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)

- 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):

I. Targeted Immunostimulatory Antibodies and Fusion Proteins:

- 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 EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQGQVG
TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC WDHDPEARLT AQCVAERFSE
LEHLDRLSGR SCSEKIPED 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 ICEKPQEV CV AVWRKNDENI
TLETVCHDPK LPYHDFILED AASP KCIMKE KKKPGETFFM CSCSSDECND NIIFSEYNT
SNPDLLLVI FQVTGISLLPP LGVAISVIII FYCYRVNRQQ KLSSTWETGK TRKLMEFSEH
CAIILED RSDISSTCANNI NHNT ELLPIE 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 SCSEKIPED 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-

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

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

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 EERKTELQKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
 LHSHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQVVG
 TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
 HPCVESMKDN VLRDRGRPEI PSFWLNHQGI QMVCETLTEC WDHDPEARL

TGF-β R-IIB (ΔC terminus): TGFβ RIB 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
 NIIFSEYNT SNPDLVIF QVTGISLLPP LGVAISVIII FYCYRVNRQQ KLSSTWETGK
 TRKLMEFSEH CAIILEDERS DISSTCANNI NHNTPELLIE LDTLVGKGRF AEVYKAKLKQ
 NTSEQFETVA VKI FP YEEYASWKTE KDIFSDINLK HENILQFLTA EERKTELQKQ
 YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH LHSHTPCGR PKMPIVHRDL
 KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSQVVG TARYMAPEVL ESRMNLENVE
 SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE HPCVESMKDN VLRDRGRPEI
 PSFWLNHQGI QMVCETLTEC WDHDPEARL

(ii) **TGF-βR-II (Δcyt):** TGFβRII 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-βR-IIB (Δcyt): TGFβRIB 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 NIIFSEYNT
 SNPDLVIF 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 NIIFSEYNT
 SNPDLVIF 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-IIB containing the extracellular domain that binds TGF- β

TIPPHVQK SDVEMEAQKD EIICPSCNRT AHPLRHINND MIVTDNNGAV KFPQLCKFCD
VRFSTCDNOK 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 VNRQOKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNT
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVRIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV 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 VNRQOKLSST 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
HPCVESMKDA SGIQMVCE TL 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
QEVCAVVRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS
DECNDNIIFS EYNTSNPD

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
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
EVTCTVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
GGGSGGGSGGGGS TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

Anti-HER2/neu light chain amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFGSG
RSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
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*
GAGCTCGAGGTGCAGCTGGTGGGAATCCGGCGGAGGACTGGTGCAGCCTGGCGGATCCCTG
1 -----+-----+-----+-----+-----+-----+-----+
CTCGAGCTCCACGTCGACCACCTTAGGCCGCCTCCTGACCACGTCGGACCGCCTAGGGAC
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 K D T Y I H W V R
19 21 23 25 27 29 31 33 35 37

CAGGCCCTGGCAAGGACTGGAATGGGTGGCCCGGATCTACCCACCAACGGCTACACC
121 -----+-----+-----+-----+-----+-----+-----+
GTCCGGGGACCGTTCCTGACCTTACCACCGGGCCTAGATGGGGTGGTTGCCGATGTGG

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
 GCCTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCTCCAGA
 241 -----+-----+-----+-----+-----+-----+
 CGGATGGACGTCTACTTGAGGGACGCCCCGGCTCCTGTGGCGGCACATGATGACGAGGTCT
 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
 TCCTCTGCCTCCACCAAGGGCCCCCTCTGTGTTCCCTCTGGCCCCCTTCCAGCAAGTCCACA
 361 -----+-----+-----+-----+-----+-----+
 AGGAGACGGAGGTGGTTCCCGGGGAGACACAAGGGAGACCGGGGAAGGTCGTTTCAGGTGT
 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

 TCTGGCGGCACCGCCGCTCTGGGCTGCCTGGTGAAAGACTACTTCCCCGAGCCCGTGACC
 421 -----+-----+-----+-----+-----+-----+
 AGACCGCCGTGGCGGCGAGACCCGACGGACCACTTTCTGATGAAGGGGCTCGGGCACTGG
 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*
 GTGTCCTGGAACCTCTGGCGCCCTGACCTCCGGCGTGACACCTTTCCAGCCGTGCTGCAG
 481 -----+-----+-----+-----+-----+-----+
 CACAGGACCTTGAGACCGCGGGACTGGAGGCCGCACGTGTGGAAAGGTCGGCACGACGTC
 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

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

 GAACCCAAGTCCTGCGACAAGACCCACACCTGTCCCCCTTGCCCTGCTCCTGAAGTGTCTG
 661 -----+-----+-----+-----+-----+-----+
 CTTGGGTTTCAGGACGCTGTTCTGGGTGTGGACAGGGGGAACGGGACGAGGACTTGACGAC
 E P K S C D K T H T C P P C P A P E L L
 219 221 223 225 227 229 231 233 235 237

 GGCGGACCCTCCGTGTTCTGTTCCTGTTCCTGTTCCTGTTCCTGTTCCTGTTCCTGTTCCTGT
 721 -----+-----+-----+-----+-----+-----+
 CCGCCTGGGAGGCACAAGGACAAGGGGGGTTTCGGGTTCCTGTGGGACTACTAGAGGGGCC
 G G P S V F L F P P K P K D T L M I S R
 239 241 243 245 247 249 251 253 255 257

BspMI
 ACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCACGAGGACCCTGAAGTGAAGTTC
 781 -----+-----+-----+-----+-----+-----+
 TGGGGGCTTCACTGGACGCACCAACACCTGCACAGGGTGCTCCTGGGACTTCACTTCAAG
 T P E V T C V V V D V S H E D P E V K F
 259 261 263 265 267 269 271 273 275 277

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

 TACAACTCCACCTACCGGGTGGTGTCTGTGCTGACCGTGCTGCACCAGGACTGGCTGAAC
 901 -----+-----+-----+-----+-----+-----+
 ATGTTGAGGTGGATGGCCACACAGACACGACTGGCACGACGTGGTCTCTGACCGACTTG
 Y N S T Y R V V S V L T V L H Q D W L N
 299 301 303 305 307 309 311 313 315 317

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

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

 GAAGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGCTTCTACCCCTCC
 1081 -----+-----+-----+-----+-----+-----+
 CTTCTCTACTGGTTCTTGGTCCACAGGACTGGACAGACCACTTCCGAAGATGGGGAGG

