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Application Number: 18883719 Document Date: 09/12/2024

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Form Revision Date: March 1, 2024

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Application Number: 18883719 Document Date: 09/12/2024

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Form Revision Date: 07 January 2022

DocCode - SCORE

Placeholder Sheet for Supplemental Content

Application Number: 18883719 Document Date: 09/12/2024

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Form Revision Date: March 1, 2024

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
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ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/883,719	**09/12/2024 05:10:37 PM Z ET**	**JHU3260-10**

Title of Invention

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	6410	FILED BY	Aldon Griffis
PATENT CENTER #	67155289	AUTHORIZED BY	-
CUSTOMER #	100899	FILING DATE	-
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Atul Bedi

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
DA / 071896	**E20249BH13126407**	**Aldon Griffis**

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
2081	UTILITY APPL. SIZE FEE > THAN 100 PAGES (FEE X PAGES >= 101 IN 50 PAGE INCREMENTS)	168.00	1	168.00
2830	PROCESSING FEE, EXCEPT IN PROVISIONAL APPLICATIONS	56.00	1	56.00
2817	REQUEST FOR PRIORITIZED EXAMINATION	1680.00	1	1680.00
2111	UTILITY PATENT APPL. SEARCH FEE	280.00	1	280.00
4011	BASIC FILING FEE- UTILITY	64.00	1	64.00
2311	EXAMINATION OF ORIGINAL PATENT APPLICATION	320.00	1	320.00

TOTAL AMOUNT:	**$2,568.00**

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (04-14)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Atul Bedi	Nonprovisional Application Number (if known):	
Title of Invention:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently $0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.

2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.

3. The applicable box is checked below:

I. ☑ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via Patent Center or EFS-Web.

---OR---

(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, **or** the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via Patent Center or EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature /Pamela E. Hwang/	Date September 12, 2024
Name (Print/Typed) Pamela E. Hwang, J.D., Ph.D.	Practitioner Registration Number 54,984

Note: *This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

☑ *Total of one forms are submitted.

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PATENT
ATTORNEY DOCKET NO.: JHU3260-10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	Not Yet Assigned
Parent Serial No.:	17/694,541	Examiner:	Not Yet Assigned
Parent Filing Date:	March 14, 2022		
Serial No.:	Not Yet Assigned		
Filed:	September 12, 2024		
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

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

INFORMATION DISCLOSURE STATEMENT

Sir:

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

The listed documents are of record in prior U.S. Application Serial No. 17/694,541 filed March 14, 2022, now pending, upon which Applicant relies on for an earlier effective filing date under 35 U.S.C. §120. Consequently, copies of those references are not provided pursuant to 37 C.F.R. § 1.98(d); however, Applicant will provide copies of those references at the request of the Examiner.

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

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

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

Respectfully submitted,

Date: September 12, 2024

/Pamela E. Hwang/

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 5

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	September 12, 2024
First Named Inventor	Atul Bedi
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	JHU3260-10

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1	7,317,091	01-08-2008	Lazar et al.	
	2	7,527,791	05-05-2009	Adams et al.	
	3	7,795,389	09-14-2010	Sun et al.	
	4	7,803,378	09-28-2010	Tranchanf-Bunel	
	5	7,820,165	10-26-2010	McKenna et al.	
	6	8,101,720	01-24-2012	Lazar et al.	
	7	8,114,845	02-14-2012	Langermann et al.	
	8	8,399,618	03-19-2013	Lazar et al.	
	9	8,815,247	08-26-2014	Govindappa et al.	
	10	2005/0054832	03-10-2005	Lazar et al.	
	11	2006/0135459	06-22-2006	Epstein et al.	
	12	2006/0193849	08-31-2006	Krauss et al.	
	13	2006/0263368	11-23-2006	Rosenblum et al.	
	14	2007/0212337	09-13-2007	Bedi et al.	
	15	2007/0244042	10-18-2007	Sun et al.	
	16	2008/0075717	03-27-2008	Tranchand-Bunel	
	17	2009/0053240	02-26-2009	Lazar et al.	
	18	2009/0214533	08-27-2017	Clynes	
	19	2009/0226435	09-10-2009	Sanjay	
	20	2011/0052585	03-03-2011	Scaria et al.	
	21	2013/0017199	01-17-2013	Langermann	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] "Number[4] "Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
	22	WO 2009/062399 A1	05-22-2009	Shanghai Jiaotong University		
	23	WO 2009/152610 A1	12-23-2009	The Royal Institution For The Advancement Of Learning/Mcgill University		
	24	WO 2010/003118 A1	01-07-2010	Trubion Pharmaceuticals, Inc.		

Examiner Signature		Date Considered	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 2 of 5

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	September 12, 2024
First Named Inventor	Atul Bedi
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	JHU3260-10

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	25	Alignment of SEQ ID No. 10 of D3 with SEQ ID No. 87 of B1	
	26	Alignment of SEQ ID No. 744 of D2 with SEQ ID No. 87 of B1	
	27	Canadian Office Action dated Mar. 28, 2018, regarding CA 2,791,383	
	28	Carter et al., "Identification and Validation of Cell Surface Antigens for Antibody Targeting in Oncology", Endocr. Relat. Cancer, December 2004, 11(4):659-687.	
	29	Coffelt et al., "Angiopoietin 2 Stimulates TIE2-Expressing Monocytes to Suppress T Cell Activation and to Promote Regulatory T Cell Expression"; The Journal of Immunology 2011, 186 (7), p. 4183-4190	
	30	Contardi et al, Int. J. Cancer: 117, 538-550; 2005	
	31	EP Communication dated Sep. 5, 2018, pursuant to Rule 114(2) EPC regarding EP 2542590	
	32	EP Third Party Observation in European Application No. EP20200205164 dated April 5, 2022, 10 pages.	
	33	European Search Report and Search Opinion Received for EP Application No. 17168491.3, mailed on November 24, 2017, 5 pages	
	34	Fernandez-Botran et al., "Soluble cytokine receptors in biological therapy"; Expert Opinion on Biological Therapy, 2002, vol. 2, Issue 6, p. 585-605	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 3 of 5

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	September 12, 2024
First Named Inventor	Atul Bedi
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	JHU3260-10

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	35	Fonseca et al., "Capitalizing on the immunogenicity of Dying Tumor Cells"; Clinical Cancer Research 2008, 14 (6), p. 1603-1608	
	36	Francisco et al, "PD-L1 Regulates the Development, Maintenance, and Function of Induced Regulatory T Cells", J. Exp. Med., December 2009, 206(13):3015-3029	
	37	Haier et al., "Cell Surface Molecules and Their Prognostic Values in Assessing Colorectal Carcinomas", Annal Surgery, January 2000, 231(1):11-24	
	38	IN Examination Report in Indian Application No. 201948021756 dated January 24, 2022, 6 pages	
	39	International Preliminary Report on Patentability received for PCT Patent Application No. PCT/US2011/027317, mailed on September 20, 2012, 9 pages	
	40	International Search Report and Written Opinion received for PCT Patent Application No. PCT/US2011/027317, mailed on February 08. 2012, 11 pages	
	41	Japanese Office Action issued on May 27, 2020, regarding JP 2018-140050	
	42	JP Office Action in Japanese Application No. 2021-094042 dated August 1, 2022, 6 pages (with English translation)	
	43	Jones et al., "Degree of CD25 expression in T-Cell lymphoma is dependent on tissue site: implications for targeted therapy", Clinical Cancer Research, 2004, 10:5587-5594	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 4 of 5

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	September 12, 2024
First Named Inventor	Atul Bedi
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	JHU3260-10

NON PATENT LITERATURE DOCUMENTS

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

Examiner Signature		Date Considered	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 5 of 5

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	September 12, 2024
First Named Inventor	Atul Bedi
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	JHU3260-10

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	53	Schrama et al.: "Antibody targeted drugs as cancer therapeutics"; Nature Reviews Drug Discovery, Jan. 20, 2006, vol. 5, p. 147-159	
	54	Supplementary European Search Report received for EP Patent Application No. 11751481.0, mailed on June 27, 2013, 16 pages	
	55	Trail et al.: "Monoclonal antibody drug conjugates in the treatment of cancer"; Current Opinion in Immunology, 1999, vol. 11, Issue 5, p. 584-588	
	56	U.S. Appl. No. 61/311;255, Mar. 5, 2010, Atul Bedi	
	57	U.S. Appl. No. 61/435,671, Jan. 24, 2011, Atul Bedi	
	58	Wang et al.: "Tumor necrosis factor and cancer, buddies or foes?"; Acta Pharmacologica Sinica, 2008, 29 (11), p. 1275-1288	
	59	Yang et al.: "The role of T.sub.reg in the cancer immunological response"; American Journal of Immunology, 2009, 5 (1), p. 17-28	
	60	Yao and Chen: "Contribution of B7-H1/PD-1 Co-inhibitory Pathway to T-Ce11 Dysfunction in Cancer"; D.I. Gabrilovich, A.A. Hurwitz (eds.), Tumor-Induced Immune Suppression. pp. 29-40 .COPYRGT. Springer 2008	
	61	Zhang et al., "Design and Optimization of a Linker for Fusion Protein Construction," Prog. Natural Sci. (2009), 19:1197-1200, Elsevier	
	62	Zhang et al: "Expression of a Soluble TGF-.beta. Receptor by Tumor Cells Enhances Dendritic Cell/Tumor FusionVaccine Efficacy.sup.1"; J. Immunology, 2008, 181, pp. 3690-3697	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/883,719	**09/12/2024 05:10:37 PM Z ET**	**JHU3260-10**

Title of Invention

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	6410	FILED BY	Aldon Griffis
PATENT CENTER #	67155289	FILING DATE	-
CUSTOMER #	100899	FIRST NAMED INVENTOR	Atul Bedi
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	-

Documents

TOTAL DOCUMENTS: 12

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
JHU3260-10ADS_EFS.pdf	9	Application Data Sheet	2179 KB
JHU3260-10 Continuation Application Transmittal EFS.pdf	3	Transmittal of New Application	197 KB
JHU3260-10 Request for Prioritized Examination.pdf	2	Track One Request	178 KB
JHU3260-10 Continuation Application EFS-APP.TEXT.docx	75	Application body structured text document	89 KB
Warning: The margins of the attached document do not meet USPTO rules. This may cause processing errors or delays. Bookmarks were found and have been removed.			
JHU3260-	75	Drawings-only black and white	2060 KB

10DrawingsEFS.pdf			line drawings	
JHU3260-10 IDS Transmittal EFS.pdf		2	Transmittal Letter	100 KB
JHU3260-10 IDS Form SB08.pdf		5	Information Disclosure Statement (IDS) Form (SB08)	261 KB
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JHU3260-10ParentInventorDeclsEFS.pdf		2	-	203 KB
JHU3260-10ParentInventorDeclsEFS-OATH.pdf	(1-1)	1	Oath or Declaration filed	106 KB
JHU3260-10ParentInventorDeclsEFS-OATH.pdf	(2-2)	1	Oath or Declaration filed	97 KB
JHU3260-10ParentPowerAttyEFS.pdf		2	Power of Attorney	95 KB
JHU3260-10_ST26.xml		-	Sequence Listing Supp (XML File)	171 KB
JHU3260-10 Continuation Application EFS.pdf		75	Auxiliary PDF of Application	641 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
JHU3260-10ADS_EFS.pdf	F2ABD6A1DADB02383B42E2978F19D7A38BA2B29B7190D342 50C3686C4ABC1B5DF29759E048072E99CEA7356D59A8C3E3 DCB092E8EAE4AFF243D0C932A9D833B1
JHU3260-10 Continuation Application Transmittal EFS.pdf	5AD429F630907A6C9F85098978FE773E0C509C644C01C842D 327EDF024B7E9D61C84FD0080433B4308F8BF80CEC5DEF816 B2B94EEA105C7313AEAB87A9D82AEC
JHU3260-10 Request for Prioritized Examination.pdf	DB15D718E25E26CBEB9771C5522A9F494E8B3E1E217E532A B165FA997CE4D860067FFCC5D27491C06C52A831B22986ED3 7C501D467006A95E8575EA1F89215F8
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JHU3260-10 IDS Form SB08.pdf	B94A076EBCD3118953CD77B5CCEC513AEB0F9035A25413FB2E74C251AC168D82553645C65547DDA0E14CFE17AC29B0DACC404EDD8F83C643898726311ED8E8D9
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JHU3260-10ParentInventorDeclsEFS-OATH.pdf	BEEEC95C6FB6D3961BD3771167CD0EF0DD901578D79D5FE1131DE0F24AF1F512FF99B3771BD6DBCF63F92FD4FF76C552703E454C650FCA6A1FA6996E64037BAD
JHU3260-10ParentInventorDeclsEFS-OATH.pdf	BC2645153627991FACD0060416824030499O1A0CE0A6BA3EF9A646E1CC687DF6FE4569E69F74EA4C181C27325C6C2D58781571CCE0C37E173AAEF52F1D2ADC60
JHU3260-10ParentPowerAttyEFS.pdf	1775DEDE498421E232676289F040BFED3B75E846F5D9CBFBC0AAFC3C89F80A065FA1C3CC8F28DF2F40357D48D421A959394B6EAEA375DF7C54802FB764FF4158
JHU3260-10_ST26.xml	62C245CED7DA2922581AF65C4E25E367CAA1395B8CDFE1F3494D93E8EC8E05710C21D8E67606B61D019AAA258A826F21D5E0FEE0FEF63732181AA0F69322F40D
JHU3260-10 Continuation Application EFS.pdf	A3D429FF97C1853DDF057E67B9F6C964F403B3BE60F61B5AD0FE21A7CB156BFB3CA829D4D9AB51646860CF89A44A38F84C59246795B3DFF2670343A5FD651F96

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New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

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September 12, 2024

ATTORNEY DOCKET NO.: JHU3260-10
Parent Application No.: 17/694,541
Parent Filing Date: March 14, 2022
Parent Inventors: Atul BEDI and Rajani RAVI

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

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

INVENTORS	**:**	**Atul BEDI and Rajani RAVI**
TITLE	**:**	**COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS**

The prior application is assigned of record to **THE JOHNS HOPKINS UNIVERSITY**, by virtue of an assignment recorded by the U.S. Patent and Trademark Office on April 20, 2020 at Reel 052447, Frame 0640.

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

Pages of Specification	72
Pages of Claims	2
Pages of Abstract	1
Sheets of Drawing	75
Pages of Power of Attorney (from Parent) (with Statement under 37 CFR 3.73(c))	2
Pages of Declaration (from Parent)	2

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

- Track 1 Prioritized Examination Request Form PTO/AIA/424 (2 pages);
- Application Data Sheet (9 pages);
- Information Disclosure Statement (2 pages);
- PTO Form SB/08 for the following unenclosed documents (2 pages); and
    - Nine (9) U.S. Patents;
    - Twelve (12) U.S. Patent Application Publications;
    - Three (3) Foreign Patent Documents; and
    - Thirty-Eight (38) Non Patent Literature Documents.
- Sequence Listing in .txt format entitled "JHU3260-10_ST26.xml" (174,955 bytes).

Under 35 USC §120, this application is a **continuation** application of U.S. Application Serial No. 17/694,541 filed March 14, 2022, now pending; which is a continuation application of U.S. Application Serial No. 16/601,347 filed October 14, 2019, now issued as U.S. patent 11,274,156; which is a continuation application of U.S. Application Serial No. 15/362,632 filed November 28, 2016, now issued as U.S. patent 10,442,860; which is a divisional application of U.S. Application Serial No. 15/231,309 filed August 8, 2016, now issued as U.S. patent 9,850,306; which is a continuation application of U.S. Application Serial No. 14/645,282 filed March 11, 2015, now issued as U.S. patent 9,441,044; which is a continuation application of U.S. Application Serial No. 13/582,717 filed October 17, 2012, now issued as U.S. patent 8,993,524; which is a 35 USC §371 National Stage application of International Application No. PCT/US2011/027317 filed March 4, 2011, now expired; which claims the benefit under 35 USC §119(e) to U.S. Application Serial No. 61/435,671 filed January 24, 2011 and to U.S. Application Serial No. 61/311,255 filed March 5, 2010, both now expired. The disclosure of each of the prior applications is considered part of and is incorporated by reference in the disclosure of this application.

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

The filing fee is calculated as follows:

	Number Filed		Number Extra		Rate			Fee	
					Large Entity	Small Entity		Large Entity	Small Entity
Total Claims	**8**-20	=	**0**	X		$40	=		$ .00
Independent Claims	**2**-3	=	**0**	X		$192	=		$ .00
Multiple Dependent Claims Present: ____ Yes __x__ No						$344			N/A
Fee for Each Additional 50 Sheets in Excess of 100 Pages						$168			$ 168.00
					FILING FEE				$ 64.00
					SEARCH FEE				$ 280.00
					EXAM FEE				$ 320.00
					TOTAL FEE				**$ 832.00**

The fees for this filing total $2,568.00, which consist of $1,680.00 for the request for prioritized exam fee, $56.00 for the processing fee, $64.00 for the electronic filing fee, $280.00 for the search fee, $320.00 for the exam fee and $168.00 for the application size fee. The Commissioner is hereby authorized to charge any additional fees associated with the filing submitted herewith, or credit any overpayment, to Deposit Account 07-1896, referencing the Attorney Docket number shown above.

If this application is found to be INCOMPLETE, or if it appears that a telephone conference would helpfully advance prosecution, please telephone the undersigned at (858) 677-1473.

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

Respectfully submitted,

DLA Piper LLP (US)

September 12, 2024
Date

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PEH:ag
Attachments

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331323.001436

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation application of U.S. Application Serial No. 17/694,541 filed March 14, 2022, now pending; which is a continuation application of U.S. Application Serial No. 16/601,347 filed October 14, 2019, now issued as U.S. patent 11,274,156; which is a continuation application of U.S. Application Serial No. 15/362,632 filed November 28, 2016, now issued as U.S. patent 10,442,860; which is a divisional application of U.S. Application Serial No. 15/231,309 filed August 8, 2016, now issued as U.S. patent 9,850,306; which is a continuation application of U.S. Application Serial No. 14/645,282 filed March 11, 2015, now issued as U.S. patent 9,441,044; which is a continuation application of U.S. Application Serial No. 13/582,717 filed October 17, 2012, now issued as U.S. patent 8,993,524; which is a 35 USC §371 National Stage application of International Application No. PCT/US2011/027317 filed March 4, 2011, now expired; which claims the benefit under 35 USC §119(e) to U.S. Application Serial No. 61/435,671 filed January 24, 2011 and to U.S. Application Serial No. 61/311,255 filed March 5, 2010, both now expired. The disclosure of each of the prior applications is considered part of and is incorporated by reference in the disclosure of this application.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under Grant No. CA123277 awarded by the National Institutes of Health. The government has certain rights in the invention.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0003] The material in the accompanying sequence listing is hereby incorporated by reference into this application. The accompanying sequence listing text file, named JHU3260-10_ST26.xml, was created on September 11, 2024 and is 174,955 bytes in size.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

[0004] The present invention relates generally to the field of targeted immunomodulatory antibodies and fusion proteins for cancer therapy and more specifically to composition and methods for targeted immunostimulatory or immunosuppressive antibodies and fusion proteins to counteract or induce immune tolerance of cancer cells.

BACKGROUND INFORMATION

[0005] The immune system provides the human body with a means to recognize and defend itself against microorganisms and substances recognized as foreign or potentially harmful. While passive immunotherapy of cancer with monoclonal antibodies and passive transfer of T cells to attack tumor cells have demonstrated clinical efficacy, the goal of active therapeutic vaccination to induce these immune effectors and establish immunological memory against tumor cells has remained challenging. Several tumor-specific and tumor-associated antigens have been identified, yet these antigens are generally weakly immunogenic and tumors employ diverse mechanisms to create a tolerogenic environment that allows them to evade immunologic attack. Strategies to overcome such immune tolerance and activating robust levels of antibody and/or T cell responses hold the key to effective cancer immunotherapy.

SUMMARY OF THE INVENTION

[0006] The present invention is based on the seminal discovery that targeted immunomodulatory antibodies and fusion proteins can counteract or reverse immune tolerance of cancer cells. Cancer cells are able to escape elimination by chemotherapeutic agents or tumor-targeted antibodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. Such immunosuppressive mechanisms include immunosuppressive cytokines (for example, Transforming growth factor beta (TGF-β)) and regulatory T cells and/or immunosuppressive myeloid dendritic cells (DCs). By counteracting tumor-induced immune tolerance, the present invention provides effective compositions and methods for cancer treatment, optional in combination with another existing cancer treatment. The present invention provides strategies to counteract tumor-induced immune tolerance and

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enhance the antitumor efficacy of chemotherapy by activating and leveraging T cell-mediated adaptive antitumor immunity against resistant or disseminated cancer cells.

[0007] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule, and the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2); (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); (iv) Transforming growth factor-beta receptor (TGF-βR); (v) Programmed death-1 (PD-1); and (vi) Receptor activator of nuclear factor-κB (RANK).

[0008] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. In one aspect, the targeting moiety specifically binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), CD20, Vascular endothelial growth factor (VEGF), insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, CD30, CD33, or CD40.

[0009] In one aspect, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: (i) CD4; (ii) CD25 (IL-2α receptor; IL-2αR); (iii) cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); (iv) Interleukin-10 (IL-10); (v) Transforming growth factor-beta receptor (TGF-βR); (vi) Transforming growth factor-beta (TGF-β); (vii) Programmed Death-1 (PD-1); (viii) Programmed death-1 ligand (PD-L1 or PD-L2); (ix) Receptor activator of nuclear factor-κB (RANK); or (x) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL).

[0010] In one aspect, the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed

death-1 ligand (PD-L1 or PD-L2); or (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL).

[0011] In one aspect, the immunomodulatory moiety includes a molecule that binds TGF-β. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Transforming growth factor-beta receptor TGF-βRII, TGF-βRIIb, or TGF-βRIII. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of TGF-β.

[0012] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

[0013] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Programmed Death-1 (PD-1), Programmed death-1 ligand 1 (PD-L1), or Programmed death-1 ligand 2 (PD-L2). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes PD-1 ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

[0014] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory

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moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

[0015] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Programmed death-1 ligand 1 (PD-L1).

[0016] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.

[0017] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Programmed Death-1 (PD-1). In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and PD-1 ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 25 or 26.

[0018] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or

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ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Receptor activator of nuclear factor-κB ligand (RANKL).

[0019] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36.

[0020] In one aspect, the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the immunomodulatory moiety increases the function of PD-1.

[0021] In one aspect, the targeting moiety specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 37. In another aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2), and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from PD-L1. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 38 or 39.

[0022] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25, or CD4, and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1

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ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 40, 41, 42, 43, 44, or 45.

[0023] In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 46 or 47.

[0024] In one aspect, the targeting moiety includes transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 48 or 49.

[0025] In one aspect, the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the immunomodulatory moiety activates the signaling function of transforming growth factor-β (TGF-β) receptor.

[0026] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from TGF-β. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 50. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 51 or 52.