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

GATATCGCCGTGGAATGGGAGTCCAACGGCCAGCCCCGAGAACAACATAAGACCACCCCC
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

CGGTGGCAGCAGGGCAACGTGTTCTCTGCTCCGTGATGCACGAGGCCCTGCACAACCAC
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 -----+-----+-----+-----+-----+-----+-----+
ATGTGGGTCTTCAGGGACAGGGACTCGGGACCTTTCCCGCCTCCGCCGAGACCACCACCT
Y T Q K S L S L S P G K 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

ATCTGCGAGAAGCCCCAGGAAGTGTGCGTGGCCGTGTGGCGGAAGAACGACGAGAACATC
1561 -----+-----+-----+-----+-----+-----+-----+
TAGACGCTCTTCGGGGTCCTTCACACGCACCGGCACACCGCCTTCTTGCTGCTCTTGTAG

I C E K P Q E V C V A V W R K N D E N I
 519 521 523 525 527 529 531 533 535 537

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

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

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

KpnI
 TCCAACCCCGACTGAGGTACC
 1801 -----+-----+-----+-----+-----+-----+-----+
 AGGTTGGGGCTGACTCCATGG
S N P D *
 599 601 603

Light chain sequence:

SacI
 GAGCTCGACATCCAGATGACCCAGTCCCCCTCCAGCCTGTCCGCCTCTGTGGGCGACAGA
 1 -----+-----+-----+-----+-----+-----+-----+
 CTCGAGCTGTAGGTCTACTGGGTTCAGGGGGAGGTTCGGACAGGCGGAGACACCCGCTGTCT
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

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

 AAGCCTGGCAAGGCCCCCAAGCTGCTGATCTACTCCGCCTCCTTCCTGTACTCCGGGGTG
 121 -----+-----+-----+-----+-----+-----+-----+
 TTCGGACCGTTTCGGGGGGTTCGACGACTAGATGAGGCGGAGGAAGGACATGAGGCCCCAC
K P G K A P K L L I Y S A S F L Y S G V
 39 41 43 45 47 49 51 53 55 57

XbaI *PstI*
 CCATCCCGGTTCTCCGGCTCTAGATCCGGCACCGACTTCACCCTGACCATCTCCAGCCTG

```

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 -----+-----+-----+-----+-----+-----+
GTCGGGCTCCTGAAGCGGTGGATGATGACGGTCGTCGTGATGTGGTGGGGGGGATGGAAG
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

GGCCAGGGCACCAAGGTGGAAATCAAGCGGACCGTGGCCGCTCCCTCCGTGTTCATCTTC
301 -----+-----+-----+-----+-----+-----+
CCGGTCCCGTGGTTCACCTTTAGTTCGCCCTGGCACCGGCGAGGGAGGCACAAGTAGAAG
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
CCACCCTCCGACGAGCAGCTGAAGTCTGGCACCGCCTCCGTGCTGCTGAACAAC
361 -----+-----+-----+-----+-----+-----+
GGTGGGAGGCTGCTCGTCGACTTCAGACCGTGGCGGAGGCAGCACACGGACGACTTGTTC
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
TTCTACCCCCGCGAGGCCAAGGTGCAGTGGGAAGGTGGACAACGCCCTGCAGTCCGGCAAC
421 -----+-----+-----+-----+-----+-----+
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

TCCCAGGAATCCGTGACCGAGCAGGACTCCAAGGACAGCACCTACTCCCTGTCCTCCACC
481 -----+-----+-----+-----+-----+-----+
AGGGTCCTTAGGCACTGGCTCGTCCTGAGGTTCTGTCGTGGATGAGGGACAGGAGGTGG
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 -----+-----+-----+-----+-----+-----+
GACTGGGACAGGTTCCGGCTGATGCTCTTCGTGTTCACATGCGGACGCTTCACTGGGTG
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 -----+-----+-----+-----+-----+-----+
GTCCCCGACAGGTCGGGGCACTGGTTCAGGAAGTTGGCCCCGCTCACGACTCCATGG
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:

QVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPTFTS
RLSINKDNSKSKQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTVTVSAASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSL
GTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKGGGGSG
GGGSGGGGS TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN
CSITSICEKP QEVCAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG
ETFFMCSCSS DECNDNIIFS EYNTSNPD

Anti-EGFR1 light chain amino acid sequence:

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGS
GSGTDFTLINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEKHKVYACE
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 -----+-----+-----+-----+-----+-----+-----+
CTCGAGGTCCACGTCGACTTCGTCAAGCCGGGTCCTGACCACGTCGGAAGGGTCAGGGAC
 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

TCCATCACCTGTACCGTGTCCGGCTTCTCCCTGACCAACTACGGCGTGCACCTGGGTCCGA
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

CAGTCCCCAGGCAAGGGCCTGGAATGGCTGGGAGTGATTTGGAGCGGCGGCAACACCGAC
121 -----+-----+-----+-----+-----+-----+-----+
GTCAGGGGTCCGTTCCCGGACCTTACCGACCTCACTAAACCTCGCCGCCGTTGTGGCTG
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 -----+-----+-----+-----+-----+-----+
ATGTTGTGGGGGAAGTGGAGGGCCGACAGGTAGTTGTTTCCTGTTGAGGTTACAGGGTCCAC
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 -----+-----+-----+-----+-----+-----+
AAGAAGTTCTACTTGAGGGACGTCAGGTTGCTGTGGCGGTAGATGATGACGCGGTCTCGG
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

CTGACCTACTATGACTACGAGTTTCGCTACTGGGGACAGGGCACCCCTGGTCACCGTGTCT
301 -----+-----+-----+-----+-----+-----+
GACTGGATGATACTGATGCTCAAGCGGATGACCCCTGTCCCGTGGGACCACTGGGCACAGA
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 -----+-----+-----+-----+-----+-----+
CGGCGGAGATGGTTCCCGGGGAGGCACAAAGGGGACCGGGGGAGGTTCGTTTCAGGTGTAGA
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 -----+-----+-----+-----+-----+-----+
CCGCCGTGGCGGCGAGACCCGACGGACCACTTCCTGATGAAGGGGCTCGGGCACTGGCAC
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 -----+-----+-----+-----+-----+-----+
AGGACCTTGAGACCGCGGGACTGGAGGCCGACGTCGTGGAAGGTTCGGCACGACGTCAGG
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 -----+-----+-----+-----+-----+-----+
AGGCCGGACATGAGGGACAGGAGGCAGCACTGGCACGGGAGGTCGAGAGACCCGTGGGTC
S G L Y S L S S V V T V P S S S L G T Q
179 181 183 185 187 189 191 193 195 197

ACCTACATCTGCAACGTGAACCACAAGCCCTCCAACACCAAGGTGGACAAGCGGGTGGAA
601 -----+-----+-----+-----+-----+-----+
TGGATGTAGACGTTGCACTTGGTGTTCGGGAGGTTGTGGTTCCACCTGTTGCCACCTT

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 CCCAAGTCCTGCGACAAGACCCACACCTGTCCCCCTGCCCTGCCCCTGAAGTGTGGGA
-----+-----+-----+-----+-----+-----+
GGGTTTCAGGACGCTGTTCTGGGTGTGGACAGGGGGGACGGGACGGGGACTTGACGACCTT
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 GGCCCTTCCGTGTTCTCTGTTCCCCCAAGCCCAAGGACACCCTGATGATCTCCCGGACC
-----+-----+-----+-----+-----+-----+
CCGGGAAGGCACAAGGACAAGGGGGGTTTCGGGTTCTGTGGGACTACTAGAGGGCCTGG
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

781 CCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCACGAGGACCCTGAAGTGAAGTTCAAT
-----+-----+-----+-----+-----+-----+
GGGCTTCACTGGACGCACCAACACCTGCACAGGGTGCTCCTGGGACTTCACTTCAAGTTA
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
-----+-----+-----+-----+-----+-----+
ACCATGCACCTGCCGCACCTTCACGTGTTGCGGTTCTGGTTCGGGTCTCTCCTTGTCATG
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
-----+-----+-----+-----+-----+-----+
AGGTTCCGGTTCCTCGGTCGGGGCGCTCGGAGTCCACATGTGAGACGGAGGGTTCGGCCCTG
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
-----+-----+-----+-----+-----+-----+
CTCGACTGGTTCCTGGTCCACAGGGACTGGACAGACCAGTTCCCGAAGATGGGGAGGCTA

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

 ATCGCCGTGGAATGGGAGTCCAACGGCCAGCCCGAGAACAACACTACAAGACCACCCCCCT
 1141 -----+-----+-----+-----+-----+-----+
 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

 GTGCTGGACTCCGACGGCTCATTCTTCTGTACTCCAAGCTGACCGTGGACAAGTCCCGG
 1201 -----+-----+-----+-----+-----+-----+
 CACGACCTGAGGCTGCCGAGTAAGAAGGACATGAGGTTGCACTGGCACCTGTTCAAGGCC
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

 TGGCAGCAGGGCAACGTGTTCTCTCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTAC
 1261 -----+-----+-----+-----+-----+-----+
 ACCGTCGTCCCCTTGCACAAGAGGACGAGGCACCTACGTGCTCCGGGACGTGTTGGTGATG
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

 ACCCAGAAGTCCCTGTCTCTGAGCCCCGGCAAGGGCGGAGGCGGATCTGGTGGTGGTGGC
 1321 -----+-----+-----+-----+-----+-----+
 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

 TCTGGTGGCGGAGGCTCTACCATCCCTCCACACGTGCAGAAATCCGTGAACAACGACATG
 1381 -----+-----+-----+-----+-----+-----+
 AGACCACCGCCTCCGAGATGGTAGGGAGGTGTGCACGTCTTTAGGCACCTTGTGCTGTAC
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*

 ATCGTGACCGACAACAACGGCGCCGTGAAGTTCCCCCAGCTGTGCAAGTTCTGCGACGTG
 1441 -----+-----+-----+-----+-----+-----+
 TAGCACTGGCTGTTGTTGCCGCGGCACTTCAAGGGGGTCGACACGTTCAAGACGCTGCAC
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

 CGGTTCTCTACCTGCGACAACCAGAAATCCTGCATGTCCAAGTCTCCATCACCTCCATC
 1501 -----+-----+-----+-----+-----+-----+
 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

 TGCGAGAAGCCCCAGGAAGTGTGCGTCGCCGTCTGGCGGAAGAACGACGAGAACATCACC
 1561 -----+-----+-----+-----+-----+-----+
 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

CTGGAACCGTGTGCCACGACCCCAAGCTGCCCTACCACGACTTCATCCTGGAAGATGCC
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

PvuII

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

AGCTGCTCCTCCGACGAGTGCAACGACAACATCATCTTCTCCGAAGAGTACAACACCTCC
1741 -----+-----+-----+-----+-----+-----+
TCGACGAGGAGGCTGCTCACGTTGCTGTTGTAGTAGAAGAGGCTTCTCATGTTGTGGAGG
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
GAGCTCGATATCCTGTGACCCAGTCCCCCGTGATCCTGTCCGTGTCTCCTGGCGAGCGG
1 -----+-----+-----+-----+-----+-----+
CTCGAGCTATAGGACGACTGGGTCAGGGGGCACTAGGACAGGCACAGAGGACCGCTCGCC
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

GTGTCTTCTCCTGCCGGCCTCCAGTCCATCGGCACCAACATCCACTGGTATCAGCAG
61 -----+-----+-----+-----+-----+-----+
CACAGGAAGAGGACGGCCCGGAGGGTCAGGTAGCCGTGGTTGTAGGTGACCATAGTCGTC
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