[0027] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25 (IL-2α receptor; IL-2αR), or CD4, and the immunomodulatory moiety includes a sequence from transforming growth factor-β

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(TGF-β). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 53, 54, 55, 56, 57 or 58.

[0028] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 59 or 60.

[0029] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from an extracellular RANKL-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 61. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from an extracellular RANK-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 62 or 63.

[0030] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

[0031] In one aspect, the targeting moiety includes a sequence from transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

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[0032] In one aspect, the targeting moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

[0033] In various aspects, the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

[0034] In another embodiment, the present invention provides a composition including the molecule of the invention and a cell, wherein the cell is a tumor cell, immune cell, or dendritic cell.

[0035] In another embodiment, the present invention provides a method of counteracting or overcoming immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

[0036] In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof one or more molecule of the invention. In various aspects, the subject is administered one or more molecule of the invention in combination with another anticancer therapy. In one aspect, the anticancer therapy includes a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent or cytotoxic agent. In another aspect, the anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

[0037] In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof an antibody that targets and depletes CD4+ regulatory T cells (Tregs) in combination with another cytotoxic anticancer therapy. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In various aspects, the cytotoxic anticancer therapy includes a chemotherapeutic molecule, tumor-targeted antibody, small molecule kinase inhibitor, hormonal agent or tumor-targeted cytotoxic agent. In another

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

[0038] In another embodiment, the subject is administered one or more molecule of the invention in combination with any vaccine. In another aspect, the vaccine includes a tumor antigen, tumor-associated antigen, tumor epitope, tumor antigen-containing fusion protein, tumor cell, or dendritic cell. In another aspect, the vaccine includes a pathogen antigen, pathogen-associated antigen, pathogen epitope, or pathogen antigen-containing fusion protein.

[0039] In another embodiment, the present invention provides a method for treating immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treatment of a neoplastic disease. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

[0040] In another embodiment, the present invention provides a method of inducing or promoting immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

[0041] In another embodiment, the present invention provides a method of preventing or treating an autoimmune or inflammatory disease including administering to a subject in need thereof one or more molecule of the invention. In one aspect, the subject is administered one or more molecule of the invention in combination with another anti-inflammatory or immunosuppressive therapy. In another embodiment, the present invention provides a method of treatment of immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treating an autoimmune or inflammatory disease or preventing rejection of grafted cells or tissue. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] Figures 1A-1C show exemplary amino acid sequences of transforming growth factor beta receptor type II (TGF-β-RII) or TGF-β-RIIB or a fragment thereof, including (i) Transforming growth factor beta receptor type II (TGF-β-RII) (SEQ ID NO: 79); and (ii) Transforming growth factor beta receptor type IIB (TGF-β-RIIB) (SEQ ID NO: 80).

[0043] Also shown in Figures 1A-1C are exemplary truncated mutants of Transforming growth factor beta Receptor II (TGF-β-RII) or TGF-β-RIIB including the Extracellular domain (ECD) region that binds TGF-β, including (i) TGF-β R-II (ΔC terminus): TGFβ RII lacking the last 38 amino acids from the C-terminus (SEQ ID NO: 81) and TGF-β R-IIB (ΔC terminus): TGFβ RIIB lacking the last 38 aa from the C-terminus (SEQ ID NO: 82); (ii) TGF-βR-II (Δcyt): TGFβRII lacking the kinase domain & juxtamembrane region (SEQ ID NO: 83) and TGF-βR-IIB (Δcyt): TGFβRIIB lacking the kinase domain & juxtamembrane region (SEQ ID NO: 84); (iii) TGF-β R-II containing the N-terminus region including the extracellular domain (SEQ ID NO: 85) and TGF-β R-IIB containing the N-terminus region including the extracellular domain (SEQ ID NO: 86); (iv) TGF-β R-II containing the extracellular domain that binds TGF-β (SEQ ID NO: 87) and TGF-β R-IIB containing the extracellular domain that binds TGF-β (SEQ ID NO: 88); and (v) TGF-β R-II containing the region of the extracellular domain that binds TGF-β (SEQ ID NO: 89).

[0044] In addition, Figures 1A-1C also show exemplary kinase-deficient mutants, deletion mutants, or point mutants of Transforming growth factor beta Receptor II (TGFβ-RII) or TGFβ-RIIB or a fragment thereof which binds TGF-β, including (i) Transforming growth factor beta Receptor II containing point mutations - amino acid sequence of TGF-β R-II (K277R) contains a point mutation in its ATP-binding site and is inactive as a kinase (SEQ ID NO: 90); and (ii) Transforming growth factor beta Receptor II containing deletions in the amino acid sequence (deletion mutants) - Transforming growth factor beta Receptor II (Δi) - TGF-β R-II (Δi2) contains a deletion of amino acids 498 to 508 and is inactive as a kinase (SEQ ID NO: 91).

[0045] Figure 2 shows exemplary fusion proteins including anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD),

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including anti-HER2/neu heavy chain + TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 1) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

[0046] Figure 3 shows exemplary fusion proteins including anti-EGFR1 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-EGFR1 heavy chain + TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 2) and anti-EGFR1 light chain amino acid sequence (SEQ ID NO: 71).

[0047] Figure 4 shows exemplary fusion proteins including anti-CD20 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-CD20 heavy chain+ TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 3) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0048] Figure 5 shows exemplary fusion proteins including anti-VEGF antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-VEGF heavy chain+ TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 4) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

[0049] Figure 6 shows exemplary fusion proteins including anti-human CTLA-4 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including Anti-CTLA-4 heavy chain + TGFβ-RII Extracellular domain fusion amino acid sequence (SEQ ID NO: 5) and Anti-CTLA-4 light chain (SEQ ID NO: 74).

[0050] Figure 7 shows exemplary fusion proteins including IL-2, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including IL-2 + Fc + TGFβ-RII Extracellular domain (SEQ ID NO: 6) and TGFβ-RII Extracellular domain + Fc + IL-2 (SEQ ID NO: 7). The linker GGGGSGGGGSGGGGS (SEQ ID NO: 104) is optional and can be replaced with EPKSCDK (SEQ ID NO: 105) or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0051] Figures 8A-8B show exemplary fusion proteins including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD),

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including anti-CD25 (Daclizumab) heavy chain and TGFβ-RII Extracellular domain (SEQ ID NO: 8) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 8A); and anti-CD25 (Basiliximab) heavy chain and TGFβ-RII Extracellular domain (SEQ ID NO: 9) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76).

[0052] Figure 9 shows exemplary fusion proteins including anti-CD4 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-CD4 heavy chain and TGFβ-RII Extracellular domain (SEQ ID NO: 10) and anti-CD4 light chain (SEQ ID NO: 77).

[0053] Figure 10 shows exemplary fusion proteins including Programmed Death-1 (PD-1) Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ectodomain), including PD-1 ectodomain + Fc + TGFβRII ectodomain (SEQ ID NO: 11) and TGFβRII ectodomain + Fc + PD-1 ectodomain (SEQ ID NO: 12). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

[0054] Figure 11 shows exemplary fusion proteins including Receptor activator of nuclear factor-kB (RANK) Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ectodomain), including RANK ectodomain + Fc + TGFβRII ectodomain (SEQ ID NO: 13) and TGFβRII ectodomain + Fc + RANK ectodomain (SEQ ID NO: 14). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

[0055] Figure 12 shows exemplary immunomodulatory moiety that binds Programmed Death-1 ligand 1 (PD-L1 or B7-H1) or Programmed Death-1 ligand 2 (PD-L2 or B7-DC), including full-length PD-1 or fragment thereof (SEQ ID NO: 92), PD-1 extracellular domain (ectodomain) or fragment thereof (SEQ ID NO: 93), and PD-1 extracellular domain (ectodomain) ligand-binding region (SEQ ID NO: 94).

[0056] Figure 13 shows exemplary fusion proteins including anti-HER2/neu antibody and PD-1 Ectodomain, including anti-HER2/neu heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 15) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

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[0057] Figure 14 shows exemplary fusion proteins including anti-EGFR1 antibody and PD-1 Ectodomain, including anti-EGFR heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 16) and anti-EGFR light chain amino acid sequence (SEQ ID NO: 71).

[0058] Figure 15 shows exemplary fusion proteins including anti-CD20 antibody and PD-1 Ectodomain, including anti-CD20 heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 17) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0059] Figure 16 shows exemplary fusion proteins including anti-VEGF antibody and PD-1 Ectodomain, including anti-VEGF heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 18) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

[0060] Figure 17 shows exemplary fusion proteins including anti-human CTLA-4 antibody and PD-1 Ectodomain, including anti-CTLA-4 heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 19) and anti-CTLA-4 light chain (SEQ ID NO: 74).

[0061] Figures 18A-18B show exemplary fusion proteins including anti-CD25 antibody and PD-1 Ectodomain, including anti-CD25 (Daclizumab) heavy chain and PD-1 ectodomain (SEQ ID NO: 20) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 18A), and anti-CD25 (Basiliximab) heavy chain and PD-1 ectodomain (SEQ ID NO: 21) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 18B).

[0062] Figure 19 shows exemplary fusion proteins including IL-2, Fc, and PD-1 ectodomain, including IL-2 + Fc + PD-1 ectodomain (SEQ ID NO: 22) and PD-1 ectodomain + Fc + IL-2 (SEQ ID NO: 23). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0063] Figure 20 shows exemplary fusion proteins including anti-CD4 antibody and PD-1 ectodomain, including anti-CD4 heavy chain and PD-1 ectodomain (SEQ ID NO: 24) and anti-CD4 light chain (SEQ ID NO: 77).

[0064] Figure 21 shows exemplary fusion proteins including Receptor activator of nuclear factor-kB (RANK) Ectodomain, Fc, and PD-1 ectodomain, including RANK ectodomain + Fc + PD-1 ectodomain (SEQ ID NO: 25) and PD-1 ectodomain + Fc + RANK ectodomain (SEQ ID NO: 26). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

[0065] Figure 22 shows exemplary immunomodulatory moiety that binds Receptor activator of nuclear factor-kB (RANK) ligand (RANKL) including full-length RANK or fragment thereof (SEQ ID NO: 95), extracellular ligand-binding domain or ectodomain of RANK (SEQ ID NO: 96), RANKL-binding sequences or residues of RANK (SEQ ID NO: 93), or RANKL-binding sequences of Osteoprotegerin (OPG) (SEQ ID NO: 98).

[0066] Figure 23 shows exemplary fusion proteins including anti-HER2/neu antibody and RANK Ectodomain, including anti-HER2/neu heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 27) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

[0067] Figure 24 shows exemplary fusion proteins including anti-EGFR1 antibody and RANK Ectodomain, including anti-EGFR heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 28) and anti-EGFR light chain amino acid sequence (SEQ ID NO: 71).

[0068] Figure 25 shows exemplary fusion proteins including anti-CD20 antibody and RANK Ectodomain, including anti-CD20 heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 29) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0069] Figure 26 shows exemplary fusion proteins including anti-VEGF antibody and RANK Ectodomain, including anti-VEGF heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 30) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

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[0070] Figure 27 shows exemplary fusion proteins including anti-human CTLA-4 antibody and RANK Ectodomain, including anti-CTLA-4 heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 31) and anti-CTLA-4 light chain (SEQ ID NO: 74).

[0071] Figures 28A-28B show exemplary fusion proteins including anti-CD25 antibody and RANK Ectodomain, including anti-CD25 (Daclizumab) heavy chain and RANK ectodomain (SEQ ID NO: 32) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 28A), and anti-CD25 (Basiliximab) heavy chain and RANK ectodomain (SEQ ID NO: 33) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 28B).

[0072] Figure 29 shows exemplary fusion proteins including IL-2, Fc, and RANK ectodomain, including IL-2 + Fc + RANK ectodomain (SEQ ID NO: 34) and RANK ectodomain + Fc + IL-2 (SEQ ID NO: 35). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0073] Figure 30 shows exemplary fusion proteins including anti-CD4 antibody and RANK ectodomain, including anti-CD4 heavy chain and RANK ectodomain (SEQ ID NO: 36) and anti-CD4 light chain (SEQ ID NO: 77).

[0074] Figure 31 shows exemplary immunomodulatory moiety that binds Programmed Death-1 (PD-1) including a PD-1 ligand 1 (PD-L1 or B7-H1) or PD-1 ligand 2 (PD-L2 or B7-DC) or a fragment thereof (for example, SEQ ID NO: 101), full-length human PD-1 ligand 1 (B7-H1; PDCD1L1; PD-L1; or CD274) protein or a fragment thereof (SEQ ID NO: 99), and PD-L1 extracellular binding domain (ectodomain) or fragment thereof (SEQ ID NO: 100).

[0075] Figure 32 shows exemplary fusion proteins including anti-tumor necrosis factor (TNFα) antibody and PD-1 ligand, including anti-TNFα heavy chain + PD-1L (SEQ ID NO: 37) and anti-TNFα light chain (SEQ ID NO: 78). The sequence KKAE (SEQ ID NO: 107) can be replaced with KRVE (SEQ ID NO: 108) or KKVE (SEQ ID NO: 109).

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[0076] Figure 33 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand, including TNFR2 ECD + IgG Cγ1 + PD-L1 (SEQ ID NO: 38) and PD-L1 + IgG Cγ1 -TNFR2 ECD (SEQ ID NO: 39).

[0077] Figure 34 shows exemplary fusion proteins including anti-CD20 antibody and PD-1 ligand 1 (PD-L1), including anti-CD20 heavy chain + PD-L1 sequence (SEQ ID NO: 40) and anti-CD20 light chain sequence (SEQ ID NO: 72).

[0078] Figures 35A-35B show exemplary fusion proteins including anti-CD25 antibody and PD-1 ligand 1 (PD-L1), including anti-CD25 (Daclizumab) heavy chain and PD-L1 (SEQ ID NO: 41) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 35A), and anti-CD25 (Basiliximab) heavy chain and PD-1 ectodomain (SEQ ID NO: 42) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 35B).

[0079] Figure 36 shows exemplary fusion proteins including IL-2, Fc, and PD-1 ligand 1 (PD-L1), including fusion protein hPD-1 ligand 1 + Fc + IL-2 (SEQ ID NO: 43) and fusion protein IL-2 + Fc + PD-1 ligand 1 (SEQ ID NO: 44). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0080] Figure 37 shows exemplary fusion proteins including anti-CD4 antibody and PD-1 ligand 1 (PD-L1), including anti-CD4 heavy chain and PD-1 ligand 1 (PD-L1) (SEQ ID NO: 45) and anti-CD4 light chain (SEQ ID NO: 77).

[0081] Figure 38 shows exemplary fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from PD-1 ligand (PD-L1) including Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + PD-L1 (SEQ ID NO: 46) and PD-1L1 + IgG Cγ1 + CTLA-4 ECD (SEQ ID NO: 47). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

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[0082] Figure 39 shows exemplary fusion proteins including a sequence of transforming growth factor-β (TGF-β), Immunoglobulin Fc (IgG Cγ1), and a sequence of PD-1 ligand (PD-L1) including TGFβ-1 + Fc + PD-L1 (SEQ ID NO: 48), and PD-1L1 + Fc + TGFβ-1 (SEQ ID NO: 49). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0083] Figure 40 shows exemplary immunomodulatory moiety that binds Transforming growth factor-beta receptor (TGF-βR) including Transforming growth factor-beta (TGF-β1, TGF-β2, or TGF-β3 or a fragment thereof, TGF-β1 full sequence (SEQ ID NO: 102), and mature (active) TGF-β1 sequence (Ala 279 – Ser 390; 112 amino acids) (SEQ ID NO: 103).

[0084] Figure 41 shows exemplary fusion proteins including an antibody that binds TNF-α, and a sequence of transforming growth factor-β (TGF-β), including anti-TNFα heavy chain + TGF-β1 (SEQ ID NO: 50) and anti-TNFα light chain (SEQ ID NO: 78). The sequence KKAE (SEQ ID NO: 107) can be replaced with KRVE (SEQ ID NO: 108) or KKVE (SEQ ID NO: 109).

[0085] Figure 42 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain (TNFR2 ECD), immunoglobulin Fc (IgG Cγ1), and a sequence from transforming growth factor-β (TGF-β) including TNFR2 ECD + IgG Cγ1 + TGF-β1 (SEQ ID NO: 51), and TGF-β1 + IgG Cγ1 + TNFR2 ECD (SEQ ID NO: 52).

[0086] Figure 43 shows exemplary fusion proteins including anti-CD20 antibody and a sequence from transforming growth factor-β (TGF-β), including anti-CD20 heavy chain + mature TGFβ1 sequence (SEQ ID NO: 53) and anti-CD20 light chain sequence (SEQ ID NO: 72).

[0087] Figures 44A-44B show exemplary fusion proteins including anti-CD25 antibody and a sequence from transforming growth factor-β (TGF-β), including anti-CD25 (Daclizumab) heavy chain and TGF-β1 (SEQ ID NO: 54) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 44A), and anti-CD25 (Basiliximab) heavy chain and

TGF-β1 (SEQ ID NO: 55) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 44B).

[0088] Figure 45 shows exemplary fusion proteins including IL-2, Fc, and a sequence from transforming growth factor-β (TGF-β), including TGF-β1 + Fc + IL-2 (SEQ ID NO: 56) and IL-2 + Fc + TGF-β1 (SEQ ID NO: 57). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0089] Figure 46 shows exemplary fusion proteins including anti-CD4 antibody and a sequence from transforming growth factor-β (TGF-β), including anti-CD4 heavy chain and TGF-β (SEQ ID NO: 58) and anti-CD4 light chain (SEQ ID NO: 77).

[0090] Figure 47 shows exemplary Fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from transforming growth factor-β (TGF-β) including Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + TGF-β1 (SEQ ID NO: 59), and TGF-β1 + IgG Cγ1 + CTLA-4 ECD (SEQ ID NO: 60). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

[0091] Figure 48 shows exemplary fusion proteins including an antibody that binds TNF-α, and a sequence of RANK ectodomain, including anti-TNFα heavy chain + RANK ectodomain (SEQ ID NO: 61) and anti-TNFα light chain (SEQ ID NO: 78). The sequence KKAE (SEQ ID NO: 107) can be replaced with KRVE (SEQ ID NO: 108) or KKVE (SEQ ID NO: 109).

[0092] Figure 49 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain (TNFR2 ECD), immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including TNFR2 ECD + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 62), and RANK ectodomain + IgG Cγ1 + TNFR2 ECD (SEQ ID NO: 63).

[0093] Figure 50 shows exemplary Fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 64), and RANK ectodomain + IgG Cγ1 + CTLA-4 ECD (SEQ ID NO: 65). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

[0094] Figure 51 shows exemplary fusion proteins including a sequence from transforming growth factor-β (TGF-β), immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including TGF-β + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 66), and RANK ectodomain + IgG Cγ1 + TGF-β (SEQ ID NO: 67).

[0095] Figure 52 shows exemplary fusion proteins including a sequence from PD-1 ligand (PD-L1), immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including PD-L1 + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 68), and RANK ectodomain + IgG Cγ1 + PD-L1 (SEQ ID NO: 69).

[0096] Figures 53A-53G show Regulatory T cells (Treg) accumulate in the tumor microenvironment and counteract the ability of chemotherapy to activate $CD8^+$ T cell-mediated antitumor immunity. (FIG. 53A) Surface exposure of calreticulin (CRT) in response to treatment of hum[a]n (SW780) and murine (MB49) cancer cells with doxorubicin (10 μM) for 4h. The surface exposure of CRT was determined by immunofluorescence cytometry of untreated control or doxorubicin-treated cells stained with Dylight-labeled anti-CRT antibody or an isotype control (IgG1) antibody. (FIG. 53B) Priming of tumor-reactive immune responses by MB49 tumor cells treated with doxorubicin *ex vivo* or *in vivo*. $5x10^6$ MB49 cells that were pre-treated *ex vivo* with doxorubicin (10 μM) for 4h were injected into one flank of syngeneic immunocompetent C57BL/6 mice. Alternatively, C57BL/6 mice were injected with $5x10^5$ live MB49 tumor cells and then administered intratumoral doxorubicin (10 μg) at 10d following tumor inoculatio[n.] Tumor-reactive immune responses were determined by measuring production of IFN-γ by draining lymph node (DLN) cells in response to *in vitro* re-challenge with either MB49 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone.

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(FIG. 53C) Vaccination with doxorubicin-treated tumor cells induces $CD8^+$ T cell-mediated antitumor immunity that prevents tumor formation following re-challenge with live tumor cells. MB49 cells ($5x10^6$) that were pre-treated *in vitro* with doxorubicin (10 μM) for 4h were injected subcutaneously into one flank of syngeneic immunocompetent C57BL/6 mice. Naïve or vaccinated mice were challenged with untreated live MB49 tumor cells injected into the opposite flank with or without pre-treatment with an anti-CD8 antibody (Clone GK2.43)(5 μg x 2 doses, iv) to deplete $CD8^+$ T cells. (FIG. 53D) Delayed administration of chemotherapy in mice with pre-established tumors decreases its immunogenicity and antitumor efficacy. C57BL/6 mice were injected with $5x10^5$ live syngeneic MB49 tumor cells and then administered intratumoral doxorubicin (10 μg) at d3, d7, or d10 following tumor inoculation. (FIG. 53E) Tumors foster the accumulation of $CD4^+CD25^+FoxP3^+$ cells (Tregs) in their microenvironment. Flow cytometric analyses of the percentage of $CD4^+CD25^+Fox^P3^+$ cells (Tregs) among $CD4^+$ T lymphocytes isolated from the spleen, draining lymph nodes (DLN), and tumors of immunocompetent C57BL/6 mice at d0 and d14 after subcutaneous inoculation of $5x10^5$ live MB49 tumor cells. (FIG. 53F) Tregs infiltrating the tumor microenvironment suppress priming of tumor-reactive immune responses by doxorubicin-treated tumor cells. Naïve C57BL/6 mice were vaccinated with $5x10^6$ doxorubicin-killed MB49 cells with or without intravenous adoptive transfer of $5x10^6$ $CD4^+CD25^+$ cells isolated from tumors and DLN of tumor-bearing mice via immunomagnetic separation. Tumor-reactive immune responses were determined by measuring production of IFN-γ by draining lymph node (DLN) cells in response to *in vitro* re-challenge with either MB49 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone. (FIG. 53G) Tregs infiltrating the tumor microenvironment suppress the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death. Naïve C57BL/6 mice were vaccinated with $5x10^6$ doxorubicin-killed MB49 cells (left flank) with or without pre-treatment with either an anti-CD8 antibody (Clone GK2.43)(5 μg x 2 doses, iv) to deplete $CD8^+$ T cells or adoptive transfer of $5x10^6$ $CD4^+CD25^+$ cells isolated from tumors and DLN of tumor-bearing mice. Protective antitumor immunity in vaccinated mice was determined by assessment of tumor growth upon challenge with untreated live MB49 tumor cells injected into the opposite flank.

[0097] Figures 54A-54F show inhibition of TGF-β in the tumor microenvironment reduces 'adaptive' FoxP3$^+$ regulatory T cells and enhances the antitumor efficacy chemotherapy. (FIG. 54A) Tumor growth results in a progressive increase in the level of serum TGF-β. Levels of TGF-β in serum of mice at d0, d14, and d28 following inoculation of $5x10^5$ live MB49 tumor cells were evaluated utilizing ELISA. (FIG. 54B) Tumor cell-autonomous expression of TGF-β is the dominant source of elevated TGF-β in tumor-bearing mice. Tumor cells or draining lymph node cells isolated from either tumor-bearing mice or their tumor-free counterparts were cultured *ex vivo* in serum-free medium for 24h and the amount of TGF-β/10^6 cells in supernatants was measured by ELISA. (FIG. 54C) TGFβRII:Fc sequesters TGF-β in supernatants of MB49 tumor cells in a concentration-dependent manner. MB49 tumor cells were cultured in the presence of graded concentrations of TGFβRII:Fc (0-400 ng/ml) for 24h followed by measurement of TGF-β (pg/ml/10^6 cells) in supernatants via ELISA. (FIG. 54D) TGF-β induces 'adaptive' FoxP3$^+$ regulatory T cells in the tumor microenvironment. At 5d following inoculation of MB49 tumor cells, mice were either left untreated (control) or treated with TGFβRII:Fc (1μg intratumoral; twice weekly) for 3 weeks followed by flow cytometric analyses of intracellular FoxP3 expression in CD4$^+$CD25$^+$ T cells infiltrating the tumors. (FIGS. 54E, 54F). Sequestration of intratumoral TGF-β with TGFβRII:Fc reduces CD4$^+$CD25$^+$FoxP3$^+$ Tregs in tumor tissue and improves the antitumor efficacy of doxorubicin. MB49 tumor-bearing mice were administered doxorubicin (5mg/kg i.p. weekly x 3) with or without twice weekly treatment with TGFβRII:Fc (1μg intratumoral). The percentage of CD4$^+$CD25$^+$FoxP3$^+$ cells (Tregs) among tumor cells was assessed by flow cytometry (FIG. 54E), and tumor volume was monitored to determine the effect of counteracting tumor-induced TGF-β-mediated immune tolerance on the *in vivo* antitumor efficacy of doxorubicin (FIG. 54F).

[0098] Figures 55A-55D show that anti-CD4 antibody-mediated depletion of CD4$^+$ regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive CD8$^+$ T cells and enhances the antitumor efficacy of chemotherapy. (FIG. 55A) *In vivo* depletion of tumor-infiltrating CD4$^+$CD25$^+$FoxP3$^+$ T cells by treatment of tumor-bearing mice with anti-CD4 antibody. C57BL/6 mice injected with 5 $x10^5$ MB49 tumor cells s.c. were left untreated (control) or administered an anti-CD4 antibody (Clone GK1.5) i.p. at 5d and 9d

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following tumor challenge. $CD4^+CD25^+FoxP3^+$ T cells infiltrating tumors isolated from mice at d16 following tumor challenge were detected by flow cytometry. (FIG. 55B) Target-specific depletion of either $CD4^+$ T cells, $CD4^+CD25^+FoxP3^+$ T cells, or $CD8^+$ T cells by treatment of tumor-bearing mice with anti-CD4 antibody or anti-CD8 antibody. C57BL/6 mice injected s.c. with 5 $x10^5$ MB49 tumor cells were left untreated or treated with doxorubicin (5mg/kg i.p. weekly x 3) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Flow cytometric analyses of peripheral blood mononuclear cells isolated from mice at d16 following tumor challenge determined the percentage of $CD4^+$ T cells or $CD8^+$ T cells among total mononuclear cells, and the percentage of $CD4^+CD25^+FoxP3^+$ T cells among total $CD4^+$ T cells. (FIG. 55C) Depletion of $CD4^+$ regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive $CD8^+$ T cells. C57BL/6 mice injected s.c. with 5 $x10^5$ MB49 tumor cells were left untreated or treated with doxorubicin (5mg/kg i.p. weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of IFN-γ expression in $CD8^+$ T cells from the tumor and draining lymph node in response to *in vitro* stimulation with MB49 cell lysates. (FIG. 55D) Depletion of $CD4^+$ regulatory T cells augments the *in vivo* antitumor efficacy of chemotherapy via activation of tumor-reactive $CD8^+$ T cells. C57BL/6 mice injected s.c. with 5 $x10^5$ MB49 tumor cells were left untreated or treated with doxorubicin (5mg/kg i.p. weekly x 3) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting either $CD4^+$ T cells or $CD8^+$ T cells on the *in vivo* antitumor efficacy of doxorubicin.

[0099] Figures 56A-56F show anti-CD4 antibody-mediated depletion of $CD4^+$ regulatory T cells augments and sustains the antitumor effect of chemotherapy by enabling activation of adaptive antitumor immunity. (FIG. 56A) Surface exposure of calreticulin (CRT) in response to treatment of MB49 cancer cells with either cisplatin or the combination of cisplatin and gemcitabine for 4h. The surface exposure of CRT was

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determined by immunofluorescence cytometry of untreated control or chemotherapy-treated cells stained with Dylight-labeled anti-CRT antibody or an isotype control (IgG1) antibody. (FIGS. 56B, 56C) Depletion of $CD4^+$ regulatory T cells enables cisplatin-induced activation of tumor-reactive IFN-γ^+CD8^+ T cells and effector memory ($CD8^+CD62L^-$) T cells. C57BL/6 mice injected s.c. with 5 $x10^5$ MB49 tumor cells were left untreated or treated with cisplatin (0.5mg/kg i.p. weekly x 4) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of IFN-γ expression in $CD8^+$ T cells from the tumor and draining lymph node (DLN) in response to *in vitro* stimulation with MB49 cell lysates (FIG. 56B). The percentage of effector memory T_{EM} cells was determined by flow cytometric analyses of $CD8^+CD62L^-$ cells (FIG. 56C). (FIGS. 56D, 56E and 56F) Depletion of $CD4^+$ regulatory T cells augments the *in vivo* antitumor efficacy of chemotherapy via activation of tumor-reactive $CD8^+$ T cells. C57BL/6 mice injected s.c. with 5 $x10^5$ MB49 tumor cells were left untreated or treated with either cisplatin (0.5mg/kg) or the combination of cisplatin and gemcitabine (i.p. weekly x 4) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting either $CD4^+$ T cells or $CD8^+$ T cells on the *in vivo* antitumor efficacy of chemotherapy and the percentage of mice exhibiting complete tumor-regression by d50 following tumor inoculation. Establishment of adaptive antitumor immunity following regression of primary tumors was determined by re-challenging mice with live MB49 tumor cells in the opposite flank.

[0100] Figures 57A-57H show that Chemotherapy-induced expression of NKG2D ligands on tumor cells cooperates with depletion of $CD4^+$ regulatory T cells to stimulate $CD8^+$ T cell-mediated tumor regression. (FIG. 57A) Genotoxic chemotherapeutic agents induce expression of mouse NKG2D ligands (Rae-1) on cancer cells. Kinetics of the upregulation of Rae1 transcripts in mouse CT26 colon cancer cells was determined by quantitative real-time PCR following treatment with irinotecan (25 μg/ml) or oxaliplatin (10 μg/ml). Quantitative RT-PCR was carried out using Rae-1 specific primers [sense, 5'-

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CTAGTGCCACCTGGGAATTCA-3' (SEQ ID NO: 111); anti-sense, 5'-CATCATTAGCTGATCTCCAGCTCA-3'(SEQ ID NO: 112)] and probe [5'-6-FAM-CATCAGTGACAGTTACTTCTTCACCTTCTACACAGAGA-Tamra-3' (SEQ ID NO: 113)]. (FIG. 57B) Genotoxic chemotherapeutic agents induce p53-independent cell surface expression of human NKG2D ligands (MHC-I-related A and B molecules – MICA/MICB) on cancer cells. Isogenic p53-proficient ($p53^{+/+}$) or p53-deficient ($p53^{-/-}$) HCT116 cells were treated with irinotecan (25 μg/ml) for 16h or left untreated. Irinotecan-induced upregulation of cell surface expression of MICA/B was determined by flow cytometryic analysis of tumor cells labeled with an anti-human MICA/B MAb (R&D Systems). (FIG. 57C) and (FIG. 57D) Induction of NKG2D ligands contributes to the antitumor effect of chemotherapy *in vivo*. Immunocompetent Balb/C mice injected s.c. with 2 $x10^5$ syngeneic CT26 tumor cells were treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d5 following tumor inoculation, with or without pre-treatment with an NKG2D blocking antibody (CX5, eBIOscience) (200 μg i.p.) at 16h before each dose of chemotherapy. Tumor volume was monitored to determine the effect of NKG2D blockade on the *in vivo* antitumor efficacy of irinotecan. (FIG. 57E) *In vivo* depletion of $CD4^+CD25^+FoxP3^+$ T cells by treatment of tumor-bearing mice with anti-CD4 antibody. Balb/C mice injected with 2 $x10^5$ CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. $CD4^+CD25^+FoxP3^+$ T cells in spleen and draining lymph node isolated from mice at d16 following tumor challenge were detected by flow cytometry. (FIG. 57F) Depletion of $CD4^+$ regulatory T cells facilitates irinotecan-induced activation of tumor-reactive IFN-γ^+CD8^+ T cells. Balb/C mice injected with 2 $x10^5$ CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of IFN-γ expression in $CD8^+$ T cells from the tumor and draining lymph node (DLN) in response to *in vitro* stimulation with either CT26 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone. (FIG. 57G) and (FIG. 57H) Chemotherapy-induced expression of NKG2D ligands on

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tumor cells cooperates with depletion of $CD4^+$ regulatory T cells to stimulate $CD8^+$ T cell-mediated tumor regression. Balb/C mice injected with 2 $x10^5$ CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) and/or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting $CD4^+$ T cells and/or $CD8^+$ T cells on the *in vivo* antitumor efficacy of irinotecan.

DETAILED DESCRIPTION OF THE INVENTION

[0101] Targeted immunostimulatory antibodies and/or fusion proteins for prevention or treatment of cancer: Chemotherapy is a cornerstone of systemic treatment of patients with most common types of advanced cancers. The vast majority of human cancers harbor genetic alterations and signaling mechanisms that impair the direct death signaling pathways entrained by chemotherapeutic agents. Although chemotherapeutic agents employ diverse mechanisms to directly kill tumor cells, the present invention provides that these agents have immuno-adjuvant effects which activate innate and adaptive antitumor immune responses that are crucial for their antitumor efficacy *in vivo*. The present invention also provides that antitumor $CD8^+$ T cells play an instrumental role in the *in vivo* response of tumors to diverse cytotoxic chemotherapeutic agents. Although chemotherapeutic agents can induce "immunogenic" tumor cell death and facilitate cross-presentation of antigens by dendritic cells, tumors create a tolerogenic environment that allows them to suppress the activation of innate and adaptive immune responses and evade immunologic attack by immune effector cells. The present invention provides that strategies to counteract tumor-induced immune tolerance in the tumor microenvironment can enhance the antitumor efficacy of chemotherapy by activating and leveraging T cell-mediated adaptive antitumor immunity against disseminated cancer cells.

[0102] The present invention is based on the seminal discovery that targeted immunomodulatory antibodies and fusion proteins can counteract or reverse immune tolerance of cancer cells. Cancer cells are able to escape elimination by chemotherapeutic agents or tumor-targeted antibodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. By counteracting tumor-induced immune tolerance, the present invention

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provides effective compositions and methods for cancer treatment, optional in combination with another existing cancer treatment.

[0103] The present invention provides compositions and methods for targeted immunostimulatory antibodies and fusion proteins that counteract immune tolerance in the tumor microenvironment and promote T cell-mediated adaptive antitumor immunity for maintenance of durable long-term protection against recurrent or disseminated cancers. These tumor-targeted immunostimulatory molecules are designed to facilitate effective long term T cell-mediated immune responses against tumor cells by at least one of the following:

(i) promoting death of tumor cells via enhancement of antibody-dependent cellular cytotoxicity (ADCC);

(ii) facilitating effective cross-presentation of tumor antigen(s) from dying tumor cells by augmenting maturation of dendritic cells (DCs); and

(iii) increasing activation and proliferation of antitumor CD8+ T cells by negating immune suppression mediated by regulatory T cells and myeloid suppressor cells. These antitumor immune responses may be activated in tandem with the sensitization of tumor cells to immune effector-mediated cytotoxicity, thereby establishing a positive feedback loop that augments tumor cytoreduction and reinforces adaptive antitumor immunity. The tumor-targeted immunostimulatory monoclonal antibodies (mAbs) of the present invention provides the ability to generate and boost antitumor immunity to multiple cross-presented tumor antigens obtained from endogenous tumor cells during the course of therapy (as an *in situ* tumor vaccine), while simultaneously leveraging the antitumor immune response to eliminate disseminated cancer cells. Accordingly, the targeted immunostimulatory antibodies and fusion proteins of the invention can integrate the hitherto distinct fields of passive and active immunotherapy and provide a novel platform for simultaneously leveraging the synergistic benefits of these strategies to entrain effective innate and adaptive immune responses against targeted cancers.

[0104] 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

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cells has remained challenging. Several tumor-specific and tumor-associated antigens have been identified, yet tumors employ diverse mechanisms to create a tolerogenic environment that allows them to suppress the activation of a T cell-mediated antitumor immune response. The tumor-targeted immunostimulatory antibodies and/or fusion proteins of the invention are designed to overcome such immune tolerance in the tumor microenvironment and activate robust levels of T cell responses for effective cancer immunotherapy or chemo-immunotherapy. Accordingly, the tumor-targeted immunostimulatory antibodies and/or fusion proteins of the invention have broad clinical relevance for advancing the treatment of many types of human cancers.

[0105] The tumor-targeted immunostimulatory mAbs and/or fusion proteins of the invention provide their ability to generate and boost antitumor immunity to multiple cross-presented tumor antigens obtained from endogenous tumor cells during the course of therapy (as an *in situ* tumor vaccine), while simultaneously leveraging the antitumor immune response to eliminate disseminated cancer cells. Accordingly, the tumor-targeted immunostimulatory antibodies and/or fusion proteins of the invention can integrate the hitherto distinct fields of passive and active immunotherapy and provide a novel platform for simultaneously leveraging the synergistic benefits of these strategies to entrain effective innate and adaptive immune responses against targeted cancers. This approach of the present invention is distinguished from and superior to conventional tumor antigen-, allogeneic tumor cell- or DC-based vaccines in at least one of the following aspects: (i) There is no *a priori* requirement to define, clone and purify individual tumor antigens, since the patient's tumor itself is the *in vivo* source of antigens; (ii) Multivalent antitumor immune responses that are naturally tailored against antigens from the patient's own tumor are less likely to allow immune escape than a pre-selected tumor antigen; (iii) The activation of antitumor immune responses by the immuno-adjuvant effects of tumor-targeted immunostimulatory antibodies or fusion proteins occurs in tandem with the sensitization of tumor cells to immune effector-mediated cytotoxicity, thereby establishing a positive feedback loop that augments tumor cytoreduction and reinforces adaptive antitumor immunity; and (iv) The molecules of the invention have broad clinical relevance for advancing the treatment of many types of human cancers.

[0106] In addition, the targeted immunostimulatory antibodies and/or fusion proteins of the invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) to counteract immune tolerance in the tumor microenvironment and promote T cell-mediated adaptive antitumor immunity for maintenance of long-term protection against recurrent or disseminated cancers (for prevention or treatment of diverse cancers); (ii) to produce immune cell compositions for adoptive cellular therapy of diverse cancers; and (iii) to serve as immune adjuvants or vaccines for prophylaxis of diverse cancers or infectious diseases.

[0107] The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to disrupt immunosuppressive networks in the tumor microenvironment. Tumors employ a wide array of regulatory mechanisms to avoid or suppress the immune response. Cancer cells actively promote immune tolerance in the tumor microenvironment via the expression of cytokines and molecules that inhibit the differentiation and maturation of antigen-presenting dendritic cells. The immunosuppressive cytokines and ligands produced by tumor cells include the following: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed death-1 ligand 1 (PD-L1; B7-H1); (iii) Vascular endothelial growth factor (VEGF); and (iv) Interleukin-10 (IL-10). In addition to blocking dendritic cell (DC) maturation, these molecules promote the development of specialized subsets of immunosuppressive $CD4^+$ T cells (regulatory T cells; Treg cells) and myeloid-derived suppressor cells (MDSC). Tregs are a minority sub-population of $CD4^+$ T cells that constitutively express CD25 [the interleukin-2 (IL-2) receptor α-chain] and the forkhead box P3 (FOXP3) transcription factor. Tregs (CD4+CD25+FoxP3+ cells) maintain immune tolerance by restraining the activation, proliferation, and effector functions of a wide range of immune cells, including $CD4^+$ and $CD8^+$ T cells, natural killer (NK) and NKT cells, B cells and antigen presenting cells (APCs) *in vitro* and *in vivo*. The accumulation of Treg cells in the tumor microenvironment re-inforces tumor immune tolerance and facilitates tumor progression and metastases. The increased expression of immunosuppressive cytokines (TGF-β; PD-L1) and tumor-infiltrating Tregs is correlated with a reduction of survival of patients with diverse types of cancers. The present invention provides that tumor-induced immune tolerance mediated via Tregs is a crucial determinant of the resistance of cancers to

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

[0108] The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to inhibit the development or function of Tregs and myeloid suppressor cells (DCs or MDSC) within the tumor microenvironment. Tregs (CD4+CD25+FoxP3+ cells) express an array of immunosuppressive cytokines and molecules which act in concert to induce immune tolerance and promote tumor progression and metastases. These include: (i) Cytotoxic T-lymphocyte associated protein 4 (CTLA-4; CD152), a co-inhibitory receptor that binds to the ligands CD80 (B7-1) or CD86 (B7-2) on the antigen presenting cell (APC) and inhibits co-stimulation of T cells; (ii) Programmed death-1 ligand 1 (PD-L1; B7-H1), a ligand which engages the co-inhibitory receptor Programmed death-1 (PD-1) and inhibits T cell activation and proliferation. (iii) Transforming growth factor-beta (TGF-β), a cytokine which regulates immune responses by restricting the maturation and antigen-presenting function of dendritic cells, inhibiting the proliferation and activation of naïve T cells, suppressing the expression of cytotoxic molecules (Granzyme A/B, FasL, Apo2L/TRAIL, IFN-γ) in immune effector cells, and promoting the development and function of Tregs; (iv) Receptor activator of nuclear factor-κB ligand (RANKL), a ligand which engages Receptor activator of nuclear factor-κB (RANK) and promotes osteoclast differentiation, Treg development, and tumor metastases. In addition, Tregs express other surface molecules; (v) LAG-3, a CD4-related molecule that binds MHC class II; (vi) glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); and (vii) IL-10. The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to bind a targeted molecule expressed by Tregs or myeloid suppressor cells while concurrently sequestering and inhibiting one or more immunosuppressive molecule that promotes their development, survival or function.

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In one aspect, the targeted immunostimulatory antibodies and/or fusion proteins directly deplete the number of Tregs.

[0109] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule on the tumor cell or tumor microenvironment (tumor stroma, tumor vasculature, or tumor infiltrating immune cell), and the immunomodulatory moiety specifically binds an immunosuppressive molecule expressed by the targeted tumor cell or tumor-infiltrating Treg cells and myeloid suppressor cells (DC or MDSC).

[0110] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule expressed by Treg cells, myeloid suppressor cells (MDSC), or dendritic cells (DC), and the immunomodulatory moiety specifically binds an immunosuppressive molecule that promotes their development, survival or function.

[0111] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule, and the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2); (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); (iv) Vascular endothelial growth factor (VEGF); (v) Transforming growth factor-beta receptor (TGF-βR); (vi) Programmed death-1 (PD-1); and (vii) Receptor activator of nuclear factor-κB (RANK).

[0112] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. In one aspect, the targeting moiety specifically binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), CD20, Vascular endothelial growth factor (VEGF), insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial

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cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, CD30, CD33, or CD40.

[0113] In one aspect, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: (i) CD4; (ii) CD25 (IL-2α receptor; IL-2αR); (iii) cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); (iv) Interleukin-10 (IL-10); (v) Transforming growth factor-beta receptor (TGF-βR); (vi) Transforming growth factor-beta (TGF-β); (vii) Programmed Death-1 (PD-1); (viii) Programmed death-1 ligand (PD-L1 or PD-L2); (ix) Receptor activator of nuclear factor-κB (RANK); (x) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); (xi) LAG-3; or (xii) glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18).

[0114] In one aspect, the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed death-1 ligand (PD-L1 or PD-L2); (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); or (iv) vascular endothelial growth factor (VEGF).

[0115] In one aspect, the immunomodulatory moiety includes a molecule that binds TGF-β. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Transforming growth factor-beta receptor TGF-βRII, TGF-βRIIb, or TGF-βRIII. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes a TGF-β-binding amino acid sequence corresponding to SEQ ID NOs: 79-91. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of TGF-β.

[0116] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the

molecule includes an amino acid sequence corresponding to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

[0117] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Programmed Death-1 (PD-1), Programmed death-1 ligand 1 (PD-L1), or Programmed death-1 ligand 2 (PD-L2). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes PD-1 ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

[0118] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

[0119] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes a PD-L1-binding amino acid sequence corresponding to SEQ ID NO: 92, 93, or 94. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Programmed death-1 ligand 1 (PD-L1).

[0120] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial

growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.

[0121] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Programmed Death-1 (PD-1). In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and PD-1 ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 25 or 26.

[0122] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes a RANKL-binding amino acid sequence corresponding to SEQ ID NO: 95, 96, 97, or 98. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Receptor activator of nuclear factor-κB ligand (RANKL).

[0123] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36.

[0124] The present invention provides novel targeted immunosuppressive antibodies and fusion proteins that induce or promote immune tolerance by at least one of the following:

(i) inhibiting the activation of dendritic cells, T cells, and/or B cells; and

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

[0125] Targeted immunosuppressive antibodies and/or fusion proteins: The aberrant activation of self-reactive T cells and/or breakdown of the mechanisms of immune tolerance promotes the development of autoimmunity that results in various diseases including type I diabetes mellitus, multiple sclerosis, systemic lupus erythematosus, inflammatory bowel disease, and rheumatoid arthritis. The targeted immunosuppressive antibodies and/or fusion proteins of the invention are designed to suppress unwanted or excessive immune or inflammatory responses and restore or promote immune tolerance. Accordingly, the compositions and methods of the invention have broad clinical relevance for the treatment of diverse autoimmune or inflammatory diseases and preventing the rejection of a transplanted cell, tissue, or organ grafts.