GAATCCGAGGATATCGCCGACTACTACTGCCAGCAGAACAACAACCTGGCCCACCACCTTC
241 -----+-----+-----+-----+-----+-----+
CTTAGGCTCCTATAGCGGCTGATGATGACGGTCGTCTTGTGTTGACCGGGTGGTGAAG
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

CCACCCTCCGACGAGCAGCTGAAGTCCGGCACC GCCTCCGTCGTGTGCCTGCTGAACAAC
361 -----+-----+-----+-----+-----+-----+
GGTGGGAGGCTGCTCGTCGACTTCAGGCCGTGGCGGAGGCAGCACACGGACGACTTGTTC
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

TTCTACCCCCGCGAGGCCAAGGTGCAGTGGGAAGGTGGACAACGCCCTGCAGTCCGGCAAC
421 -----+-----+-----+-----+-----+-----+
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

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

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 -----+-----+-----+-----+-----+-----+
GTCCCCGACAGGTCCGGGCACTGGTTCAGGAAGTTGGCCCCGCTCACGACTCCATGG
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

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

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

661 GCCGAGCCCAAGTCCTGCGACAAGACCCACACCTGTCCCCCTGCCCTGCCCTGAACTG
-----+-----+-----+-----+-----+-----+
CGGCTCGGGTTCAGGACGCTGTTCTGGGTGTGGACAGGGGGGACGGGACGGGACTTGAC
A E P K S C D K T H T C P P C P A P E L
219 221 223 225 227 229 231 233 235 237

721 CTGGGAGGCCCTTCTGTGTTCTGTTCCTGTTCCCCCAAAGCCCAAGGACACCCTGATGATCTCC
-----+-----+-----+-----+-----+-----+
GACCTCCGGGAAGACACAAGGACAAGGGGGGTTTCGGGTTCTGTGGGACTACTAGAGG
L G G P S V F L F P P K P K D T L M I S
239 241 243 245 247 249 251 253 255 257

BspMI

781 CGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCACGAGGACCCTGAAGTGAAG
-----+-----+-----+-----+-----+-----+
GCCTGGGGGCTTCACTGGACGCACCACCACCTGCACAGGGTGCTCCTGGGACTTCACTTC
R T P E V T C V V V D V S H E D P E V K
259 261 263 265 267 269 271 273 275 277

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

901 CAGTACAACCTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTG
-----+-----+-----+-----+-----+-----+
GTCATGTTGAGGTGGATGGCCCACCACAGGCACGACTGGCACGACGTGGTCCTGACCGAC
Q Y N S T Y R V V S V L T V L H Q D W L
299 301 303 305 307 309 311 313 315 317

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

1021 ACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTAACTCTGCCTCCCAGC
-----+-----+-----+-----+-----+-----+
TGGTAGAGGTTCCGGTTCCCGGTCGGGGCGCTCGGAGTCCACATGTGAGACGGAGGGTCG

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

 CGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTCAAGGGCTTCTACCCC
 1081 -----+-----+-----+-----+-----+-----+
 GCCCTGCTCGACTGGTTCCTTGGTCCACAGGGACTGGACAGACCAGTTCCTCGAAGATGGGG
 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 -----+-----+-----+-----+-----+-----+
 AGGCTATAGCGGCACCTTACCCTCAGGTTGCCGGTCGGGCTCTTGTGATGTTCTGGTGG
 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

 CCCCCTGTGCTGGACTCCGACGGCTCATTCTTCCTGTACTCCAAGCTGACCGTGGACAAG
 1201 -----+-----+-----+-----+-----+-----+
 GGGGGACACGACCTGAGGCTGCCGAGTAAGAAGGACATGAGGTTGCGACTGGCACCTGTTC
 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

 TCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAAC
 1261 -----+-----+-----+-----+-----+-----+
 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

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

 GGAGGATCAGGCGGCGGAGGCTCTACCATCCCCCACACGTGCAGAAATCCGTGAACAAC
 1381 -----+-----+-----+-----+-----+-----+
 CCTCCTAGTCCGCGCCTCCGAGATGGTAGGGGGTGTGCACGTCTTTAGGCACCTTGTG
 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
 KsiI PvuII
 GACATGATCGTGACCGACAACAACGGCGCCGTGAAGTTCCCCCAGCTGTGCAAGTTCTGC
 1441 -----+-----+-----+-----+-----+-----+
 CTGTACTAGCACTGGCTGTTGTTGCCGCGCACTTCAAGGGGGTGCACACGTTCAAGACG
 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
 GACGTGCGGTTCTCTACCTGCGACAACCAGAAATCCTGCATGTCCAACGTCTCCATCACC
 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

 ATCACCTGGAAACCGTGTGCCACGACCCCAAGCTGCCCTACCACGACTTCATCCTGGAA
 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
 GATGCCGCCTCCCCAAGTGCATCATGAAGGAAAAGAAGAAGCCCGCGAGACTTTCTTC
 1681 -----+-----+-----+-----+-----+-----+
 CTACGGCGGAGGGGGTTCACGTAGTACTTCTTTCTTCTTCGGGCCGCTCTGAAAGAAG
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*
 GAGCTCCAGATCGTGCTGTCCCAGTCCCCGCCATCCTGTCTGCTAGCCCTGGCGAGAAA
 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
 GTGACAATGACCTGCCGGGCCTCCTCCTCCGTGTCCTACATCCACTGGTTCCAGCAGAAG
 61 -----+-----+-----+-----+-----+-----+
 CACTGTTACTGGACGGCCCGGAGGAGGCACAGGATGTAGGTGACCAAGGTCGTCTTC
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 -----+-----+-----+-----+-----+-----+
 GGGCCGAGGTCGGGGTTCGGGACCTAGATGCGGTGGAGGTTGGACCGGAGCCGCACGGT

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 -----+-----+-----+-----+-----+-----+
CGGCTCCTGCGGCGGTGGATGATGACGGTCGTCACCTGGAGGTGGGGGGGTGGAAACCG
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 -----+-----+-----+-----+-----+-----+
CCTCCGTGGTTCGACCTTTAGTTTCGCCTGGCACCGGCGAGGGAGGCACAAGTAGAAGGGT
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

CCCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTCGTGTGCCTGCTGAACAACTTC
361 -----+-----+-----+-----+-----+-----+
GGGAGGCTGCTCGTCTGACTTCAGGCCGTGGCGGAGGCAGCACGACGACTTGTTGAAG
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

TACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGGCAACTCC
421 -----+-----+-----+-----+-----+-----+
ATGGGGGCGCTCCGGTTCCACGTACCTTCCACCTGTTGCGGGACGTACGGCCGTTGAGG
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

CAGGAATCCGTCACCGAGCAGGACTCCAAGGACAGCACCTACAGCCTGTCCTCCACCCTG
481 -----+-----+-----+-----+-----+-----+
GTCCTTAGGCAGTGGCTCGTCCTGAGGTTCCGTGTCGTGGATGTCGGACAGGAGGTGGGAC
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 -----+-----+-----+-----+-----+-----+
TGGGACAGGTTCCGGCTGATGCTCTTCGTGTTCACATGCGGACGCTTCACTGGGTGGTC
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 -----+-----+-----+-----+-----+-----+
CCGACAGGTCGGGGCACTGGTTTCAGGAAGTTGGCCCCGCTCACGACTCCATGG

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
PTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP
QEVCAVVRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS
DECNDNIIFS EYNTSNPD

Anti-VEGF Light chain sequence:

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSR
FSGSGSGTDFTLTISLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSPVTKSFNRGEC

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*
GAGCTCGAGGTGCAGCTGGTGGAAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGATCCCTG
1 -----+-----+-----+-----+-----+-----+
CTCGAGCTCCACGTCCGACACCTTAGGCCGCTCCGGACCAGGTCCGACCGCTAGGGAC
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

AGACTGTCCTGTGCCGCTCCGGCTACACCTTCACCAACTACGGCATGAAGTGGGTCCGA
61 -----+-----+-----+-----+-----+-----+
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

CAGGCCCTGGCAAGGGCCTGGAATGGGTCGGATGGATCAACACCTACACCGGCGAGCCC
 121 -----+-----+-----+-----+-----+-----+-----+
 GTCCGGGGACCGTTCCTCGGACCTTACCCAGCCTACCTAGTTGTGGATGTGGCCGCTCGGG
 Q A P G K 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

 ACCTACGCCGCCGACTTCAAGCGGCGGTTACCTTCTCCCTGGACACCTCCAAGTCCACC
 181 -----+-----+-----+-----+-----+-----+-----+
 TGGATGCGGCGGCTGAAGTTGCGCGCCAAGTGGAGAGGGACCTGTGGAGGTTTCAGGTGG
 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 -----+-----+-----+-----+-----+-----+-----+
 CGGATGGACGTCTACTTGAGGGACGCGCGGCTCCTGTGGCGGCACATGATGACGCGGTTT
 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
 TACCCCCACTACTACGGCTCCTCCCACTGGTACTTCGACGTGTGGGGCCAGGGCACCCCTG
 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 -----+-----+-----+-----+-----+-----+-----+