[0126] The targeted immunosuppressive antibodies and/or fusion proteins of the invention provides their ability to inhibit the activity of targeted pro-inflammatory cytokines or immune cells while simultaneously promoting immune tolerance via the targeted delivery of immunosuppressive molecules that facilitate the development and/or function of regulatory T cells. These molecules of the present invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) The molecules of the invention enable targeted delivery of immunosuppressive molecules to immune cells or pro-inflammatory molecules in the milieu of the affected cell, tissue or organ; (ii) The molecules of the invention can couple the inhibition of the targeted pro-inflammatory molecule or immune cell with the

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

[0127] Further, the targeted immunosuppressive antibodies and/or fusion proteins of the invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) To suppress unwanted or excessive immune or inflammatory responses in order to treat autoimmune or inflammatory diseases; and (ii) To prevent the rejection of a transplanted cell, tissue, or organ grafts.

[0128] In one aspect, the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes a PD-1-binding amino acid sequence corresponding to SEQ ID NO: 99, 100, or 101. In an additional aspect, the immunomodulatory moiety increases the function of PD-1.

[0129] In one aspect, the targeting moiety specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 37. In another aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2), and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from PD-L1. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 38 or 39.

[0130] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25, or CD4, and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1

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ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 40, 41, 42, 43, 44, or 45.

[0131] In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 46 or 47.

[0132] In one aspect, the targeting moiety includes transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 48 or 49.

[0133] In one aspect, the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 99, 100, or 101. In an additional aspect, the immunomodulatory moiety activates the signaling function of transforming growth factor-β (TGF-β) receptor.

[0134] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from TGF-β. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 50. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 51 or 52.

[0135] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25 (IL-2α receptor; IL-2αR), or CD4, and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 53, 54, 55, 56, 57 or 58.

[0136] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 59 or 60.

[0137] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) or Osteoprotegerin (OPG). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from an extracellular RANKL-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 61. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from an extracellular RANK-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 62 or 63.

[0138] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) or Osteoprotegerin (OPG). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

[0139] In one aspect, the targeting moiety includes a sequence from transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), and the

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immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

[0140] In one aspect, the targeting moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

[0141] In various aspects, the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

[0142] In another embodiment, the present invention provides a composition including the molecule of the invention and a cell, wherein the cell is a tumor cell, immune cell, or dendritic cell.

[0143] In another embodiment, the present invention provides a method of counteracting or overcoming immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

[0144] In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. In one aspect, the neoplastic disease is a non-T cell malignancy which does not express CD4 on the tumor cell. In one embodiment, the method includes administration to a subject in need thereof an antibody that targets and depletes CD4+ regulatory T cells (Tregs) in combination with a cytotoxic anticancer therapy. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In various aspects, the cytotoxic anticancer therapy includes a chemotherapeutic molecule, tumor-targeted antibody, small molecule kinase inhibitor, hormonal agent, or tumor-targeted cytotoxic agent, anti-angiogenic agent or any combination thereof. In another aspect, the cytotoxic anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

[0145] In another embodiment, the method includes administration to a subject in need thereof an antibody or molecule that targets and depletes CD4+ regulatory T cells (Tregs) in combination with an immunostimulatory antibody, fusion protein, peptide or ligand that targets CTLA-4, PD1, PD-1L, RANKL, TGF- β, GITR, 4-1BB, OX-40, or Toll-like receptors (TLR 1-10). In one aspect, the TLR-agonist comprises an activator of TLR-8 or TLR-9. In one aspect, the TLR agonist comprises an immunostimulatory nucleic acid sequence containing CpG nucleotides. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody.

[0146] In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof one or more molecule of the invention. In various aspects, the subject is administered one or more molecule of the invention in combination with another anticancer therapy. In one aspect, the anticancer therapy includes a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent, cytotoxic agent, targeted therapeutic agent, or anti-angiogenic agent. In another aspect, the anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation. In another aspect, the subject is administered one or more molecule of the invention in combination with an antibody or molecule that inhibits the production or function of regulatory T cells (Tregs) or depletes the number of Tregs. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In another aspect, the molecule that counteracts the function of Tregs is an antibody, fusion protein, peptide or ligand that targets CTLA-4, PD1, PD-1L, RANKL, TGF- β, GITR. In another aspect, the molecule that counteracts the function of Tregs is an antibody or fusion protein or ligand that targets 4-1BB or OX-40. In another aspect, the molecule that counteracts the function of Tregs is an agonist of Toll-like receptors (TLR 1-10). In one aspect, the TLR-agonist comprises an activator of TLR-8 or TLR-9. In one aspect, the TLR agonist is an immunostimulatory nucleic acid sequence containing CpG nucleotides.

[0147] In one aspect the chemotherapeutic agent is a topoisomerase-interacting agent, anthracycline, doxorubicin, mitoxantrone, camptothecin, camptothecin analogue, irinotecan, epipodophyilotoxin, etoposide, alkylating agent, cyclophosphamide, cisplatin,

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cisplatin analogue, oxaliplatin, antimetabolite, fluoropyrimidine analogue, 5-fluorouracil, gemcitabine, azacytidine, antimicrotubule agent, taxane, paclitaxel, or docetaxel.

[0148] In another embodiment, the subject is administered one or more molecule of the invention in combination with any vaccine. In another aspect, the vaccine includes a tumor antigen, tumor-associated antigen, tumor epitope, tumor antigen-containing fusion protein, tumor cell, or dendritic cell. In another aspect, the vaccine includes a pathogen antigen, pathogen-associated antigen, pathogen epitope, or pathogen antigen-containing fusion protein. In one aspect, the vaccine includes a surrogate CD4+ T cell helper epitope from tetanus toxin. In one aspect, the CD4+ T helper sequence contains a domain of tetanus toxin fragment C (pDOM1). In one aspect, the pDOM sequence is fused to a cell-permeabilizing cationic polypeptide (*e.g.,* Arginine-9). In another aspect, the Arg9-pDOM sequence is fused to a specific antigen comprising the vaccine.

[0149] In another embodiment, the present invention provides a method for treating immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treatment of a neoplastic disease. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

[0150] In another embodiment, the present invention provides a method of inducing or promoting immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

[0151] In another embodiment, the present invention provides a method of preventing or treating an autoimmune or inflammatory disease including administering to a subject in need thereof one or more molecule of the invention. In one aspect, the subject is administered one or more molecule of the invention in combination with another anti-inflammatory or immunosuppressive therapy. In another embodiment, the present invention provides a method of treatment of immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treating an autoimmune or inflammatory disease or preventing rejection of grafted cells or tissue. The method includes administering to a

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subject in need thereof a composition of immune cells contacted with a molecule of the invention.

[0152] 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.

[0153] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, references to "the method" includes one or more methods, and/or steps of the type described herein which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0154] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described.

[0155] As used herein "immune cells" or "immune effector cells" include T lymphocytes, B lymphocytes, natural killer (NK) cells, NKT cells, monocytes, macrophages, dendritic cells (DC), antigen presenting cells (APC).

[0156] As used herein, "neoplasm" or "tumor" including grammatical variations thereof, means new and abnormal growth of tissue, which may be benign or cancerous. In a related aspect, the neoplasm is indicative of a neoplastic disease or disorder, including but not limited, to various cancers. For example, such cancers can include prostate, pancreatic, biliary, colon, rectal, liver, kidney, lung, testicular, breast, ovarian, pancreatic, brain, and head and neck cancers, melanoma, sarcoma, multiple myeloma, leukemia, lymphoma, and the like.

[0157] A used herein, "subject," including grammatical variations thereof, means a human or vertebrate animal including a dog, cat, horse, cow, pig, sheep, goat, chicken, monkey, rat, and mouse.

[0158] As used herein, "targeting moiety" refers to a molecule that has the ability to localize and bind to a specific molecule or cellular component. The targeting moiety can be an antibody, antibody fragment, scFv, Fc-containing polypeptide, fusion antibody, polypeptide, peptide, aptamer, ligand, nucleic acid, or any combination thereof. In one embodiment, a targeting moiety can bind to a molecule present in a cell or tissue. In one aspect, the targeting moiety can bind a molecule in a diseased cell or tissue, such as a cancer cell or tumor. In, another aspect, the targeting molecule can bind a normal cell or tissue, such as an immune cell. In another aspect, the targeting moiety can bind a cellular or extracellular molecule that modulates the immune response. In another aspect, the targeting moiety binds a growth factor receptor, growth factor, cytokine receptor, cytokine, or cell surface molecule.

[0159] In another embodiment, the targeting moiety is a tumor-targeting moiety. The tumor-targeting moiety can bind a component of a tumor cell or bind in the vicinity of a tumor cell (*e.g.*, tumor vasculature or tumor microenvironment). In one embodiment, the tumor targeting moiety binds to a component of a tumor cell, tumor microenvironment, tumor vasculature, tumor-associated lymphocyte, tumor antigen, tumor-associated antigen, tumor cell surface molecule, tumor antigenic determinant, tumor antigen-containing fusion protein, tumor-associated cell, tumor-associated immune cell, or tumor vaccine.

[0160] For example, in various embodiments, a targeting moiety is specific for or binds to a molecule or component, which includes but is not limited to, epidermal growth factor receptor (EGFR, EGFR1, ErbB-1, HER1), ErbB-2 (HER2/neu), ErbB-3/HER3, ErbB-4/HER4, EGFR ligand family; insulin-like growth factor receptor (IGFR) family, IGF-binding proteins (IGFBPs), IGFR ligand family (IGF-1R); platelet derived growth factor receptor (PDGFR) family, PDGFR ligand family; fibroblast growth factor receptor (FGFR) family, FGFR ligand family, vascular endothelial growth factor receptor (VEGFR) family, VEGF family; HGF receptor family: TRK receptor family; ephrin (EPH) receptor family; AXL receptor family; leukocyte tyrosine kinase (LTK) receptor

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family; TIE receptor family, angiopoietin 1, 2; receptor tyrosine kinase-like orphan receptor (ROR) receptor family; discoidin domain receptor (DDR) family; RET receptor family; KLG receptor family; RYK receptor family; MuSK receptor family; Transforming growth factor alpha (TGF-α), TGF-α receptor; Transforming growth factor-beta (TGF-β), TGF-β receptor; Interleukin 13 receptor alpha2 chain (lL13Ralpha2), Interleukin-6 (IL-6), 1L-6 receptor, Interleukin-4, IL-4 receptor, Cytokine receptors, Class I (hematopoietin family) and Class II (interferon/lL-10 family) receptors, tumor necrosis factor (TNF) family, TNF-α, tumor necrosis factor (TNF) receptor superfamily (TNTRSF), death receptor family, TRAIL-receptor; cancer-testis (CT) antigens, lineage-specific antigens, differentiation antigens, alpha-actinin-4, ARTC1, breakpoint cluster region-Abelson (Bcr-abl) fusion products, B-RAF, caspase-5 (CASP-5), caspase-8 (CASP-8), beta-catenin (CTNNB1), cell division cycle 27 (CDC27), cyclin-dependent kinase 4 (CDK4), CDKN2A, COA-1, dek-can fusion protein, EFTUD-2, Elongation factor 2 (ELF2), Ets variant gene 6/acute myeloid leukemia 1 gene ETS (ETC6-AML1) fusion protein, fibronectin (FN), GPNMB, low density lipid receptor/GDP-L fucose: beta-Dgalactose 2-alpha-Lfucosyltraosferase (LDLR/FUT) fusion protein, HLA-A2, arginine to isoleucine exchange at residue 170 of the alpha-helix of the alpha2-domain in the HLA-A2 gene (HLA-A*201-R170I), MLA-A11, heat shock protein 70-2 mutated (HSP70-2M), KIAA0205, MART2, melanoma ubiquitous mutated 1,2, 3 (MUM-1, 2,3), prostatic acid phosphatase (PAP), neo-PAP, Myosin class 1, NFYC, OGT, OS-9, pml-RARalpha fusion protein, PRDX5, PTPRK, K-ras (KRAS2), N-ras (NRAS), HRAS, RBAF600, SIRT2, SNRPD1, SYT-SSX1 or -SSX2 fusion protein, Triosephosphate Isomerase, BAGE, BAGE-1, BAGE-2,3,4,5, GAGE-1,2,3,4,5,6,7,8, GnT-V (aberrant N-acetyl giucosaminyl transferase V, MGAT5), HERV-K-MEL, KK-LC, KM-HN-1, LAGE, LAGE-1, CTL-recognized antigen on melanoma (CAMEL), MAGE-Al (MAGE-1), MAGE-A2, MAGE-A3, MAGE-A4, MAGE-AS, MAGE-A6, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-3, MAGE-B1, MAGE-B2, MAGE-B5, MAGE-B6, MAGE-C1, MAGE-C2, mucin 1 (MUCl), MART-1/Melan-A (MLANA), gp100, gp100/Pme117 (S1LV), tyrosinase (TYR), TRP-1, HAGE, NA-88, NY-ESO-1, NY-ESO-l/LAGE-2, SAGE, Sp17, SSX-1,2,3,4, TRP2-1NT2, carcino-embryonic antigen (CEA), Kallikfein 4, mammaglobm-A, OA1, prostate specific antigen (PSA), prostate specific membrane

antigen, TRP-l/gp75, TRP-2, adipophilin, interferon inducible protein absent in nielanorna 2 (AIM-2), BING-4, CPSF, cyclin D1, epithelial cell adhesion molecule (Ep-CAM), EpbA3, fibroblast growth factor-5 (FGF-5), glycoprotein 250 (gp250intestinal carboxyl esterase (iCE), alpha-feto protein (AFP), M-CSF, mdm-2, MUCI, p53 (TP53), PBF, FRAME, PSMA, RAGE-1, RNF43, RU2AS, SOX10, STEAP1, survivin (BIRCS), human telomerase reverse transcriptase (hTERT), telomerase, Wilms' tumor gene (WT1), SYCP1, BRDT, SPANX, XAGE, ADAM2, PAGE-5, LIP1, CTAGE-1, CSAGE, MMA1, CAGE, BORIS, HOM-TES-85, AF15q14, HCA66I, LDHC, MORC, SGY-1, SPO11, TPX1, NY-SAR-35, FTHLI7, NXF2 TDRD1, TEX 15, FATE, TPTE, immunoglobulin idiotypes, Bence-Jones protein, estrogen receptors (ER), androgen receptors (AR), CD40, CD30, CD20, CD19, CD33, CD4, CD25, CD3, cancer antigen 72-4 (CA 72-4), cancer antigen 15-3 (CA 15-3), cancer antigen 27-29 (CA 27-29), cancer antigen 125 (CA 125), cancer antigen 19-9 (CA 19-9), beta-human chorionic gonadotropin, 1-2 microglobulin, squamous cell carcinoma antigen, neuron-specific enoJase, heat shock protein gp96, GM2, sargramostim, CTLA-4, 707 alanine proline (707-AP), adenocarcinoma antigen recognized by T cells 4 (ART-4), carcinoembryogenic antigen peptide-1 (CAP-1), calcium-activated chloride channel-2 (CLCA2), cyclophilin B (Cyp-B), human signet ring tumor-2 (HST-2), Human papilloma virus (HPV) proteins (HPV-E6, HPV-E7, major or minor capsid antigens, others), Epstein-Barr vims (EBV) proteins (EBV latent membrane proteins—LMP1, LMP2; others), Hepatitis B or C virus proteins, and HIV proteins. A composition of the invention can further include the foregoing as a peptide/polypeptide and/or encoding the same.

[0161] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. In one aspect, the targeting moiety specifically binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), CD20, Vascular endothelial growth factor (VEGF), insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, CD30, CD33, CD40, or a combination thereof.

[0162] Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as trastuzumab (anti-HER2/neu antibody); Pertuzumab (anti-HER2 mAb); cetuximab (chimeric monoclonal antibody to epidermal growth factor receptor EGFR); panitumumab (anti-EGFR antibody); nimotuzumab (anti-EGFR antibody); Zalutumumab (anti-EGFR mAb); Necitumumab (anti-EGFR mAb); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-447 (humanized anti-EGF receptor bispecific antibody); Rituximab (chimeric murine/human anti-CD20 mAb); Obinutuzumab (anti-CD20 mAb); Ofatumumab (anti-CD20 mAb); Tositumumab-I131 (anti-CD20 mAb); Ibritumomab tiuxetan (anti-CD20 mAb); Bevacizumab (anti-VEGF mAb); Ramucirumab (anti-VEGFR2 mAb); Ranibizumab (anti-VEGF mAb); Aflibercept (extracellular domains of VEGFR1 and VEGFR2 fused to IgG1 Fc); AMG386 (angiopoietin-1 and -2 binding peptide fused to IgG1 Fc); Dalotuzumab (anti-IGF-1R mAb); Gemtuzumab ozogamicin (anti-CD33 mAb); Alemtuzumab (anti-Campath-1/CD52 mAb); Brentuximab vedotin (anti-CD30 mAb); Catumaxomab (bispecific mAb that targets epithelial cell adhesion molecule and CD3); Naptumomab (anti-5T4 mAb); Girentuximab (anti-Carbonic anhydrase ix); or Farletuzumab (anti-folate receptor). Other examples include antibodies such as Panorex™ (17-1A) (murine monoclonal antibody); Panorex (@ (17-1A) (chimeric murine monoclonal antibody); BEC2 (ami-idiotypic mAb, mimics the GD epitope) (with BCG); Oncolym (Lym-1 monoclonal antibody); SMART M195 Ab, humanized 13' 1 LYM-1 (Oncolym), Ovarex (B43.13, anti-idiotypic mouse mAb); 3622W94 mAb that binds to EGP40 (17-1A) pancarcinoma antigen on adenocarcinomas; Zenapax (SMART Anti-Tac (IL-2 receptor); SMART M195 Ab, humanized Ab, humanized); NovoMAb-G2 (pancarcinoma specific Ab); TNT (chimeric mAb to histone antigens); TNT (chimeric mAb to histone antigens); Gliomab-H (Monoclonals—Humanized Abs); GNI-250 Mab; EMD-72000 (chimeric-EGF antagonist); LymphoCide (humanized IL.L.2 antibody); and MDX-260 bispecific, targets GD-2, ANA Ab, SMART IDIO Ab, SMART ABL 364 Ab or ImmuRAIT-CEA. Examples of antibodies include those disclosed in US5736167, US7060808, and US5821337.

[0163] In one embodiment, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: CD4; CD25 (IL-2α receptor; IL-2αR); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF-βR); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); Programmed death-1 ligand (PD-L1 or PD-L2); Receptor activator of nuclear factor-κB (RANK); Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); or Interleukin-4 receptor (IL-4R). In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

[0164] In one aspect, the targeting moiety binds a specific cytokine, cytokine receptor, co-stimulatory molecule, co-inhibitory molecule, or immunomodulatory receptor that modulates the immune system. In another aspect, the targeting moiety specifically binds one of the following molecules: tumor necrosis factor (TNF) superfamily; tumor necrosis factor-α (TNF-α); tumor necrosis factor receptor (TNFR) superfamily; Interleukin-12 (IL-12); IL-12 receptor; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNR4); OX40 ligand (OX40L; CD40; CD40 ligand (CD40L); CTLA-4; Programmed death-1 (PD-1); PD-1 ligand 1 (PD-L1; B7-H1); or PD-1 ligand 2 (PD-L2; B7-DC); B7 family; B7-1 (CD80); B7-2 (CD86); B7-H3; B7-H4; GITR/AITR; GITRL/AITRL; BTLA; CD70; CD27; LIGHT; HVEM; Toll-like receptor (TLR)(TLR 1,2,3,4,5,6,7,8,9, 10). In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

[0165] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide, or peptide that specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a cytokine, cytokine receptor, co-stimulatory molecule,

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or co-inhibitory molecule that modulates the immune system. In another aspect, the targeting moiety specifically binds one of the following molecules: CD4; CD25 (IL-2α receptor; IL-2αR); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF-βR); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); PD-1 ligand 1 (PD-L1; B7-H1); PD-1 ligand 2 (PD-L2; B7-DC); Receptor activator of nuclear factor-κB (RANK); Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); Interleukin-4 receptor (IL-4R); tumor necrosis factor (TNF) superfamily; tumor necrosis factor-α (TNF-α); tumor necrosis factor receptor (TNFR) superfamily; Interleukin-12 (IL-12); IL-12 receptor; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNR4); OX40 ligand (OX40L; CD40; CD40 ligand (CD40L); CTLA-4; B7 family; B7-1 (CD80); B7-2 (CD86); B7-H3; B7-H4; GITR/AITR; GITRL/AITRL; BTLA; CD70; CD27; LIGHT; or HVEM. In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

[0166] Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as Zanulimumab (anti-CD4 mAb), Keliximab (anti-CD4 mAb); Ipilimumab (MDX-101; anti-CTLA-4 mAb); Tremilimumab (anti-CTLA-4 mAb); (Daclizumab (anti-CD25/IL-2R mAb); Basiliximab (anti-CD25/IL-2R mAb); MDX-1106 (anti-PD1 mAb); antibody to GITR; GC1008 (anti-TGF-β antibody); metelimumab/CAT-192 (anti- TGF-β antibody); lerdelimumab/CAT-152 (anti- TGF-β antibody); ID11 (anti- TGF-β antibody); Denosumab (anti-RANKL mAb); BMS-663513 (humanized anti-4-1BB mAb); SGN-40 (humanized anti-CD40 mAb); CP870,893 (human anti-CD40 mAb); Infliximab (chimeric anti-TNF mAb; Adalimumab (human anti-TNF mAb); Certolizumab (humanized Fab anti-TNF); Golimumab (anti-TNF); Etanercept (Extracellular domain of TNFR fused to IgG1 Fc); Belatacept (Extracellular domain of CTLA-4 fused to Fc); Abatacept (Extracellular domain of CTLA-4 fused to Fc); Belimumab (anti-B Lymphocyte stimulator); Muromonab-CD3 (anti-CD3 mAb); Otelixizumab (anti-CD3 mAb); Teplizumab (anti-CD3 mAb); Tocilizumab (anti-IL6R mAb); REGN88 (anti-IL6R mAb); Ustekinumab

(anti-IL-12/23 mAb); Briakinumab (anti-IL-12/23 mAb); Natalizumab (anti-α4 integrin); Vedolizumab (anti-α4 β7 integrin mAb); T1h (anti-CD6 mAb); Epratuzumab (anti-CD22 mAb); Efalizumab (anti-CD11a mAb); and Atacicept (extracellular domain of transmembrane activator and calcium-modulating ligand interactor fused with Fc).

[0167] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an "immunomodulatory moiety". As used herein, "immunomodulatory moiety" refers to a ligand, peptide, polypeptide, or Fc-containing polypeptide that binds a specific component of a regulatory T cell, myeloid suppressor cell, or dendritic cell and modulates the number or function of Tregs or myeloid suppressor cells. In an additional aspect, the "immunomodulatory moiety" specifically binds a cytokine, cytokine receptor, co-stimulatory molecule, or co-inhibitory molecule that modulates the immune system. In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: Transforming growth factor-beta receptor (TGF-βR); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); PD-1 ligand 1 (PD-L1; B7-H1); PD-1 ligand 2 (PD-L2; B7-DC); Receptor activator of nuclear factor-κB (RANK); or Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); or vascular endothelial growth factor (VEGF). In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR; TNFRSF18); GITRL/AITRL; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNR4); OX40 ligand (OX40L); B7-H3; B7-H4; BTLA; CD40; CD40 ligand (CD40L); CD70; CD27; LIGHT; or HVEM. In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: tumor necrosis factor-α (TNF-α); Interleukin-12 (IL-12); IL-12R; Interleukin-10 (IL-10); IL-10R. In another aspect, the immunoodulatory moiety comprises an extracellular domain of CTLA-4. In one aspect, the immunomodulatory moiety is an agonist that increases the function of the bound molecule. In another aspect, the immunomodulatory moiety is an antagonist that inhibits the function of the targeted molecule.

[0168] In another aspect, the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming

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

[0169] In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: Transforming growth factor-beta (TGF-β); PD-1 ligand 1 (PD-L1); PD-1 ligand 2 (PD-L2); or IL-10. In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL.

[0170] In another aspect, the immunomodulatory moiety is fused to the C-terminus of the targeting moiety. In another aspect, the immunomodulatory moiety is fused to the N-terminus of the targeting moiety. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is the targeting moiety, Fc is an immunoglobulin Fc region, and Y is the immunomodulatory moiety. In another aspect, the fusion molecule is represented by Y-Fc-X, wherein X is the targeting moiety, and Y is the immunomodulatory moiety. In one aspect, the targeting moiety may additionally be an immunomodulatory moiety.

[0171] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell, and the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF-βRII, TGF-βRIIb, or TGF-βRIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor-κB (RANK); vascular endothelial growth factor receptor (VEGFR1 or VEGFR2); or IL-10R.

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[0172] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell, and the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL.

[0173] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds a specific component of a regulatory T cell, myeloid suppressor cell, or dendritic cell, and the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF-βRII, TGF-βRIIb, or TGF-βRIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor-κB (RANK); or IL-10R. In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL. In another aspect, the specific targeted component of a regulatory T cell, myeloid suppressor cell, or dendritic cell is one of the following molecules: CD4; CD25 (IL-2α receptor; IL-2αR); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); Programmed death-1 ligand (PD-L1 or PD-L2); Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); or Interleukin-4 receptor (IL-4R).

[0174] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds one of the following: CTLA-4; 4-1BB (CD137); OX40 (CD134; TNR4); CD40; IL-12R; or glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR; TNFRSF18); and the immunomodulatory moiety comprises a different molecule selected from the following: (i) an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF-βRII, TGF-βRIIb, or TGF-βRIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor-κB (RANK); or IL-

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10R; or (ii) Fc-containing polypeptide or ligand that binds one of the following: CTLA-4; 4-1BB (CD137); OX40 (CD134; TNR4); CD40; IL-12R; or GITR (AITR; TNFRSF18).

[0175] In another aspect, the targeting moiety and immunomodulatory moiety are two different molecules selected from any of the following: an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds TGF-β, CTLA-4, PD-1, 4-1BB (CD137), OX40 (CD134; TNR4), CD40; IL-12R, or GITR/AITR (TNFRSF18), or Toll-like receptor (TLR); an extracellular domain or ligand-binding sequence of Transforming growth factor-beta receptor (TGF-βRII, TGF-βRIIb, or TGF-βRIII), Programmed Death-1 (PD-1), Receptor activator of nuclear factor-κB (RANK), or IL-10R. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is an immunomodulatory targeting moiety and Y is a different immunomodulatory moiety.

[0176] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds one of the following molecules: CD4; CD25 (IL-2α receptor; IL-2αR); or CD20; and the immunomodulatory moiety comprises one of the following ligands or active ligand fragments: Transforming growth factor-beta (TGF-β); PD-1 ligand 1 (PD-L1); PD-1 ligand 2 (PD-L2); or IL-10.

[0177] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds tumor necrosis factor-α (TNF-α), Interleukin-12 (IL-12), IL-6R, B-lymphocyte stimulator, CD11a, CD6, or CD22; and the immunomodulatory moiety comprises one of the following: (i) ligands or active ligand fragments of Transforming growth factor-beta (TGF-β), PD-1 ligand 1 (PD-L1), or IL-10; or (ii) an extracellular domain or ligand-binding fragment of RANK, 4-1BB (CD137), OX40 (CD134; TNR4), CD40, IL-12R or GITR/AITR (TNFRSF18).

[0178] In another aspect, the targeting moiety comprises the extracellular domain of CTLA-4 fused to immunoglobulin Fc, and the immunomodulatory moiety comprises one of the following: (i) ligands or active ligand fragments of Transforming growth factor-beta (TGF-β), PD-1 ligand 1 (PD-L1), or IL-10; or (ii) ligand-binding fragment of TNFR2, RANK, 4-1BB (CD137), OX40 (CD134; TNR4), CD40, IL-12R or GITR/AITR (TNFRSF18).

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[0179] In another aspect, the targeting moiety and immunomodulatory moiety are two different molecules selected from any of the following: an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds tumor necrosis factor-α (TNF-α), Interleukin-12 (IL-12), IL-6R, B-lymphocyte stimulator, CD11a, CD6, or CD22; a ligand-binding fragment of TNFR2, RANK, 4-1BB (CD137), OX40 (CD134; TNR4), CD40, IL-12R or GITR/AITR (TNFRSF18); ligands or active ligand fragments of Transforming growth factor-beta (TGF-β), PD-1 ligand 1 (PD-L1), or IL-10; or CTLA-4-Fc. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is the immunomodulatory targeting moiety and Y is a different immunomodulatory moiety.

[0180] Antibodies: In one embodiment, the targeting moiety or fusion protein is an immunoglobulin. As used herein, the term "immunoglobulin" includes natural or artificial mono- or polyvalent antibodies including, but not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments. F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin ion can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgGl, IgG2, IgG3, IgG4, IgAl, and IgA2) or subclass of immunoglobulin molecule.

[0181] An antibody as disclosed herein includes an antibody fragment, such as, but not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdfv) and fragments including either a VL or VH domain. In one embodiment, the targeting moiety is an antibody or scFv.

[0182] An antigen-binding antibody fragment, including single-chain antibody, may include the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. An antigen-binding fragment can also include any combination of variable region(s) with a hinge region, CHI, CH2, and CH3 domains. Also includes is a Fc fragment, antigen-Fc fusion proteins, and Fc-

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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.

[0183] The antibody herein specifically include a "chimeric" antibody in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al. (1984) Proc. Natl. Acad. Sci. USA. 81:6851-6855). A chimeric antibody of interest herein includes "primatized" antibodies including variable domain antigen-binding sequences derived from a non-human primate (*e.g.*, Old World Monkey, Ape etc.) and human constant region sequences.

[0184] 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.

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[0185] In one embodiment, a hybridoma can produce a targeted fusion protein comprising a targeting moiety and an immunomodulatory moiety. In one embodiment, a targeting moiety comprising an antibody, antibody fragment, or polypeptide is linked or fused to an immunomodulatory moiety consisting of a polypeptide, with a linker or without a linker. The linker can be an amino acid linker. In one embodiment, a linker is (GGGGS)n (SEQ ID NO: 123) wherein n is 1, 2, 3, 4, 5, 6, 7, or 8. For example, GGGGSGGGGSGGGGS (SEQ ID NO: 104). In another embodiment, a linker is EPKSCDK (SEQ ID NO: 105). In another embodiment, a linker is IEGRDMD (SEQ. ID. NO: 106). In various aspects, the length of the linker may be modified to optimize binding of the target moiety or the function of the immunomodulatory moiety. In various aspects, the immunomodulatory moiety is a polypeptide that is fused to the C-terminus of the Fc region of the heavy chain of a targeting antibody or Fc-containing fusion protein. In another aspect, the immunomodulatory moiety is a polypeptide that is fused to the C-terminus of the light chain of a targeting antibody. In another aspect, the fusion protein comprises an X-Fc-Y sequence, wherein X is a targeting polypeptide and Y is an immunomodulatory polypeptide.

[0186] For example, a hybridoma can produce the polypeptides corresponding to SEQ. ID. NO: 1-69.

[0187] An antibody fragment can include a portion of an intact, antibody, *e.g.,* including the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')2, and Fv fragments; Fc fragments or Fc-fusion products; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragment(s).

[0188] An intact antibody is one which includes an antigen-binding variable region as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variant thereof tor any other modified Fc (*e.g.,* glycosylation or other engineered Fc).

[0189] The intact antibody may have one or more "effector functions" which refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region or any other modified Fc region) of an antibody. Examples of antibody effector functions include Clq binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor (BCR); and cross-presentation of antigens by antigen presenting cells or dendritic cells. In one embodiment, the targeting antibody or Fc-containing fusion protein facilitates focused or preferential delivery of a immunomodulatory moiety to a target cell. In another aspect, a targeting antibody can induce death of the targeted cell or sensitize it to immune cell-mediated cytotoxicity. In another aspect, the Fc-fusion protein or antibody can facilitate delivery of the immunomodulatory moiety or immunogenic apoptotic material from antibody-bound tumor targets, or both, to an antigen presenting cells (APC) via interactions between their Fc and Fc receptors (on APC).

[0190] Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different "classes." There arc five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" (isotypes), *e.g.*, IgG1, IgG2, IgC3, IgG4, IgA, and IgA2. The heavy-chain constant domains (hat correspond to the different classes of antibodies are called alpha (α), delta (δ), epsilon (ε), gamma (γ), and mu (μ) respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0191] Peptides: In some aspects of the invention the targeting moiety or immunomodulatory moiety is a peptide or polypeptide. A peptide includes any analog, fragment or chemical derivative of a peptide whose amino acid residue sequence is shown herein. Therefore, a present peptide can be subject to various changes, substitutions, insertions, and deletions where such changes provide for certain advantages in its use. In this regard, a peptide of this invention corresponds to, rather than is identical to, the sequence of a recited peptide where one or more changes are made and it retains the ability to function as the unmodified peptide in one or more of the assays.

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[0192] The term "analog" includes any peptide having an amino acid residue sequence substantially identical to a sequence specifically shown herein in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the activity as described herein. Examples of conservative substitutions include the substitution of one non-polar (hydrophobic) residue such as isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another.

[0193] 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.

[0194] 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.

[0195] 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.

[0196] Aptamers: In one aspect of the invention, the targeting moiety is an aptamer. In various embodiments, an aptamer is specific for a molecule on a tumor cell, tumor vasculature, and/or a tumor microenvironment. The term "aptamer" includes DNA, RNA or peptides that are selected based on specific binding properties to a particular molecule. For example, an aptamer(s) can be selected for binding a particular gene product in a tumor cell, tumor vasculature, tumor microenvironment, and/or an immune cell, as disclosed herein, where selection is made by methods known in the art and familiar to one of skill in the art. Subsequently, said aptamer(s) can be administered to a subject to modulate or regulate an immune response.

[0197] Some aptamers having affinity to a specific protein, DNA, amino acid and nucleotides have been described (*e.g.*, K. Y. Wang, et al., Biochemistry 32:1899-1904 (1993); Pitner et al., U.S. Pat. No. 5,691,145: Gold, et al., Ann. Rev. Biochem. 64:763-797 (1995); Szostak et al., U.S. Pat. No. 5,631,146). High affinity and high specificity binding aptamers have been derived from combinatorial libraries (supra, Gold, et al.). Aptamers may have high affinities, with equilibrium dissociation constants ranging from micromolar to sub-nanomolar depending on the selection used, aptamers may also exhibit high selectivity, for example, showing a thousand fold, discrimination between 7-methyl G and G (Haller and Sarnow, Proc. Natl. Acad. Sci. USA 94:8521-8526 (1997)) or between D and L-tryptophan (supra, Gold et al.). An aptamer can be selected based on the particular molecule targeted (*e.g.*, aptamer targeting EGFR or other cancer markers). Standard procedures for in vitro selection are known, such as SELEX experiments, described at Science 249 (4968) 505-510 (1990), and Nature (London), 346 (6287) 818-822 (1990) which can be followed throughout, or with modifications and improvements known in the art.

[0198] 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.

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[0199] 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.

[0200] 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.

[0201] 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.

[0202] 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.

[0203] The following examples are provided to further illustrate the embodiments of the present invention, but are not intended to limit the scope of the invention. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

EXAMPLE 1

[0204] Counteracting Tumor Immune Tolerance via Antibody-mediated Depletion of $CD4^+$ Regulatory T Cells Facilitates the Activation of Tumor-Reactive $CD8^+$ T Cells and Enhances the *In Vivo* Antitumor Efficacy of Cytotoxic Anticancer Agents.

[0205] Immunogenic death of tumor cells by chemotherapeutic agents can induce $CD8^+$ T cell-mediated antitumor immunity. In response to specific chemotherapeutic agents, tumor cells exhibit the rapid translocation of intracellular calreticulin (CRT) to the cell surface where its aggregation provides a signal for the recognition and engulfment of dying tumor cells by antigen presenting dendritic cells (DCs). Treatment of mouse MB49 or human SW780 bladder cancer cells with doxorubicin, an anthracycline chemotherapeutic agent, induced rapid surface exposure of CRT that was detected by immunofluorescence cytometry of cells stained with Dylight 488-labeled anti-CRT antibody (FIG. 53A). To determine whether *ex vivo* treatment with doxorubicin induced an immunogenic death of tumor cells, either untreated live MB49 cells or an equivalent number of MB49 cells that were pre-treated *in vitro* with doxorubicin were injected into one flank of syngeneic immunocompetent C57BL/6 mice. Unlike mice injected with live tumor cells, mice injected with doxorubicin-treated tumor cells exhibited increased production of IFN-γ by draining lymph node (DLN) cells in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 53B). Vaccination with doxorubicin-killed MB49 cells generated a tumor-specific immune response since no corresponding increase in IFN-γ secretion by DLN cells was observed following *in vitro* exposure to an irrelevant peptide (Hemagglutinin-HA). Injection of doxorubicin-treated MB49 tumor cells protected mice against tumor growth upon challenge with untreated live MB49 tumor cells injected into the opposite flank. (FIG. 1C). The protection against tumor growth by vaccination with doxorubicin-treated tumor cells was not observed in mice that were depleted of $CD8^+$ T cells with an anti-CD8 antibody before challenge with live tumor cells (FIG. 53C). These

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observations indicate that *ex vivo* treatment with chemotherapeutic agents can induce an immunogenic death of tumor cells that generates $CD8^+$ T cell-mediated adaptive antitumor immunity.

[0206] Tumor-induced immune tolerance inhibits activation of $CD8^+$ T cells in response to chemotherapy. To examine whether *in vivo* treatment with chemotherapeutic agents can activate $CD8^+$ T cell-mediated immune responses in mice with pre-established tumors, C57BL/6 mice were injected with live syngeneic MB49 tumor cells and then administered intratumoral doxorubicin at various time points following tumor inoculation. In contrast to vaccination of naïve mice with doxorubicin-killed MB49 cells, *in vivo* treatment of mice with established MB49 tumors at d10 following tumor inoculation failed to induce a corresponding increase in IFN-γ secretion by DLN cells in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 53B). Whereas treatment with doxorubicin on d3 following tumor inoculation was able to arrest tumor growth, delayed administration of the same dose of doxorubicin on d10 failed to inhibit the progressive growth of established MB49 tumors (FIG. 53D). These results indicate that tumor-induced immune tolerance in the microenvironment of established cancers counteracts the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death.

[0207] Regulatory T cells (Treg) accumulate in the tumor microenvironment and counteract the ability of chemotherapy to activate $CD8^+$ T cell mediated antitumor immunity. To investigate whether $FoxP3^+$ Tregs are involved in enforcing immune tolerance in the tumor microenvironment, we examined the percentage of $CD4^+CD25^+FoxP3^+$ cells (Tregs) among $CD4^+$ T lymphocytes in the spleen, draining lymph nodes (DLN), and tumors of immunocompetent C57BL/6 mice at d0 and d14 after tumor inoculation. Whereas tumor-bearing mice exhibited only a minor increase in the percentage of Tregs among $CD4^+$ T cells in the spleen and DLN at d14 following tumor inoculation, a majority of tumor-infiltrating $CD4^+$ T cells at this time were $CD4^+CD25^+FoxP3^+$ cells (FIG. 53E). To investigate whether Tregs infiltrating the tumor microenvironment can suppress the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death, $CD4^+CD25^+$ cells isolated from tumors and DLN of tumor-bearing mice were adoptively transferred into syngeneic C57BL/6 naïve

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mice before vaccination with doxorubicin-killed MB49 cells. The adoptive transfer of tumor-infiltrating $CD4^+CD25^+$ cells into naïve mice inhibited the ability of subsequent *in vivo* vaccination with doxorubicin-treated MB49 tumor cells to increase production of IFN-γ by draining lymph node (DLN) cells in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 53F). Consistent with the ability of tumor-infiltrating $CD4^+CD25^+$ cells to suppress the tumor-specific immune response, the adoptive transfer of these cells counteracted the protection conferred by vaccination with doxorubicin-treated MB49 cells against tumor growth upon challenge with untreated live MB49 tumor cells (FIG. 53G). These results indicate that the tumor microenvironment fosters the accumulation of $FoxP3^+$ Tregs which counteract the activation of $CD8^+$ T cell mediated antitumor immunity in response to chemotherapy-induced tumor cell death.

[0208] Inhibition of TGF-β in the tumor microenvironment reduces tumor-infiltrating $FoxP3^+$ regulatory T cells and enhances the antitumor efficacy chemotherapy. TGF-β induces FoxP3 expression in naïve peripheral $CD4^+CD25^-FoxP3^-$ T cells and facilitates their conversion into 'adaptive' $FoxP3^+$ Tregs that share the immunosuppressive ability of natural $FoxP3^+$ Tregs generated in the thymus. Since human cancers frequently become refractory to the growth-inhibitory effect of TGF-β and acquire an ability to increase expression and secretion of TGF-β, we investigated whether this switch enables tumor cells to increase the number of adaptive Tregs in the tumor microenvironment. Examination of serum levels of TGF-β in mice at d0, d14, and d28 following inoculation of live MB49 tumor cells demonstrated that tumor growth resulted in a progressive increase in the level of serum TGF-β (FIG. 54A). To assess the precise source of TGF-β in tumor-bearing mice, the total amount of TGF-β in supernatants of tumor cells or draining lymph node cells isolated from tumor-bearing mice were measured following *ex vivo* culture in serum-free medium for 24h. Measurement of the level of TGF-β/10^6 cells showed that tumor cells were the dominant source of the increased level of TGF-β in tumor-bearing mice (FIG. 54B). In addition to tumor cell-autonomous expression of TGF-β, T cells from tumor-bearing mice also expressed higher levels of TGF-β compared to their counterparts from tumor-free mice (FIG. 54B). To determine whether the elevation of TGF-β is responsible for the upregulation of Tregs in the tumor microenvironment, tumor-bearing mice were treated with a soluble chimeric protein comprising the

extracellular domain of TGFβRII and the Fc portion of the murine IgG1 heavy chain (TGFβRII:Fc). This fusion protein interferes with the binding of TGF-β to endogenous TGFβRII and functions as a stable TGF-β antagonist. ELISA assays confirmed the ability of TGFβRII:Fc to sequester TGF-β in supernatants of MB49 tumor cells in a concentration-dependent manner (FIG. 54C). At 5d following inoculation of MB49 tumor cells, mice were either left untreated or treated with TGFβRII:Fc (1μg intratumoral; twice weekly) for 3 weeks followed by flow cytometric analyses of intracellular FoxP3 expression in $CD4^+CD25^+$ T cells infiltrating the tumors. *In vivo* treatment of tumors with TGFβRII:Fc resulted in a significant decline in FoxP3 expression in tumor-infiltrating $CD4^+$ T cells (FIG. 54D) and a dramatic reduction of $CD4^+CD25^+FoxP3^+$ Tregs in tumor tissue (FIG. 54E). To determine whether inhibition of TGF-β in the tumor microenvironment can improve the antitumor efficacy of chemotherapy, MB49 tumor-bearing mice were administered doxorubicin (5mg/kg i.p. weekly x 3) with or without twice weekly treatment with TGFβRII:Fc (1μg intratumoral). In contrast to treatment with either doxorubicin or TGFβRII:Fc alone, combined treatment with both agents was able to arrest the growth of MB49 tumors. These results indicate that tumor cell autonomous expression of TGF-β in the tumor microenvironment induces 'adaptive' $FoxP3^+$ Tregs and that counteracting tumor-induced TGF-β-mediated immune tolerance enhances the antitumor efficacy of chemotherapy.

[0209] Anti-CD4 antibody-mediated depletion of $CD4^+$ regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive $CD8^+$ T cells and enhances the antitumor efficacy of chemotherapy. To determine whether depletion of $CD4^+$ regulatory T cells can improve the antitumor efficacy of chemotherapy by enhancing the activity of $CD8^+$ T cells in the tumor microenvironment, immunocompetent mice bearing syngeneic tumors were administered an anti-CD4 antibody (Clone GK1.5) to deplete $CD4^+$ T cells or an anti-CD8 antibody (Clone GK2.43) to deplete $CD8^+$ T cells and then treated with specific chemotherapeutic agents. Flow cytometric analyses of peripheral blood mononuclear cells from MB49 tumor-bearing mice at d7 following administration of anti-CD4 antibody or anti-CD8 antibody confirmed the target-specific depletion of either $CD4^+$ T cells or $CD8^+$ T cells, respectively (FIG. 55A). Mice treated with anti-CD4 antibody showed loss of $CD4^+CD25^+FoxP3^+$ T cells in the peripheral blood as well as among

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tumor-infiltrating cells (FIGS. 55A, 55B). To determine whether antibody-mediated depletion of $CD4^+CD25^+FoxP3^+$ cells facilitates chemotherapy-induced activation of tumor-reactive $CD8^+$ T cells in the tumor microenvironment, we evaluated the expression of IFN-γ in $CD8^+$ T cells extracted from the tumor and draining lymph node of MB49 tumor-bearing mice that were left untreated or treated with doxorubicin (with or without anti-CD4 antibody). Flow cytometric analyses showed that $CD8^+$ T cells from untreated mice did not express IFN-γ in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 55C). Whereas IFN-γ^+CD8^+ T cells became evident in mice treated with doxorubicin alone, antibody-mediated depletion of $CD4^+$ T cells further enhanced the percentage of tumor-reactive $CD8^+$ T cells that expressed IFN-γ in doxorubicin-treated animals (FIG. 55C). To directly evaluate whether the activation of tumor-reactive $CD8^+$ T cells determines the *in vivo* antitumor efficacy of chemotherapy, we examined the effect of antibody-mediated depletion of $CD8^+$ T cells or $CD4^+$ T cells on the response of MB49 tumor-bearing mice to systemic treatment with doxorubicin (5mg/kg). Treatment with doxorubicin alone inhibited the growth of MB49 tumors but failed to arrest tumor progression. Whereas depletion of $CD8^+$ T cells completely impaired the *in vivo* antitumor efficacy of doxorubicin, depletion of $CD4^+$ T cells enhanced the response to doxorubicin and resulted in tumor regression (FIG. 55D).