 CAGTGGCACAGGAGGCGGAGATGGTTCCTCGGGGAGGCACAAGGAGACCGGGGGAGGTGC
 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 -----+-----+-----+-----+-----+-----+-----+
 TTCAGGTGTAGACCGCGTGGCGGCGAGACCGACGGACCACTTCTGATGAAGGGGCTC
 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
 CCCGTGACCGTGTCTGGAACCTCTGGCGCCCTGACCTCCGGCGTGACACCTTTCCAGCC
 481 -----+-----+-----+-----+-----+-----+-----+
 GGGCACTGGCACAGGACCTTGAGACCGCGGGACTGGAGGCCGCACGTGTGGAAGGTCGG
 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
 GTGCTGCAGTCTCCGGCCTGTACTCCCTGTCTCCGTGACCGTGCCCTCCAGCTCT
 541 -----+-----+-----+-----+-----+-----+-----+
 CACGACGTCAGGAGGCCGACATGAGGGACAGGAGGCAGCACTGGCACGGGAGGTCGAGA

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

 CTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCTCCAACACCAAGGTGGAC
 601 -----+-----+-----+-----+-----+-----+-----+
 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

 AAGAAGGTGGAACCCAAGTCCTGCGACAAGACCCACACCTGTCCCCCTGCCCTGCCCT
 661 -----+-----+-----+-----+-----+-----+-----+
 TTCTTCCACCTTGGGTTTCAGGACGCTGTTCTGGGTGTGGACAGGGGGGACGGGACGGGGA
 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

 GAACTGCTGGGAGGCCCTAGCGTGTTCCTGTTCCCCCAAAGCCCAAGGACACCCCTGATG
 721 -----+-----+-----+-----+-----+-----+-----+
 CTTGACGACCCCTCCGGGATCGCACAAGGACAAGGGGGGTTTCGGGTTCTGTGGGACTAC
 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
 ATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCACGAGGACCCCTGAA
 781 -----+-----+-----+-----+-----+-----+-----+
 TAGAGGGCCTGGGGGCTTCACTGGACGCACCAACACCTGCACAGGGTGCTCCTGGGACTT
 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

 GTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCCAGA
 841 -----+-----+-----+-----+-----+-----+-----+
 CACTTCAAGTTAACCATGCACCTGCCGCACCTTACGTTGTCGGTTCTGGTTCCGGGTCT
 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

 GAGGAACAGTACAACCTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
 901 -----+-----+-----+-----+-----+-----+-----+
 CTCCTTGTTCATGTTGAGGTGGATGGCCCCACACAGGCACGACTGGCACGACGTGGTCCTG
 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

 TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCCATC
 961 -----+-----+-----+-----+-----+-----+-----+
 ACCGACTTGCCGTTTCTCATGTTTCACGTTCCACAGGTTGTTCCGGGACGGACGGGGGTAG
 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

 GAAAAGACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACTCTGCCC
 1021 -----+-----+-----+-----+-----+-----+-----+
 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 CCTAGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTCAAGGGCTTC
 -----+-----+-----+-----+-----+-----+
 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 TACCCCTCCGATATCGCCGTGGAATGGGAGTCCAACGGCCAGCCCAGAGAACAACACTACAAG
 -----+-----+-----+-----+-----+-----+
 ATGGGGAGGCTATAGCGGCACCTTACCCTCAGGTGCCGGTCTGGGCTCTTGTGATGTTTC
 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 ACCACCCCCCTGTGCTGGACTCCGACGGCTCATTCCTCCTGTACTCCAAGCTGACCGTG
 -----+-----+-----+-----+-----+-----+
 TGGTGGGGGGGACACGACCTGAGGCTGCCGAGTAAGAAGGACATGAGGTTCGACTGGCAC
 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 GACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTG
 -----+-----+-----+-----+-----+-----+
 CTGTTCAAGGCCACCGTCGTCCCGTTGCACAAGAGGACGAGGCACTACGTGCTCCGGAC
 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
 -----+-----+-----+-----+-----+-----+
 GTGTTGGTGATGTGGGTCTTCAGGGACAGGGACTCGGGTCCGTTCCTCGCTCCGCCTAGA
 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
 -----+-----+-----+-----+-----+-----+
 CCACCACCTCCTAGTCCGCCGCTCCGAGATGGTAGGGGGGTGTGCACGTCTTTAGGCAC
 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 AACACGACATGATCGTGACCGACAACAACGGCGCCGTGAAGTTCCTCCAGCTGTGCAAG
 -----+-----+-----+-----+-----+-----+
 TTGTTGCTGTACTAGCACTGGCTGTTGTTGCCGCGGCACTTCAAGGGGGTTCGACACGTT
 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 -----+-----+-----+-----+-----+-----+
 CTCTTGTAGTGGGACCTTTGGCACACGGTGTGGGGTTCGACGGGATGGTGTGAAGTAG
 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

CTGGAAGATGCCGCCTCCCCCAAGTGCATCATGAAGGAAAAGAAGAAGCCCGCGGAGACT
 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 -----+-----+-----+-----+-----+-----+
 AAGAAGTACACGTGACGAGGAGGCTGCTCACGTTGCTGTTGTAGTAGAAGAGGCTTCTC
 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 -----+-----+-----+-----+-----+-----+
 CTCGAGCTATAGGTCTACTGGGTCTAGGGGGAGGTCGGACAGGCGGAGACACCCGCTGTCT
 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 -----+-----+-----+-----+-----+-----+
 TTCGGGCCGTTCCGGGGATTCCACGACTGATGAAGTGGAGGAGGGACGTGAGGCCGCAC
 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 CAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACTCCACCGTGCCCTGGACCTTC
 -----+-----+-----+-----+-----+-----+
 GTCGGGCTCCTGAAGCGGTGGATGATGACGGTCGTCATGAGGTGGCACGGGACCTGGAAG
 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 GGCCAGGGCACCAAGGTGGAAATCAAGCGGACCGTGCGCGCTCCCTCCGTGTTTCATCTTC
 -----+-----+-----+-----+-----+-----+
 CCGGTCCCGTGGTTCCACCTTTAGTTCCGCTGGCACC GGCGAGGGAGGCACAACTAGAAG
 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
 -----+-----+-----+-----+-----+-----+
 GGTGGGAGGCTGCTCGTCTGACTTCAGGCCGTGGCGGAGGCAGCACACGGACGACTTGTG
 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 TTCTACCCCGCGAGGCCAAGGTGCAGTGAAGGTGGACAACGCCCTGCAGTCCGGCAAC
 -----+-----+-----+-----+-----+-----+
 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

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
 -----+-----+-----+-----+-----+-----+
 GTCCCCGACAGGTCGGGGCACTGGTTCAGGAAGTTGGCCCCGCTCACGACTCCATGG
 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 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
GNVFSCSVMH EALHNHYTQK SLSLSPGKGGGGSGGGGSGGGGSTIPPHVQK SVNNDMIVTD
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETY
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 DSTYSLSSTL
TLISKADYEH KUYACEVTHQ 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
 QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS
 DECNDNIIFS EEEYNTSNPD GGGGSGGGSGGGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTL
 MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
 KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
 ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG
 KGGGSGGGSGGGGS

(b)

APTSSSTKKTQLQLEHLLLDLQMI LNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEEELKP
 LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIIS
 TLTGGGSGGGSGGGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
 PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 SDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK GGGGSGGGSGGGGS
 TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP
 QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS
 DECNDNIIFSEEEYNTSNPD

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 GVFDYWGQGT LVTVSSASTK
GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
LSSVTVPSL 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 GGGGSGGGGSGGGGSTIPPHVQK SVNNDMIVTD
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETY
CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EYNTSNPD

Black: Anti-CD25 antibody heavy chain

Green: Linker

Red: TGFβRII ectodomain

anti-CD25 - Light chain

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

(b)

anti-CD25 and PD-1 ectodomain - Heavy chain

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDIYGY YFDYWGQGT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVTVPSL LGTQTYICNV NHHKPSNTKVD KRVEPPKSCD KTHTCPPCPA PELLGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMEHA LHNHYTQKSL SLSPGK GGGGSGGGGSGGGGSTIPPHVQK SVNNDMIVTD