[0210] Anti-CD4 antibody-mediated depletion of $CD4^+$ regulatory T cells augments and sustains the antitumor effect of chemotherapy by enabling activation of adaptive antitumor immunity. Whereas tumor cells treated with anthracyclins, such as doxorubicin, are particularly effective in eliciting an antitumor immune response, other chemotherapeutic agents are less effective in inducing immunogenic tumor cell death. The surface exposure of calreticulin is a key determinant of the immunogenicity of tumor cell death in response to chemotherapeutic agents. Compared to the efficient translocation of CRT to the cell surface in response to treatment with doxorubicin (FIG. 1A), treatment of MB49 tumor cells with equitoxic doses of either cisplatin or the combination of cisplatin and gemcitabine was less effective in increasing CRT exposure (FIG. 56A). Whereas tumor-reactive IFN-γ^+CD8^+ T cells were evident in tumors of MB49 tumor-bearing mice treated with doxorubicin (FIG. 55C), treatment with cisplatin was unable to induce a corresponding elevation of IFN-γ expression in $CD8^+$ T cells in response to *in*

vitro re-challenge with MB49 cell lysates (FIG. 56B). To examine whether counteraction of Treg-mediated immune tolerance enables the activation of antitumor immunity by cisplatin, immunocompetent tumor-bearing mice were treated with cisplatin following depletion of Tregs with anti-CD4 antibody. Antibody-mediated depletion of $CD4^+$ T cells enhanced the percentage of tumor-reactive IFN-γ^+CD8^+ T cells as well as $CD8^+CD62L^-$ T cells in cisplatin-treated animals (FIGS. 56B, 56C). Treatment of MB49 tumor-bearing mice with cisplatin partially inhibited tumor growth but failed to arrest tumor progression. Whereas depletion of $CD8^+$ T cells completely negated the *in vivo* antitumor effect of cisplatin, depletion of $CD4^+$ T cells enhanced the response to cisplatin and arrested tumor growth (FIG. 56D). Although treatment of tumor-bearing mice with the combination of cisplatin and gemcitabine was also able to arrest tumor growth, tumor growth rapidly resumed following termination of therapy with none of the animals (0/8) being tumor-free at d50 following tumor inoculation (FIG. 56E). In contrast, mice depleted of $CD4^+$ T cells exhibited a more sustained response to either single agent or combination chemotherapy, with 7/16 mice exhibiting complete tumor regression. The complete regression of tumors was attended with establishment of adaptive antitumor immunity since none of the cured mice (7/7) developed tumors when re-challenged with live MB49 tumor cells in the opposite flank.

[0211] Chemotherapy-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD^{4+} regulatory T cells to stimulate CD^{8+} T cell-mediated tumor regression. NKG2D (NK group 2, member D) is a lectin-like type II transmembrane stimulatory receptor used by NK cells, $\gamma\delta$-TC^{R+} T cells and $\alpha\beta$-TC^{R+} T cells for immune surveillance of tumors. Expression of mouse and human ligands for NKG2D is upregulated in transformed epithelial cell lines in response to genotoxic stress or stalled DNA replication, via activation of a DNA damage checkpoint pathway initiated by ATM (ataxia telengiectasia, mutated) or ATR (ATM- and Rad3-related) protein kinases. Treatment of CT26 mouse colon cancer cells with genotoxic chemotherapeutic agents resulted in upregulation of mouse NKG2D ligands of the retinoic acid inducible gene family (Rae1) (FIG. 57A). RT-PCR showed that Rae1 mRNA was induced in CT26 cells by 2-4h, reached a plateau after 16-24h, and began to decline after 48h of treatment with either the irinotecan or oxaliplatin (FIG. 57A). Flow cytometric analysis

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demonstrated that cell surface expression of human NKG2D ligands (MHC-I-related A and B molecules – MICA, MICB) was also upregulated on human colorectal cancer cells (HCT116) in response to treatment with irinotecan (FIG. 53B). Isogenic HCT116 cells that differ only in their p53 status demonstrated that p53 is not required for irinotecan-induced upregulation of MICA/B (FIG. 53B). To examine whether the induction of NKG2D ligands contributes to the antitumor effect of chemotherapy *in vivo*, immunocompetent Balb/C mice inoculated with syngeneic CT26 tumor cells were treated with irinotecan (50 mg/kg i.p) with or without pre-treatment with an NKG2D blocking antibody (200 μg i.p.). Whereas treatment with irinotecan alone inhibited the growth of CT26 tumors, the antitumor effect of irinotecan was negated by pre-treatment with the NKG2D blocking antibody (FIG. 1C). Since engagement of NKG2D by its ligands provides a costimulatory signal for the activation of CD^{8+} T cells, we investigated whether DNA damage-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD^{4+} regulatory T cells to stimulate CD^{8+} T cell-mediated tumor regression. Balb/C mice bearing CT26 tumors were administered an anti-CD4 antibody (Clone GK1.5) to deplete CD^{4+} T cells and/or an anti-CD8 antibody (Clone GK2.43) to deplete CD^{8+} T cells and then treated with irinotecan. Flow cytometric analyses confirmed the loss of $CD^{4+}CD2^{5+}FoxP^{3+}$ T cells in the spleen and draining lymph node of mice treated with anti-CD4 antibody (FIG. 57D). Antibody-mediated depletion of CD^{4+} T cells enhanced the percentage of tumor-reactive IFN-$\gamma^{+}CD^{8+}$ T cells in irinotecan-treated animals (FIG. 57E). Whereas treatment of CT26 tumor-bearing mice with irinotecan only slowed tumor growth, depletion of CD^{4+} T cells enhanced the response to irinotecan and arrested tumor growth (FIG. 57F). The ability of CD^{4+} T cell depletion to augment the antitumor efficacy of irinotecan was mediated by CD^{8+} T cells since antibody-mediated depletion of CD^{8+} T cells completely negated the *in vivo* antitumor effect of chemotherapy in CD^{4+} T cell-depleted mice (FIG. 57F).

[0212] These data provide the following insights: (i) activation of tumor-reactive $CD8^{+}$ T cells in response to immunogenic tumor cell death is a crucial determinant of the antitumor efficacy of chemotherapy *in vivo;* (ii) tumor-induced Tregs impair the antitumor efficacy of chemotherapy by inhibiting the activation of $CD8^{+}$ T cells in the tumor microenvironment; and (iii) Counteracting tumor-induced immune tolerance via antibody-

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

EXAMPLE 2

[0213] Exemplary Targeted Immunomodulatory Antibodies and Fusion Proteins

[0214] A targeting moiety, including an antibody, can be coupled to an immunomodulatory moiety including a polypeptide derived from the extracellular domain of TGFBR2. Crosslinkers or activating agents for such coupling or conjugation are well known in the art. Alternatively, the fusion proteins of the invention can be synthesized using recombination DNA technology well known in the art where the coding sequences of various portions of the fusion proteins can be linked together at the nucleic acid level. Subsequently the fusion proteins of the invention can be produced using a host cell well known in the art. Examples of targeted immunomodulatory antibodies and fusion proteins are shown in Figures 1-33 and briefly described below.

[0215] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety specifically binds to Transforming growth factor-beta (TGF-β). SEQ ID NO: 1 provides a fusion protein including anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 2). SEQ ID NO: 2 provides a fusion protein including anti-EGFR1 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 3). SEQ ID NO: 3 provides a fusion protein including anti-CD20 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 4). SEQ ID NO: 4 provides a fusion protein including anti-VEGF antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 5). SEQ ID NO: 5 provides a fusion

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

[0216] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety is a molecule that specifically binds to Programmed Death-1 ligand 1 (PD-L1 or B7-H1) or Programmed Death-1 ligand 2 (PD-L2 or B7-DC). SEQ ID NO: 15 provides a fusion protein including anti-HER2/neu antibody and PD-1 Ectodomain (FIG. 13). SEQ ID NO: 16 provides a fusion protein including anti-EGFR1 antibody and PD-1 Ectodomain (FIG. 14). SEQ ID NO: 17 provides a fusion protein including anti-CD20 antibody and PD-1 Ectodomain

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(FIG. 15). SEQ ID NO: 18 provides a fusion protein including anti-VEGF antibody and PD-1 Ectodomain (FIG. 16). SEQ ID NO: 19 provides a fusion protein including anti-human CTLA-4 antibody and PD-1 Ectodomain (FIG. 17). SEQ ID NO: 20 provides a fusion protein including anti-CD25 antibody and PD-1 Ectodomain (FIG. 18A). SEQ ID NO: 21 provides a fusion protein including anti-CD25 antibody and PD-1 Ectodomain (FIG. 18B). SEQ ID NO: 22 provides a fusion protein including IL-2, Fc, and PD-1 ectodomain (IL-2 + Fc + PD-1 ectodomain; FIG. 19). SEQ ID NO: 23 provides a fusion protein including PD-1 ectodomain, Fc, and IL-2 (PD-1 ectodomain + Fc + IL-2; FIG. 19). SEQ ID NO: 24 provides a fusion protein including anti-CD4 antibody and PD-1 Ectodomain (FIG. 20). SEQ ID NO: 25 provides a fusion protein including RANK Ectodomain, Fc, and PD-1 ectodomain (RANK ectodomain + Fc + PD-1 ectodomain; FIG. 21). SEQ ID NO: 26 provides a fusion protein including PD-1 ectodomain, Fc, and RANK Ectodomain (PD-1 ectodomain + Fc + RANK ectodomain; FIG. 21).

[0217] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety is a molecule that specifically binds to Receptor activator of NF-kB ligand (RANKL). SEQ ID NO: 27 provides a fusion protein including anti-HER2/neu antibody and RANK Ectodomain (FIG. 23). SEQ ID NO: 28 provides a fusion protein including anti-EGFR1 antibody and RANK Ectodomain (FIG. 24). SEQ ID NO: 29 provides a fusion protein including anti-CD20 antibody and RANK Ectodomain (FIG. 25). SEQ ID NO: 30 provides a fusion protein including anti-VEGF antibody and RANK Ectodomain (FIG. 26). SEQ ID NO: 31 provides a fusion protein including anti-human CTLA-4 antibody and RANK Ectodomain (FIG. 27). SEQ ID NO: 32 provides a fusion protein including anti-CD25 antibody and RANK Ectodomain (FIG. 28A). SEQ ID NO: 33 provides a fusion protein including anti-CD25 antibody and RANK Ectodomain (FIG. 28B). SEQ ID NO: 34 provides a fusion protein including IL-2, Fc, and RANK ectodomain (IL-2 + Fc + RANK ectodomain; FIG. 29). SEQ ID NO: 35 provides a fusion protein including RANK ectodomain, Fc, and IL-2 (RANK ectodomain + Fc + IL-2; FIG. 29). SEQ ID NO: 36 provides a fusion protein including anti-CD4 antibody and RANK Ectodomain (FIG. 30).

[0218] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety includes a molecule that specifically binds to Programmed death-1 (PD-1). SEQ ID NO: 37 provides a fusion protein including anti-tumor necrosis factor (TNFα) antibody and PD-1 ligand 1 (FIG. 32). SEQ ID NO: 38 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand: (TNFR2 ECD + IgG Cγ1 + PD-L1; FIG. 33). SEQ ID NO: 39 provides a fusion protein including PD-1 ligand, Fc, and TNFR2 Extracellular ligand binding domain: (PD-L1 + IgG Cγ1 -TNFR2 ECD; FIG. 33). SEQ ID NO: 40 provides a fusion protein including anti-CD20 antibody and PD-1 ligand 1 (PD-L1) (FIG. 34). SEQ ID NO: 41 provides a fusion protein including anti-CD25 antibody and PD-1 ligand 1 (PD-L1) (FIG. 35A). SEQ ID NO: 42 provides a fusion protein including anti-CD25 antibody and PD-1 ligand 1 (PD-L1) (FIG. 35B). SEQ ID NO: 43 provides a fusion protein including PD-1 ligand 1 (PD-L1), Fc, and IL-2 (PD-L1 – Fc – IL2; FIG. 36). SEQ ID NO: 44 provides a fusion protein including IL-2, Fc, and PD-1 ligand 1 (PD-L1) (IL-2 – Fc – PD-L1; FIG. 36). SEQ ID NO: 45 provides a fusion protein including anti-CD4 antibody and PD-1 ligand 1 (PD-L1) (FIG. 37). SEQ ID NO: 46 provides a fusion protein including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from PD-1 ligand (PD-L1) (Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + PD-L1; FIG. 38). SEQ ID NO: 47 provides a fusion protein including the extracellular domain of PD-1 ligand (PD-L1), immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4: (PD-L1 + IgG Cγ1 + CTLA-4 ECD; FIG. 38). SEQ ID NO: 48 provides a fusion protein including Transforming growth factor-β (TGF-β), immunoglobulin Fc (IgG Cγ1), and a sequence from PD-1 ligand 1 (PD-L1) (TGFβ-1 + Fc + PD-L1; FIG. 39). SEQ ID NO: 49 provides a fusion protein including a sequence from PD-1 ligand 1 (PD-L1), immunoglobulin Fc (IgG Cγ1), and Transforming growth factor beta (TGF-□β) (PD-L1 + Fc + TGFβ-1; FIG. 39).

[0219] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety includes a

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molecule that specifically binds to Transforming growth factor-beta receptor (TGF-βR). SEQ ID NO: 50 provides a fusion protein including an antibody that binds TNF-α, and a sequence from Transforming growth factor-β (TGF-β) (FIG. 41). SEQ ID NO: 51 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and a sequence from Transforming growth factor-β (TGF-β) (TNFR2 ECD + IgG Cγ1 + TGF-β; FIG. 42). SEQ ID NO: 52 provides a fusion protein including a sequence from Transforming growth factor-β (TGF-β), Fc, and TNFR2 Extracellular ligand binding domain: (TGF-β + IgG Cγ1 + TNFR2 ECD; FIG. 42). SEQ ID NO: 53 provides a fusion protein including anti-CD20 antibody and a sequence from Transforming growth factor-β (TGF-β) (FIG. 43). SEQ ID NO: 54 provides a fusion protein including anti-CD25 antibody and a sequence from transforming growth factor-β (TGF-β) (FIG. 44A). SEQ ID NO: 55 provides a fusion protein including anti-CD25 antibody and a sequence from transforming growth factor-β (TGF-β) (FIG. 44B). SEQ ID NO: 56 provides a fusion protein including a sequence from Transforming growth factor-β (TGF-β), Fc, and IL-2 (TGF-β + Fc + IL-2; FIG. 45). SEQ ID NO: 57 provides a fusion protein including IL-2, Fc, and Transforming growth factor-β (TGF-β)(IL-2 + Fc + TGF-β; FIG. 45). SEQ ID NO: 58 provides a fusion protein including anti-CD4 antibody and a sequence from transforming growth factor-β (TGF-β) (FIG. 46). SEQ ID NO: 59 provides a fusion protein including the extracellular domain of CTLA-4, immunoglobulin Fc (IgG Cγ1), and a sequence from a sequence from transforming growth factor-β (TGF-β) (Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + TGF-β1; FIG. 47). SEQ ID NO: 60 provides a fusion protein including a sequence from Transforming growth factor-β (TGF-β), immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4: (TGF-β1 + IgG Cγ1 + CTLA-4 ECD) (FIG. 47).

[0220] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety is a molecule that specifically binds to Receptor activator of NF-kB ligand (RANKL). SEQ ID NO: 61 provides a fusion protein including an antibody that binds TNF-α, and a sequence from RANK Ectodomain (FIG. 48). SEQ ID NO: 62 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and a sequence from RANK Ectodomain

(TNFR2 ECD + IgG Cγ1 + RANK Ectodomain; FIG. 49). SEQ ID NO: 63 provides a fusion protein including a sequence from RANK Ectodomain, Fc, and TNFR2 Extracellular ligand binding domain: (RANK Ectodomain + IgG Cγ1 + TNFR2 ECD; FIG. 49). SEQ ID NO: 64 provides a fusion protein including the extracellular domain of CTLA-4, immunoglobulin Fc (IgG Cγ1), and a sequence from a sequence from RANK Ectodomain (Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + RANK Ectodomain; FIG. 50). SEQ ID NO: 65 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4: (RANK Ectodomain + IgG Cγ1 + CTLA-4 ECD) (FIG. 50). SEQ ID NO: 66 provides a fusion protein including a sequence from transforming growth factor-β (TGF-β), immunoglobulin Fc region (IgG Cγ1), and an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) (TGF-β + IgG Cγ1 + RANK Ectodomain; FIG. 51). SEQ ID NO: 67 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG Cγ1), and a sequence from transforming growth factor-β (TGF-β): (RANK Ectodomain + IgG Cγ1 + TGF-β) (FIG. 51). SEQ ID NO: 68 provides a fusion protein including a sequence from Programmed death-1 ligand 1 (PD-L1), immunoglobulin Fc region (IgG Cγ1), and an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) (PD-L1 + IgG Cγ1 + RANK Ectodomain; FIG. 52). SEQ ID NO: 69 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG Cγ1), and a sequence from Programmed death-1 ligand 1 (PD-L1): (RANK Ectodomain + IgG Cγ1 + PD-L1) (FIG. 52).

[0221] Although the invention has been described with reference to the above example, it will be understood that modifications and variations are encompassed within the spirit and scope of the invention. Accordingly, the invention is limited only by the following claims.

What is claimed is:

1. An isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:

(a) the targeting moiety comprises an antibody, antibody fragment, scFv, or Fc-containing polypeptide comprising amino acids 1-119 of SEQ ID NO: 2 and amino acids 1-107 of SEQ ID NO: 71, and that specifically binds EGFR; and

(b) the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor II (TGF-βRII) comprising SEQ ID NO: 87.

2. The molecule of claim 1, wherein the molecule comprises a light chain, wherein the light chain comprises, from N-terminus to C-terminus, an amino acid sequence comprising SEQ ID NO: 71, a linker comprising SEQ ID NO: 104, and an amino acid sequence comprising SEQ ID NO: 87.

3. The molecule of claim 2, wherein the molecule further comprises a heavy chain polypeptide, wherein the heavy chain comprises amino acids 1-119 of SEQ ID NO: 2.

4. A method of treating cancer in a subject in need thereof, comprising:

administering a therapeutically effective amount of a molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein:

(a) the targeting moiety comprises an antibody, antibody fragment, scFv, or Fc-containing polypeptide comprising amino acids 1-119 of SEQ ID NO: 2 and amino acids 1-107 of SEQ ID NO: 71, and that specifically binds EGFR; and

(b) the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor II (TGF-βRII) comprising SEQ ID NO: 87.

5. The method of claim 4, wherein the molecule comprises a light chain, wherein the light chain comprises, from N-terminus to C-terminus, an amino acid sequence comprising SEQ ID NO: 71, a linker comprising SEQ ID NO: 104, and an amino acid sequence comprising SEQ ID NO: 87.

6. The method of claim 5, wherein the molecule further comprises a heavy chain polypeptide, wherein the heavy chain comprises amino acids 1-119 of SEQ ID NO: 2.

7. The method of claim 4, wherein the cancer is selected from the group consisting of: prostate cancer, pancreatic cancer, biliary cancer, colon cancer, rectal cancer, liver cancer, kidney cancer, lung cancer, testicular cancer, breast cancer, ovarian cancer, pancreatic cancer, brain cancer, head and neck cancer, melanoma, sarcoma, multiple myeloma, leukemia and lymphoma.

8. The method of claim 7, wherein the cancer is head and neck squamous cell cancer (HNSCC).

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

ABSTRACT OF THE DISCLOSURE

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

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	JHU3260-10
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	

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

Secrecy Order 37 CFR 5.2:

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

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Atul		Bedi	

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

City	Timonium	State/Province	MD	Country of Residence	US

Mailing Address of Inventor:

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Address 2			
City	Timonium	State/Province	MD
Postal Code	21093	Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Rajani		Ravi	

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

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

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

Correspondence Information:

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

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	JHU3260-10
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	

☐ An Address is being provided for the correspondence Information of this application.			
Customer Number	100899		
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Application Information:

Title of the Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		
Attorney Docket Number	JHU3260-10	**Small Entity Status Claimed**	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	75	**Suggested Figure for Publication (if any)**	

Filing By Reference:

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

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

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

Publication Information:

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

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

Representative Information:

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

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

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	JHU3260-10
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	

Domestic Benefit/National Stage Information:

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

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	17/694541	2022-03-14

Prior Application Status	Patented			Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
17/694541	Continuation of	16/601347	2019-10-14	11274156	2022-03-15
Prior Application Status	Patented			Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
16/601347	Continuation of	15/362632	2016-11-28	10442860	2019-10-15
Prior Application Status	Patented			Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
15/362632	Division of	15/231309	2016-08-08	9850306	2017-12-26
Prior Application Status	Patented			Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
15/231309	Continuation of	14/645282	2015-03-11	9441044	2016-09-13
Prior Application Status	Patented			Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
14/645282	Continuation of	13/582717	2012-10-17	8993524	2015-03-31

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

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	JHU3260-10
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	

Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2011/027317	Claims benefit of provisional	61/311255	2010-03-05
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.			Add

Foreign Priority Information:

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

			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)
Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.			Add

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

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

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	JHU3260-10
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	

Authorization or Opt-Out of Authorization to Permit Access:

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

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

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

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

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

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

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

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

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

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

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

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	JHU3260-10
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	

Applicant Information:

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

Applicant 1 Remove

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

Clear

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

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

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

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

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

Assignee Information including Non-Applicant Assignee Information:

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

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

Assignee 1

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

Remove

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

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

Mailing Address Information For Assignee including Non-Applicant Assignee:

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

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

Signature:

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

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

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

Signature	/Pamela E. Hwang/			Date (YYYY-MM-DD)	2024-09-12
First Name	Pamela	Last Name	Hwang	Registration Number	54,984

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	JHU3260-10
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS	

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

FIG. 1A

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

FIG. 1B

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

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

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

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

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

TGF-β R-II (Δi2) contains a deletion of amino acids 498 to 508 and is inactive as a kinase (SEQ ID NO: 91):

MGRGLLRGLW PLHIVLWTRI AS*TIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST*
CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK
*CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDL LLVIFQ*VTGI SLLPPLGVAI
SVIIIFYCYR VNRQQKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNTE
LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK
HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH
LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSGQVG
TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSKVRE
HPCVESMKD**A** **S**GIQMVCETL TECWDHDPEA RLTAQCVAER FSELEHLDRL SGRSCSEEKI
PEDGSLNTTK

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

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

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

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

GGGGSGGGGSGGGGS

FIG. 1C

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

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

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

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

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSR
SGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

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

FIG. 2

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

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

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

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

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSG
SGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

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

FIG. 3

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

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

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

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

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

FIG. 4

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

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

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

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

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSG
SGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

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

FIG. 5

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

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

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

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

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

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

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

Italic: *Linker*

Bold: **TGFβRII ectodomain**

FIG. 6

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

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

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

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

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

Bold: **TGFβRII ectodomain**
Italic: *Linker (Optional; other linker sequences)*
Plain: Fc
Underlined: IL-2 fragment
(Note: Can replace linker *GGGGSGGGGSGGGGS* SEQ ID NO: 104 with *EPKSCDK* SEQ ID NO: 105)
(Note: Can replace underlined aa in Fc: **E** with D and **M** with L in Fc)

FIG. 7

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

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

QVQLVQSGAE VKKPGSSVKV SCKASGYTFT SYRMHWVRQA PGQGLEWIGY INPSTGYTEY
NQKFKDKATI TADESTNTAY MELSSLRSED TAVYYCARGG GVFDYWGQGT LVTVSSASTK
GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN
VFSCSVMHEA LHNHYTQKSL SLSPGK *GGGGSGGGGSGGGGS* **TIPPHVQK SVNNDMIVTD**
NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV
CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPD

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

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

Plain: Anti-CD25 antibody
Italic: *Linker*
Bold: **TGFβRII ectodomain**

FIG. 8A

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

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

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

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

Plain: Anti-CD25 antibody

Italic: *Linker*

Bold: **TGFβRII ectodomain**

FIG. 8B

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

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

Heavy chain fusion protein:

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

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

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

Italic: *Linker*

Bold: **TGFβRII ectodomain**

FIG. 9

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

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

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

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

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

Underlined: PD-1 ectodomain

Italic: *Linkers*

Plain: IgG1 Fc

Bold: **TGFβRII ectodomain**

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

FIG. 10

Fusion proteins comprising Transforming growth factor-beta receptor II (TGFβ-RII) ectodomain, Fc, and Receptor activator of nuclear factor–κB (RANK) Ectodomain:

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

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

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

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

Bold: TGFβRII ectodomain

Plain: IgG1 Fc

Italics: Linker

Underlined: RANK ectodomain

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

FIG. 11

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

1 MQIPQAPWPV VWAVLQLGWR *PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS*

61 ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT

121 YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV VGVVGGLLGS

181 LVLLVWVLAV ICSRAARGTI GARRTGQPLK EDPSAVPVFS VDYGELDFQW REKTPEPPVP

241 CVPEQTEYAT IVFPSGMGTS SPARRGSADG PRSAQPLRPE DGHCSWPL

Italic: *PD-1 ectodomain*
Underlined Italic: *ligand-binding domain*

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

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

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

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

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

FIG. 12

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

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

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

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

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSR
SGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-HER2/neu Variable region

Plain: Anti-HER2/neu Constant region

Italic: *Linker*

Bold: **PD-1 ectodomain**

FIG. 13

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

Anti-EGFR heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 16):

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPFTSR
LSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVSAASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS**
ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT
YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV

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

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSG
SGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-EGFR Variable region

Plain: Anti-EGFR Constant region

Italic: *Linker*

Bold: **PD-1 ectodomain**

FIG. 14

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

Anti-CD20 heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 17):

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKG
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS **PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQTLV**

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Underlined: Anti-CD20 Variable region
Plain: Anti-CD20 Constant region
Italic: *Linker*
Bold: **PD-1 ectodomain**

FIG. 15

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

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

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

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

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSG
SGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: Anti-VEGF Variable region
Plain: Anti-VEGF Constant region
Italic: *Linker*
Bold: **PD-1 ectodomain**

FIG. 16

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

Anti-(human CTLA-4) (human γ1-chain)-PD-1 ectodomain fusion protein, disulfide with human κ-chain, dimer

Anti-CTLA-4 heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 19):

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

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

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

Underlined: Anti-CTLA-4 Variable region

Plain: Anti-CTLA-4 Constant region

Italic: *Linker*

Bold: **PD-1 ectodomain**

FIG. 17

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

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

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

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

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

Plain: Anti-CD25 antibody

Italic: *Linker*

Bold: **PD-1 ectodomain**

FIG. 18A

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

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

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

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

Plain: Anti-CD25 antibody

Italic: *Linker*

Bold: **PD-1 ectodomain**

FIG. 18B

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

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

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

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

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

Underlined: IL-2 fragment
Italic: *Linker*
Plain: Fc
Bold: **human PD-1 ectodomain**
(Note: Can replace optional linker 1 *GGGGSGGGGSGGGGS* SEQ ID NO: 104 with *EPKSCDK* SEQ ID NO: 105)
(Note: Can replace underlined aa in Fc: **E** with D and **M** with L)

FIG. 19

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

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

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSISIDTSKNLFSLKLRSVTAADTAVYYCASNILKYLHWLLYWGQGVLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR
SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRPAGQFQTLV

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

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

Italic: *Linker*

Bold: **PD-1 ectodomain**

FIG. 20

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

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

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

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

PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDR SQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVP TAHPSPSPRPAGQFQTLV*GGGGSGGGGSGGGGS***

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

Bold: PD-1 ectodomain

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

Plain: IgG1 Fc

Underlined: RANK ectodomain

FIG. 21

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

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

1 MAPRARRRRP LFALLLLCAL LARLQVAL**QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
61 TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW
121 SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
181 RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPGLIILLLF ASVALVAAII FGVCYRKKGK
241 ALTANLWHWI NEACGRLSGD KESSGDSCVS THTANFGQQG ACEGVLLLTL EEKTFPEDMC
301 YPDQGGVCQG TCVGGGPYAQ GEDARMLSLV SKTEIEEDSF RQMPTEDEYM DRPSQPTDQL
361 LFLTEPGSKS TPPFSEPLEV GENDSLSQCF TGTQSTVGSE SCNCTEPLCR TDWTPMSSEN
421 YLQKEVDSGH CPHWAASPSP NWADVCTGCR NPPGEDCEPL VGSPKRGPLP QCAYGMGLPP
481 EEEASRTEAR DQPEDGADGR LPSSARAGAG SGSSPGGQSP ASGNVTGNSN STFISSGQVM
541 NFKGDIIVVY VSQTSQEGAA AAAEPMGRPV QEETLARRDS FAGNGPRFPD PCGGPEGLRE
601 PEKASRPVQE QGGAKA

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

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

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

1 MAPRARRRRP LFALLLLCAL LARLQVAL**QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
61 TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VC*DTGK*ALVA VVAGNSTTPR RCACTAGYHW
121 S*QDCECCRRN* TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
181 RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

Bold: RANK extracellular domain (RANK ligand-binding domain)
RANK peptide sequences (Underlined) and residues (Italics) involved in binding RANKL.

(iv). Mutant of RANK or a fragment thereof which binds Receptor activator of nuclear factor–κB (RANK) ligand (RANKL)

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

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

Bold: RANK ligand-binding domain

FIG. 22

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

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

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

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

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFT
LTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Underlined: Anti-HER2 antibody variable region

Plain: Anti-HER2 antibody constant region

Italics: Linker

Bold: RANK ectodomain

FIG. 23

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

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

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

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

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFT
LSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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

FIG. 24

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

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

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKGKATLTA
DKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGK *GGGGSGGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC**
TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR RCACTAGYHW
SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK
RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGSGTSYSL
TISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Underlined: Anti-CD20 antibody variable region

Plain: Anti-CD20 antibody constant region

Italics: Linker

Bold: RANK ectodomain

FIG. 25

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

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

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

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

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
LSSPVTKSFNRGEC

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

FIG. 26

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

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

QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYTMHWVRQA PGKGLEWVTF ISYDGNNKYY
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGQ GTLVTVSSAS
TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL
YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPELLGGPS
VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
GNVFSCSVMH EALHNHYTQK SLSLSPGK *GGGGSGGGGSGGGGS* **QI APPCTSEKHY EHLGRCCNKC**
EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK VCDTGKALVA VVAGNSTTPR
RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV CKPCLAGYFS DAFSSTDKCR
PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG

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

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

Underlined: Anti-CTLA-4 antibody variable region

Plain: Anti-CTLA-4 antibody constant region

Italics: Linker

Bold: RANK ectodomain

FIG. 27

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

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

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

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

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

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

FIG. 28A

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

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

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

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

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

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

FIG. 28B

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

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

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

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

QI APPCTSEKHY EHLGRCCNKC EPGKYMSSKC TTTSDSVCLP CGPDEYLDSW NEEDKCLLHK
VCDTGKALVA VVAGNSTTPR RCACTAGYHW SQDCECCRRN TECAPGLGAQ HPLQLNKDTV
CKPCLAGYFS DAFSSTDKCR PWTNCTFLGK RVEHHGTEKS DAVCSSSLPA RKPPNEPHVY LPG
*GGGGSGGGGSGGGGS*THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGS*APTSSSTKKTQLQLEHLLLDLQMILNGINNYKN
PKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMC
EYADETATIVEFLNRWITFCQSIISTLT

Bold: RANK ectodomain

Italics: Linker (Optional; any linker sequence)

Plain: Fc

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

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

FIG. 29

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

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

Heavy chain fusion protein:

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

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

Underlined: Anti-CD4 antibody variable region

Italic: *Linker*

Bold: **PD-1 ectodomain**

FIG. 30

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

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

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

AFT VTVPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV NAPYNKINQR ILVVDPVTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL FNVTSTLRIN TTTNEIFYCT FRRLDPEENH TAELVI

(iii) Human PD-1 ligand 2 [PD-L2 (B7-DC)] or a fragment thereof.

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

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

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

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

Underlined Bold: **Ligand Binding domain**

FIG. 31

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

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

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

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

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLPGKAPKLLIYAASTLQSGVPSRFSGSG
SGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: anti-TNFα antibody variable region

Plain: anti-TNFα antibody constant region

Italic: *Linker*

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

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

FIG. 32

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

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

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

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

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

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

Underlined: TNFR2 ligand-binding domain
Plain: human IgG1 heavy chain constant region (IgG Cγ1 domain = H, CH2, CH3)
Italic: *Linker (Linker 1 is optional and may be replaced with another linker such as EPKSCDK* (SEQ. ID. NO: 105) *or GGGGSGGGGSGGGGS* (SEQ. ID. NO: 104))
Bold: **human PD-1 ligand 1 or extracellular binding domain of PD-L1**

FIG. 33

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

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

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKG
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Underlined: anti-CD20 antibody variable region

Plain: anti-CD20 antibody constant region

Italic: *Linker*

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

FIG. 34

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

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

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

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

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

Plain: anti-CD25 antibody

Italic: *Linker* (optional and may be replaced)

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

FIG. 35A

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

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

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

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

Plain: anti-CD25 antibody

Italic: *Linker*

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

FIG. 35B

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

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

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

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

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNL
AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
GGGGSGGGGSGGGGS
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR**E**E**M**TKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK *GGGGSGGGGSGGGGS*
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEED
LKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD
PVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEE
T

Underlined: IL-2 fragment
Italic: *Linker (optional)*
Plain: Fc
Bold: **human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)**
(Note: Can replace linker 1 *GGGGSGGGGSGGGGS* SEQ ID NO: 104 with *EPKSCDK* SEQ ID NO: 105)
(Note: Can replace underlined aa in Fc: **E** with D and **M** with L)

FIG. 36

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

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

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSISIDTSKNLFSLKLRSVTAADTAVYYCASNILKYLHWLLYWGQGVLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
RIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQF
VHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAP
YNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRI
NTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGR
MMDVKKCGIQDTNSKKQSDTHLEET

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

Underlined: Anti-CD4 antibody variable region

Plain: Anti-CD4 antibody constant region

Italic: *Linker*

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

FIG. 37

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

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

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

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

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

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

Underlined Italic: *Oncostatin M Signal Peptide (-25 to -1)*
Underlined: CTLA-4 extracellular domain (1-125)
Plain: human IgG1 heavy chain constant region (IgG Cγ1 domain = H, CH2, CH3)
Italic: *Linker (optional)*
Bold: **human PD-1 ligand 1 (PD-1L) or extracellular binding domain of PD-1L (underlined)**
(Note: Optional C to S conversion in IgG sequence (bold underlined))
(Note: The linker *QEPKSCDK* SEQ ID NO: 110 can be replaced with *EPKSCDK* SEQ ID NO: 105 or another linker sequence)

FIG. 38

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

TGFβ-1 + Fc + PD-L1 (SEQ ID NO: 48):

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

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

PD-L1 + Fc + TGFβ-1 (SEQ ID NO: 49):

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

Underlined: TGFβ-1
Italic: *Linker (optional)*
Plain: Fc
Bold: **human PD-1 ligand 1 or extracellular binding domain of PD-1L (underlined)**
(Note: Can replace linker *GGGGSGGGGSGGGGS* SEQ ID NO: 104 with *EPKSCDK* SEQ ID NO: 105)
(Note: Can replace underlined aa in Fc: **E** with D and **M** with L)

FIG. 39

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

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

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

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

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

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

FIG. 40

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

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

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

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

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLPGKAPKLLIYAASTLQSGVPSRFSGSG
SGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: anti-TNFα antibody variable region

Plain: anti-TNFα antibody constant region

Italic: *Linker*

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

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

FIG. 41

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

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

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

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

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

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

Underlined: TNFR2 ligand-binding domain

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

Italic: *Linker*

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

(Note: Can replace linker 1 with *GGGGSGGGGSGGGGS* SEQ ID NO: 104 or *EPKSCDK* SEQ ID NO: 105)
(Note: Can replace underlined aa in Fc: **E** with D and **M** with L)

FIG. 42

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

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

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKG
KATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

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

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGS
GTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Underlined: anti-CD20 antibody variable region

Plain: anti-CD20 antibody constant region

Italic: *Linker*

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

FIG. 43

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

Anti-CD25 (Daclizumab) heavy chain and TGF-β1 (SEQ ID NO: 54):

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

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

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

Plain: anti-CD25 antibody

Italic: *Linker*

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

FIG. 44A

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

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

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

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

Plain: anti-CD25 antibody

Italic: *Linker*

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

FIG. 44B

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

TGF-β1 + Fc + IL-2 (SEQ ID NO: 56):

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

IL-2 + Fc + TGF-β1 (SEQ ID NO: 57):

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

Underlined: IL-2 fragment
Italic: *Linker (optional)*
Plain: Fc
Bold: **Mature (active) human TGF-β1**
(Note: Can replace linker 1 *GGGGSGGGGSGGGGS* SEQ ID NO: 104 with *EPKSCDK* SEQ ID NO: 105)
(Note: Can replace underlined aa in Fc: **E** with D and **M** with L)

FIG. 45

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

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

Heavy chain fusion protein:

QVQLQEAGPGLVKPSETLSLTCSVSGGSISGDYYWFWIRQSPGKGLEWIGYIYGSGGGTNYNPSLN
NRVSISIDTSKNLFSLKLRSVTAADTAVYYCASNILKYLHWLLYWGQGVLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
GGGGSGGGGSGGGGS
ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

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

Light chain:

SYELSQPRSVSVSPGQTAGFTCGGDNVGRKSVQWYQQKPPQAPVLVIYADSERPSGIPARFSGSNS
GNTATLTISGVEAGDEADYYCQVWDSTADHWVFGGGTRLTVLGRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

Underlined: Anti-CD4 antibody variable region

Plain: Anti-CD4 antibody constant region

Italic: *Linker*

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

FIG. 46

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

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

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

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

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

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

Underlined Italic: *Oncostatin M Signal Peptide (-25 to -1)*
Underlined: CTLA-4 extracellular domain (1-125)
Plain: human IgG1 heavy chain constant region (IgG Cγ1 domain = H, CH2, CH3)
Italic: *Linker (optional)*
Bold: **Mature (active) human TGF-β1**
(Note: Optional C to S conversion in IgG sequence (bold underlined))
(Note: The linker *QEPKSCDK* SEQ ID NO: 110 can be replaced with *EPKSCDK* SEQ ID NO: 105 or another linker sequence)

FIG. 47

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

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

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

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

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQLPGKAPKLLIYAASTLQSGVPSRFSGSG
SGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

Underlined: anti-TNFα antibody variable region

Plain: anti-TNFα antibody constant region

Italic: *Linker*

Bold: **RANK ectodomain**

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

FIG. 48

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

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

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

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

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

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

Underlined: TNFR2 ligand-binding domain

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

Italic: *Linker*

Bold: **RANK ectodomain**

(Note: Can replace linker 1 with *GGGGSGGGGSGGGGS* SEQ ID NO: 104 or *EPKSCDK* SEQ ID NO: 105)
(Note: Can replace underlined aa in Fc: **E** with D and **M** with L)

FIG. 49

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

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

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

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

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

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

Underlined Italic: *Oncostatin M Signal Peptide (-25 to -1)*
Underlined: CTLA-4 extracellular domain (1-125)
Plain: human IgG1 heavy chain constant region (IgG Cγ1 domain = H, CH2, CH3)
Italic: *Linker (optional)*
Bold: **RANK ectodomain**
(Note: Optional C to S conversion in IgG sequence (bold underlined))
(Note: The linker *QEPKSCDK* SEQ ID NO: 110 can be replaced with *EPKSCDK* SEQ ID NO: 105 or another linker sequence)

FIG. 50

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

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

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

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

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

Underlined: Mature (active) human TGF-β1
Italic: *Linker (optional)*
Plain: Fc
Bold: **RANK ectodomain**
(Note: Can replace linker 1 *GGGGSGGGGSGGGGS* SEQ ID NO: 104 with *EPKSCDK* SEQ ID NO: 105)
(Note: Can replace underlined aa in Fc: **E** with D and **M** with L)

FIG. 51

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

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

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

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

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

Bold: PD-1L1 (May use PD-1L1 extracellular domain – underlined)
Italic: *Linker (optional)*
Plain: Fc
Underlined: RANK ectodomain
(Note: Can replace linker 1 *GGGGSGGGGSGGGGS* SEQ ID NO: 104 with *EPKSCDK* SEQ ID NO: 105)
(Note: Can replace underlined aa in Fc: **E** with D and **M** with L)