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETY
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 SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE
 QLKSGTASVV CLLNNFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSSTLTLSK
 ADYEKHKVYA CEVTHQGLSS PVTKSFNRGE

* **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

PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKL
 AAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTLYCGAISLAPKAQIKESL
 RAELRVTERRAEVPTAHPSPPRPAGQFQTLVGGGGSGGGGSGGGGSTHTCPPCPAPEL
 LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
 EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
 PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
 VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSTIPPHVQK
 SVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWR
 KNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNII
 FSEEYNTSNPD

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

PGWFLDSPDRPWNPTTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKL
 AAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTLYCGAISLAPKAQIKESL
 RAELRVTERRAEVPTAHPSPPRPAGQFQTLV (EPKSCDK) THTCPPCPAPPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
 YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM
 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
 QQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSTIPPHVQKSVNNDM
 IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENI
 TLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNII FSEEYN
 TSNPD

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:

1 MQIPQAPWPV VWAVLQLGWR PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS
61 ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT
121 YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV VGVVGLLGS
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 PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA
AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA
ELRVTERRAE VPTAHPSPSP RPAGQFQTLV

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

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)]

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

PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA
AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA
ELRVTERRAE VPTAHPSPSP 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
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSS
LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGS
GGGGSGGGGS PGWFLDSPDR PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM
SPSNQTDKLA AFPEDRSQPG QDCRFRTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP
KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV

Anti-HER2/neu light chain amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGS
RSGTDFTLTISSLPEDFATYYCQHHYTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACE
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:

QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGNTDYNTFPFTS
RLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTVTVSAASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSL
GTQTYICNVNHKPSNTKVDKRVPEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSG
GGGSGGGGS PGWFLDSPDR PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM
SPSNQTDKLA AFPEDRSQPG QDCRFRTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP
KAQIKESLRA ELRVTERRAE VPTAHPSPPSP RPAGQFQTLV

Anti-EGFR light chain amino acid sequence:

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGS
GSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACE
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
GKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSS
SLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGG
SGGGGSGGGGSPGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM
SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP
KAQIKESLRA ELRVTERRAE VPTAHPSPPSP RPAGQFQTLV

Anti-CD20 light chain amino acid sequence:

QIVLSQSPAILASAPGEEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSG
SGTSYSLTISRVEAEDAATYYCQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

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
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK GGGGSGGGGSGGGGS
PGWFLDSPDR PWNPTTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA
AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA
ELRVTERRAE VPTAHPSPSP RPAGQFQTLV

Anti-VEGF Light chain sequence:

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSR
FSGSGSGTDFTLTISLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
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
GNVFSCSVMH EALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSPGWFLDSPDR PWNPPTFSPA
LLVVTEDGNA TFTCSFSNTS ESFVLNWMYR SPSNQTDKLA AFPEDRSQPG QDCRFRTVQL
PNGRDFHMSV VRARRNSGT YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPPSP
RPAGQFQTLV

Anti-CTLA-4 light chain

EIVLTQSPGT LSLSPGERAT LSCRASQSVG SSYLAWYQQK PGQAPRLLIY GAFSRATGIP
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPWTFG QGTKVEIKRT VAAPSVFIFP
PSDEQLKSGT ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYLSSTL
TLISKADYEKH KVVACEVTHQ 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
EVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSS
DECNDNIIFSEEYNTSNPDGGGGSGGGSGGGGSTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGSGPGWFLDSPDRPWNPTTFSPAL
LVVTEGDNATFTCSFSNTSESFVLNWMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQL
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
EVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSS
DECNDNIIFSEEYNTSNPD (EPKSCDK) THTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGSGPGWFLDSPDRPWNPTTFSPALLVVTEG
DNATFTCSFSNTSESFVLNWMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLPNGRDF
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 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 GGGGSGGGGSGGGSPGWFLDSPDRPWNPTTFSPAL
LVVTEGDNATFTCSFSNTSEFVLNWMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLPNGRDF
HMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSRPAGQFQTLV

Black: anti-CD25 heavy chain

Red: Linker

Green: PD-1 ectodomain

anti-CD25 (Daclizumab) - Light chain

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

(b)

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

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDIYGY YFDFWGQGT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAAL 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 GGGGSGGGGSGGGSPGWFLDSPDRPWNPTTFSPAL
LVVTEGDNATFTCSFSNTSEFVLNWMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLPNGRDF
HMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSRPAGQFQTLV

Black: anti-CD25 heavy chain

Red: Linker

Green: PD-1 ectodomain

anti-CD25 (Basiliximab) – Light chain

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

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

Protein Sequence:

(a)

PGWFLDSPDRPWNPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWMSPSNQTDKLAAPFED
 RSQPGQDCFRVLTQLPNGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRVTERRAE
 VPTAHPSPPRPAGQFQTLVGGGGSGGGSGGGSTHTCPPCPAPPELLGGPSVFLFPPKPKDTLM
 ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
 EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE
 SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
 GGGSGGGSGGGGS

(b)

APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLCLEELKP
 LEEVLNLAQSKNFHLRPDLISNINVIIVLELKGSETTFMCEYADETATVEFLNRWITFCQSIIS
 TLTGGGGSGGGSGGGSTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
 PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 SDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGSPG
 WFLDSPDRPWNPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWMSPSNQTDKLAAPFEDRS
 QPGQDCFRVLTQLPNGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRVTERRAEVP
 TAHPSPSPRPAGQFQTLV

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 TYWHL~~LN~~AFT VTPKDL~~Y~~VV EYGSNMTIEC KFPVEKQLDL AALIVYWEME
DKNIIQFVHG EEDLK~~V~~QHSS YRQARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG
ADYKRITVKV NAPYNKINQR ILVVD~~P~~TSE HELTCQAE~~G~~Y PKAEVIWTSS DHQVLSGKTT
TTNSKREEKL FNV~~T~~STLRIN TTTNEIFYCT FRRLDPEENH TAE~~L~~VIPELP LAHPPNER~~T~~H
LVILGAILLC LGVALTFIFR LRKGRMDVK KCGIQDTNSK KQSDTHLEET

Extracellular Binding domain = underlined sequence

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

~~AFT~~ VTPKDL~~Y~~VV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG EEDLK~~V~~QHSS
YRQARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV NAPYNKINQR
ILVVD~~P~~TSE HELTCQAE~~G~~Y PKAEVIWTSS DHQVLSGKTT TTNSKREEKL FNV~~T~~STLRIN
TTTNEIFYCT FRRLDPEENH TAE~~L~~VI

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 TYWHL~~LN~~AFT VTPKDL~~Y~~VV EYGSNMTIEC KFPVEKQLDL AALIVYWEME
DKNIIQFVHG EEDLK~~V~~QHSS YRQARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG
ADYKRITVKV NAPYNKINQR ILVVD~~P~~TSE HELTCQAE~~G~~Y PKAEVIWTSS DHQVLSGKTT
TTNSKREEKL FNV~~T~~STLRIN TTTNEIFYCT FRRLDPEENH TAE~~L~~VIPELP LAHPPNER~~T~~H
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
GRFTISRDNKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGLTVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
SLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGG
SGGGSGGGGSRIFAVFIFMTYWHLLNAFTVTVPKDLVVEYGSNMTIECKFPVEKQLDLAALIV
YWEMEDKNI IQFVHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGG
ADYKRITVKVNAPYKNINQRILVVDVPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSK
REEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLC
LGVALTFIFRLRKGRMDVKKCGIQDTNSKKQSDTHLEET

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:

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLPGLKAPKLLIYAASLTQSGVPSRFS
GSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACE
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 C γ 1 – PD-1L1).

TNFR2 ECD - IgG C γ 1 – PD-1L1

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLW
NWWPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKEGRCRLCAPLRKCRPGFGVARP
GTETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLP
QPVSTRSQHTQPTPEPSTAPSTSFLPMGPSPPAEGSTGDEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNH
YTQKSLSLSPGKGGGSGGGGSGGGGSRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIE
CKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDV
KLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRIILVVDVPTSEHELTCQAEGYPKAEVIWTS
SDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHP
PNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET

Black color: TNFR2 ligand-binding domain

Blue Color: human IgG1 heavy chain constant region (IgG C γ 1 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 C γ 1 -TNFR2 ECD)

PD1-L1 - IgG C γ 1 -TNFR2 ECD

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNII
QFVHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKV
NAPYNKINQRIILVVDVPTSEHELTCQAEGYPKAEVIWTS
SDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHP
PNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET
GGGSGGGGSGGGGSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHE
ALHNH
YTQKSLSLSPGKGGGSGGGGSGGGGSLPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCS
KCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLW
NWWPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKEGRCRLCAPLRKCRPGFGVARP
GTETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLPMGPSP
PAEGSTGD

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

Blue Color: human IgG1 heavy chain constant region (IgG C γ 1 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
SLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAIEKTIKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGSGGGGSRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLA
ALIVYWEMEDKNI IQFVHGEEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMI
SYGGADYKRITVKVNAPYNKINQRILVVDVPTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTT
TNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGA
ILLCLGVALTFIFRLRKGRMDVKKCGIQDTNSKKQSDTHLEET

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:

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSG
SGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

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 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 GGGGSGGGGSGGGGSRIFAVFIFMTYWHLNAFTVT
VPKDLVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQARL
LKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPTSEHE
LTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQ
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 YFDWVGQGT 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 KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK GGGGSGGGGSGGGGSRIFAVFIFMTYWHLNAFTVT
VPKDLVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQARL
LKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPTSEHE
LTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQ
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 SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE
 QLKSGTASVV CLLNNFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK
 ADYEKHKVYA CEVTHQGLSS PVTKSFNRGE

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

MRIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNII
QFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKV
 NAPYNKINQRILVVDVPTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVT
 TLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFR
 LRKGRMDVKKCGIQDTNSKKQSDTHLEETGGGGSGGGSGGGSTHTCPPCPAPELLGGPSVFL
 FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
 VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF
 YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYT
 QKSLSLSPGKGGGGSGGGSGGGGS

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

APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEELKP
 LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIIS
 TLTGGGGSGGGSGGGSGGGSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
 EPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLD
 SDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGGSRI
 FAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEMEDKNIIQFV
HGEEDDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
 YNKNINQRILVVDVPTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLR
 INTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRK
 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
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGS
GGGSGGGGSRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVY
WEMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGA
DYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKR
EEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCL
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

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNI
IQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKV
NAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVT
TLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFR
LRKGRMMDVKKCGIQDTNSKKQSDTHLEETGGGSGGGSGGGGSEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGKGGGSGGGSGGGGSAMHVAQPAVV
LASSRGIASFVCEYASPGKATEV
RVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLT
IQGLRAMDTGLYICKVELM
YPPPYLGIGNGTQIYVIDPEPCPDS

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
NVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGGSRIFAVFIFMTYWHLLNAFTVTVP
KDLYVVEYGSNM¹IECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQARLLK
DQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPY²NKINQRI³LVVDPVTSEHELT
CQAE⁴GYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEEN
HTAELVIP⁵ELPLAHPN⁶ERTHLVILGAILLCLGVALTFIFRLRKG⁷RMDVKKCGIQDTNSKKQSD
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:

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNM¹IECKFPVEKQLDLAALIVYWEMEDKNII
QFVHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKV
NAPY²NKINQRI³LVVDPVTSEHELTCAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVT
TLRINTTTNEIFYCTFRRLDPEENHTAELVIP⁵ELPLAHPN⁶ERTHLVILGAILLCLGVALTFIFR
LRKG⁷RMDVKKCGIQDTNSKKQSDTHLEETGGGGSGGGSGGGGSTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV⁸FSCSVMHEALHNHYT
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-B1 full sequence

1 MPPSGLRLLP LLLPLLRLLV LTPGRPAAGL STCKTIDMEL VKRKRIEAIR GQILSKLRLS
61 SPSPQGEVPP VPLPEAVLAL YNSTRDRVAG ESAEPEPEPE ADYYAKEVTR VLMVENTNKI
121 YEKVKKSPHS IYMLFNTSEL REAVPEPVLL SRAELRLLRL KLKAEQHVEL YQKYSNDSWR
181 YLSNRL LAPS DTPWLSFDV TGVVRQWL SH GGEVEGFRLS AHCSCDSKDN TLQVDINGFS
241 SSRRGDLATI 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
GRFTISRDNKNSLYLQMNSLRAEDTAVYYCALVSYLSTASSLDYWGQGLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
SLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGG
SGGGSGGGGSALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSL
DTQYSKVLALYNQHNP GASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

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

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

Light Chain:

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLP GKAPKLLIYAASLTQSGVPSRFSGS
GSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNIFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACE
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
NWVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKEGCRLCAPLRKCRPGFGVARP
GTETSDVVCPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLP
QPVSTRSQHTQPTPEPSTAPSTSFLPMGPSPPAEGSTGDEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNH
YTQKSLSLSPGKGGGGSGGGSGGGGSALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKG
YHANFCLGPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPLPIVYYYVGRKPKVEQLSN
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
NQHNPGASAAPCCVPQALEPLPIVYYYVGRKPKVEQLSNMIVRSCKCSGGGGSGGGSGGGGSEPK
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGKGGGGSGGGSGGGGSLPAQVAFTPYAPEPGS
TCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWNWVPECLSCGSRCSDD
QVETQACTREQNRICTCRPGWYCALSKEGCRLCAPLRKCRPGFGVARPGTETSDVVCPCAPGT
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
PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
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:

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSG
SGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYEEKHKVYACEV
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
NQKFVKGKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LVTVSSASTK
GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
LSSVTVVPSS 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 YMHWYQQKPG KAPKLLIYTT SNLASGVPAR
FSGSGSGTEF TLTISLQPD DFATYYCHQR STYPLTFGQG TKVEVKRTVA APSVFIFPPS
DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLT
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

(ii)

anti-CD25 and TGF- β 1 - Heavy chain

QLQQSGTVLA RPGASVKMSC KASGYSFTRY WMHWIKQRPQ QGLEWIGAIY PGNSDTSYNQ
KFEGKAKLTA VTSASTAYME LSSLTHEDSA VYYCSRDIYGY YFDFWGQGT LTVSSASTKG
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
SSVTVVPSSS 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 MSASPGEKVT MTCSASSSRS YMQWYQQKPG TSPKRWIYDT SKLASGVPAR
FSGSGSGTSY SLTISSMEAE DAATYYCHQR SSYTFGGGTK LEIKRTVAAP SVFIFPPSDE

QLKSGTASVV CLLNMFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY SLSSTLTLSK
ADYEKHKVYA CEVTHQGLSS PVTKSFNERGE

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
NQHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCSGGGGSGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGGS

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

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKP
LEEVNLNAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIIS
TLTGGGGSGGGSGGGSGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGGS
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

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRLQ
ADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYL
GIGNGTQIYVIDPEPCPDSD (Q) EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV
TCTVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKGGGGS
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
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVD
KSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKGGGSGGGSGGGGSAMHVAQPAVVLASSRG
IASFVCEYASPGKATEVRVTVLRLQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQ
GLRAMDTGLYICKVELMYPPPYLGGIGNGTQIYVIDPEPCPDSD

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 Patent Application Fee Transmittal				
Application Number:				
Filing Date:				
Title of Invention:		TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		
First Named Inventor/Applicant Name:		Atul Bedi		
Filer:		Lisa A. Haile/Cara Grifone		
Attorney Docket Number:		JHU3260-3		
Filed as Small Entity				
Provisional Filing Fees				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Provisional Application filing fee	2005	1	110	110
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
Total in USD (\$)				110

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
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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		JHU3260-3Provisional.pdf	7399213	yes	132
			b41195ebcc3522cdac158cc8d9b10d25278d8ff0		
	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		
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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY DOCKET NO	TOT CLAIMS	IND CLAIMS
61/435,671	01/24/2011		110	JHU3260-3		

CONFIRMATION NO. 7162

FILING RECEIPT



CC000000046085701

28213
DLA PIPER LLP (US)
4365 EXECUTIVE DRIVE
SUITE 1100
SAN DIEGO, CA 92121-2133

Date Mailed: 02/22/2011

Receipt is acknowledged of this provisional patent application. It will not be examined for patentability and will become abandoned not later than twelve months after its filing date. 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**

Applicant(s)

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

Power of Attorney:

Lisa Haile--38347

If Required, Foreign Filing License Granted: 02/17/2011

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 61/435,671**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

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page 1 of 3

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Title 37, Code of Federal Regulations, 5.11 & 5.15

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