FIG. 52

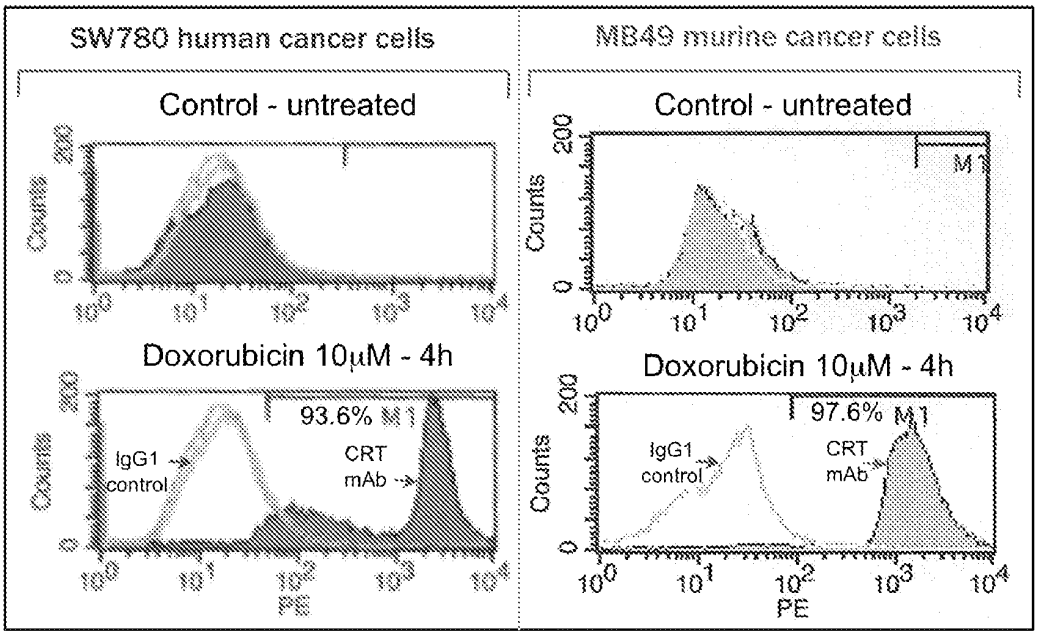

FIG. 53A

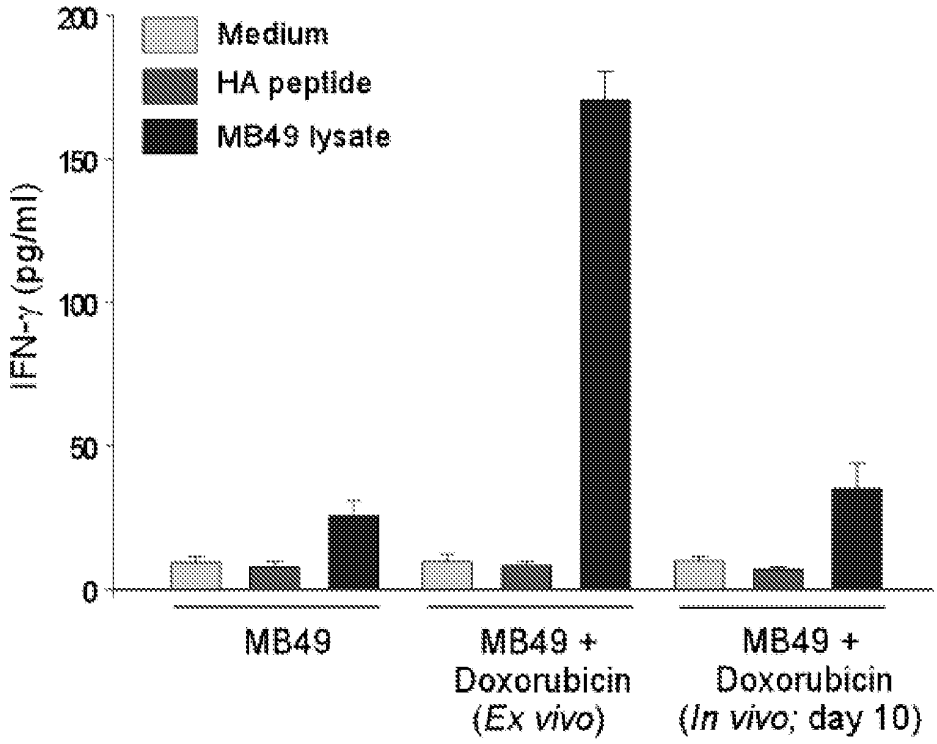

FIG. 53B

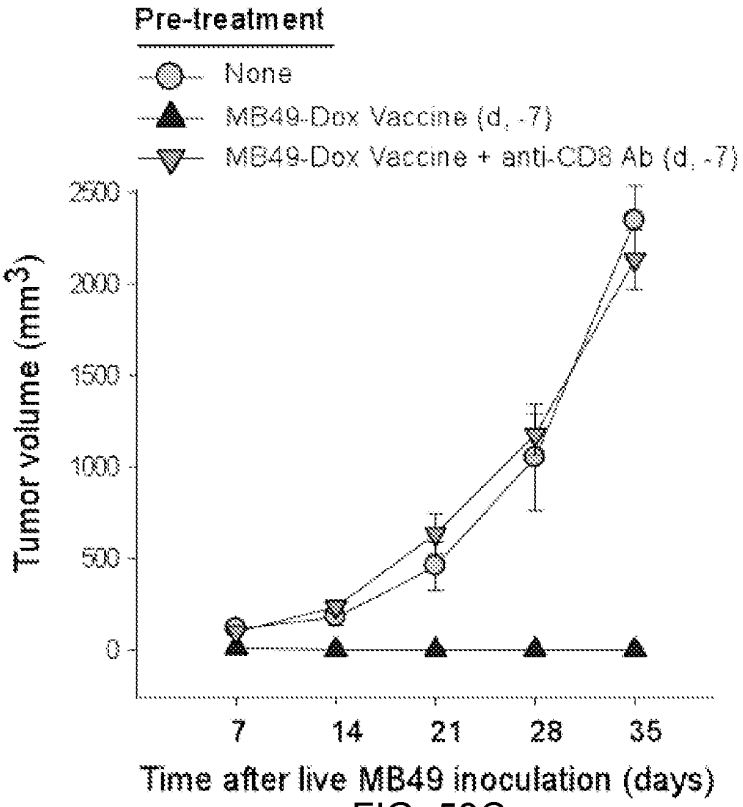

FIG. 53C

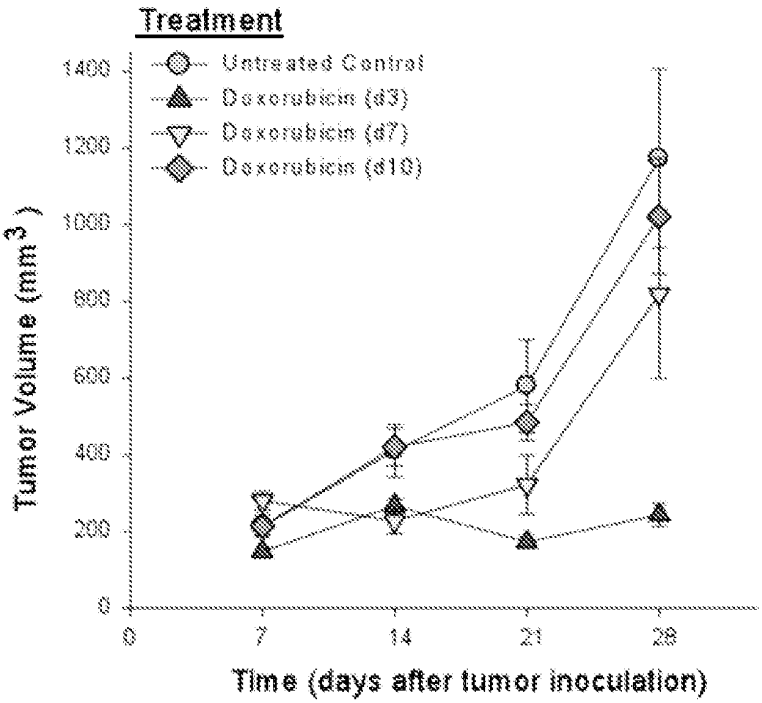

FIG. 53D

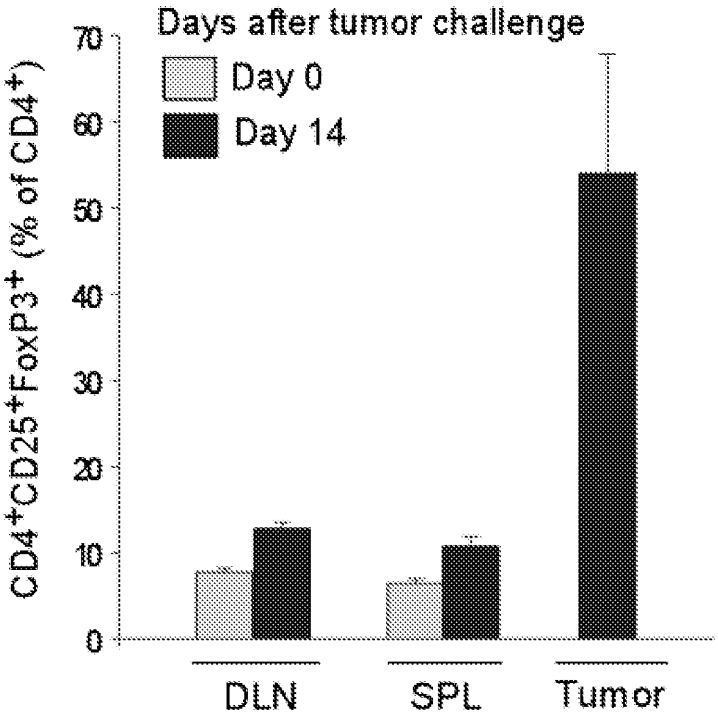

FIG. 53E

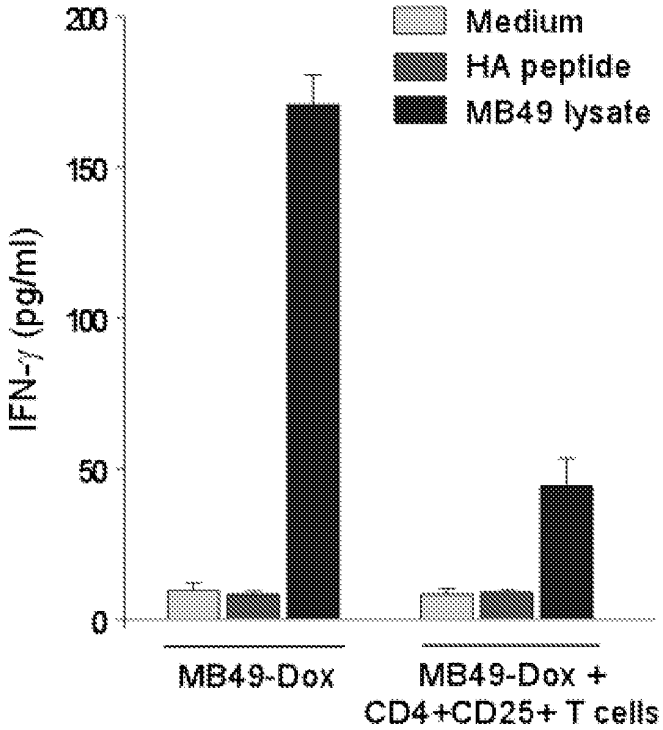

FIG. 53F

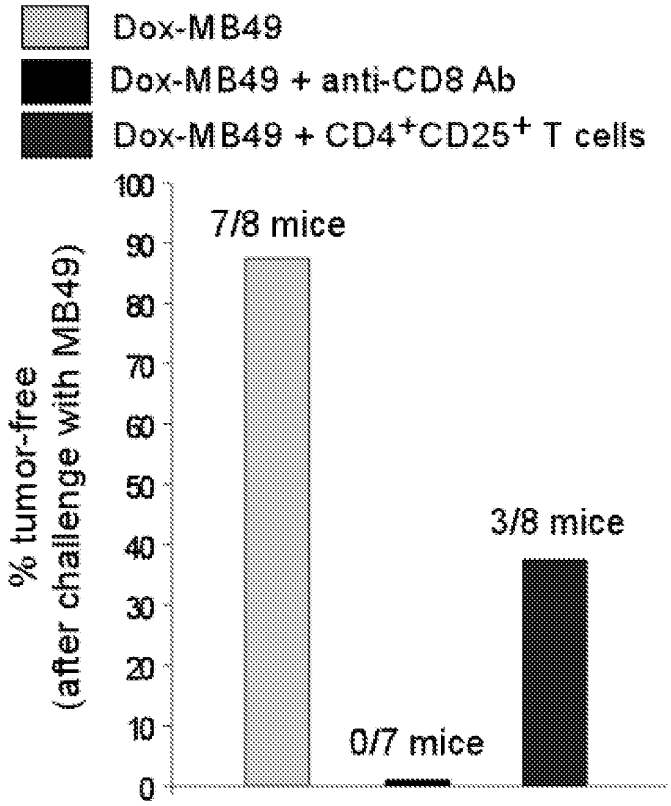
Dox-MB49
Dox-MB49 + anti-CD8 Ab
Dox-MB49 + CD4+CD25+ T cells
% tumor-free
(after challenge with MB49)
100
90
80
70
60
50
40
30
20
10
0
7/8 mice
0/7 mice
3/8 mice

FIG. 53G

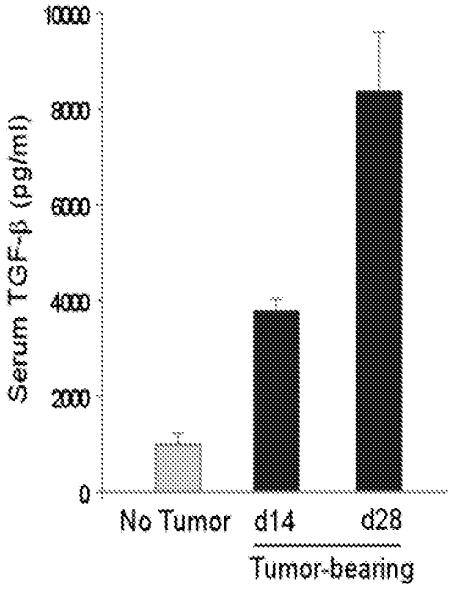

FIG. 54A

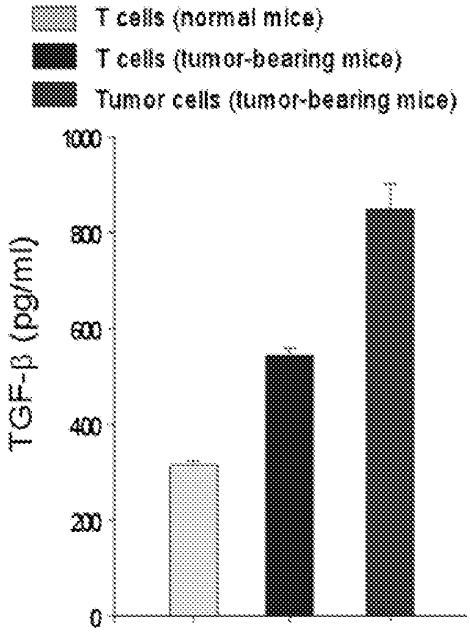

FIG. 54B

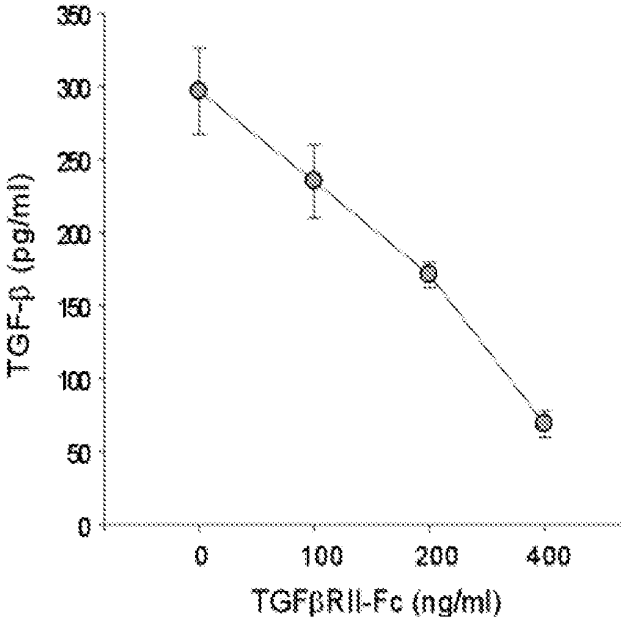

FIG. 54C

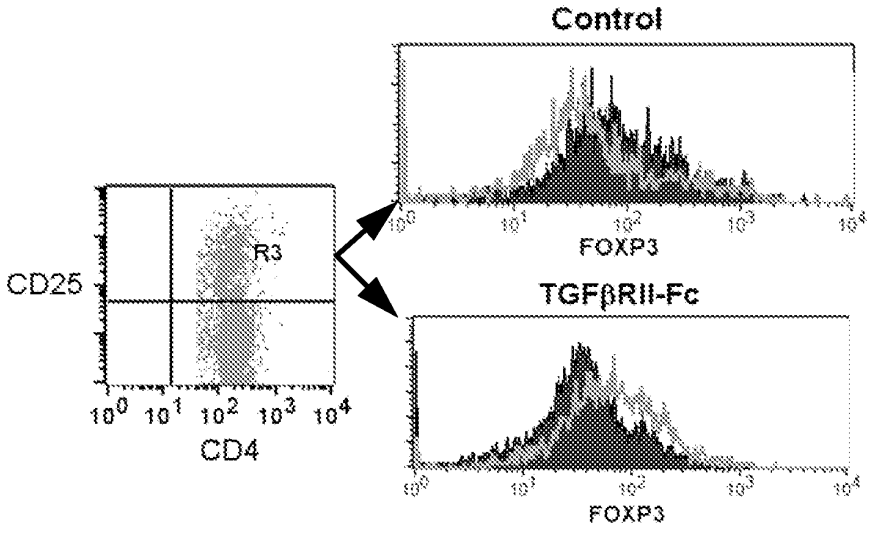

FIG. 54D

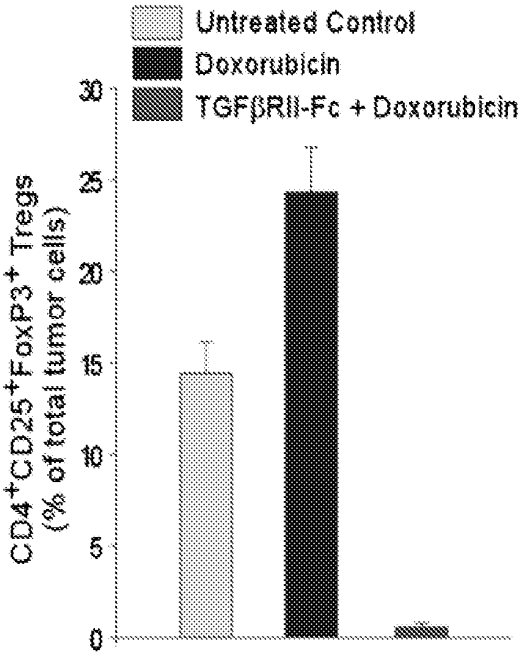

FIG. 54E

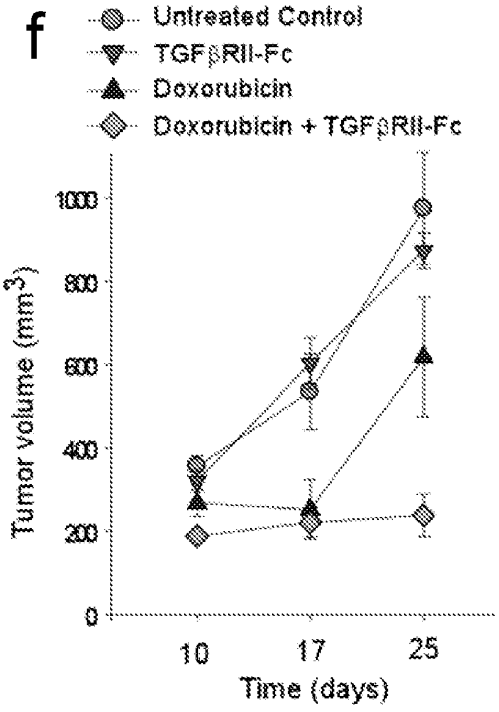

FIG. 54F

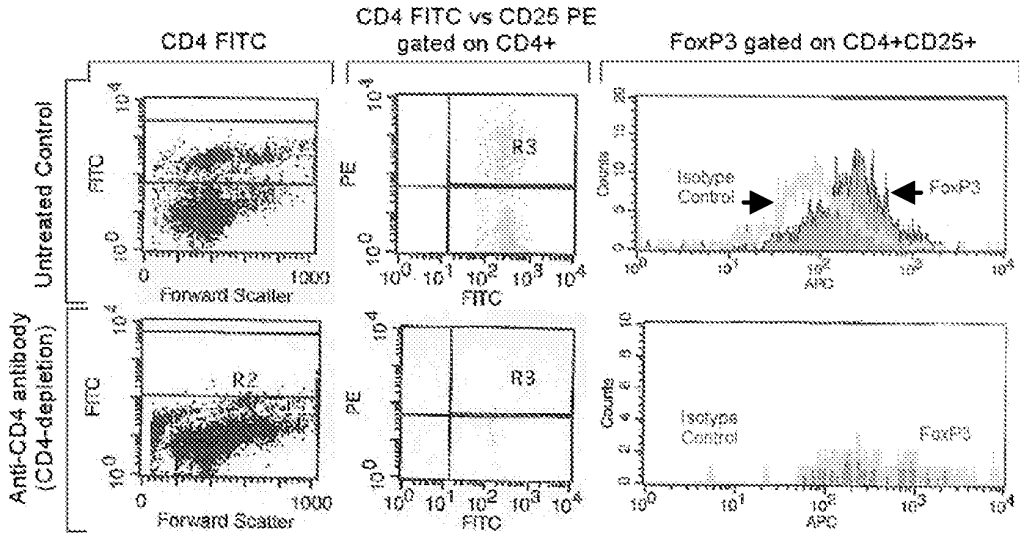

FIG. 55A

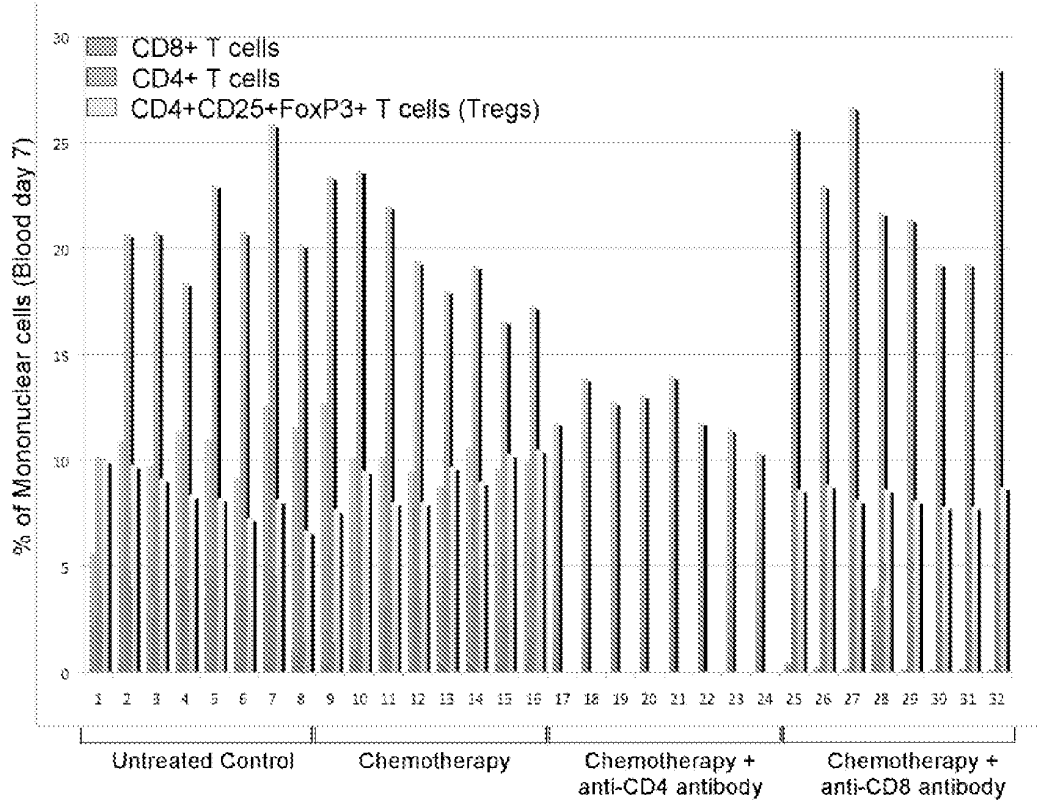

FIG. 55B

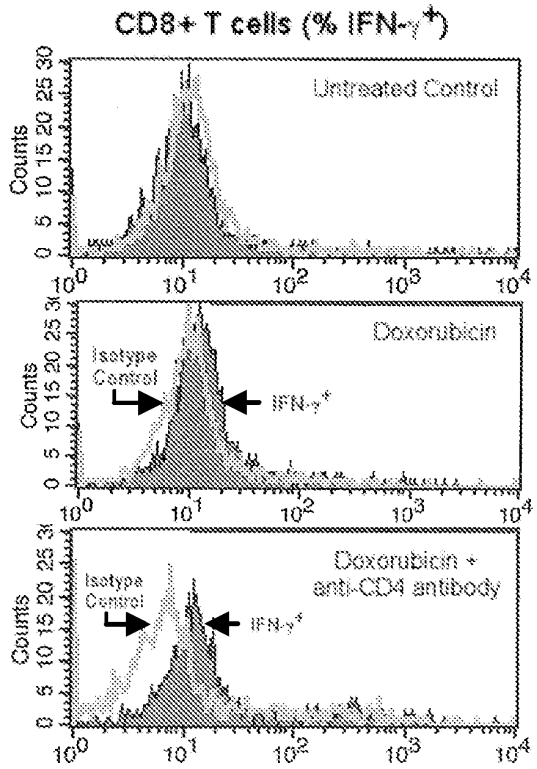

FIG. 55C

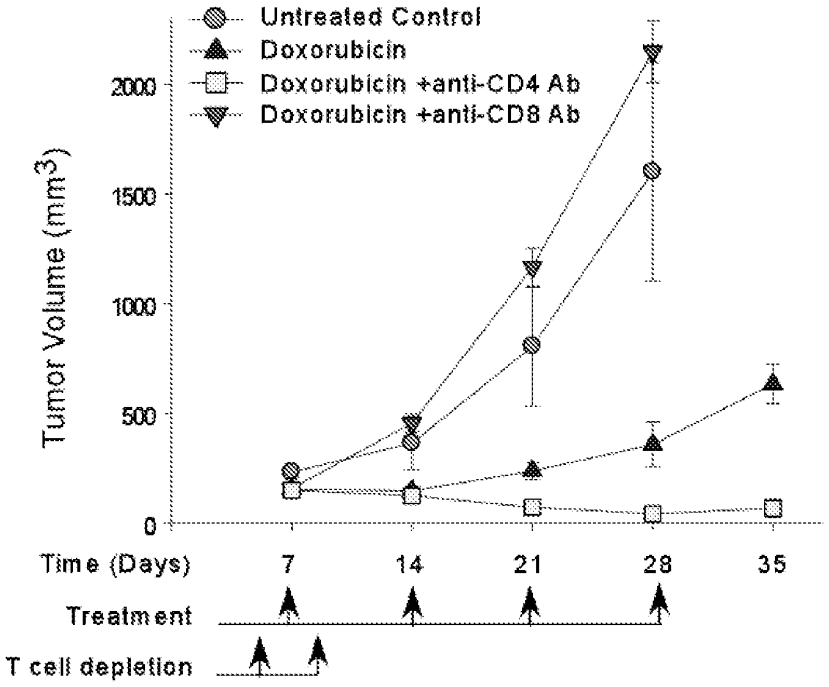

FIG. 55D

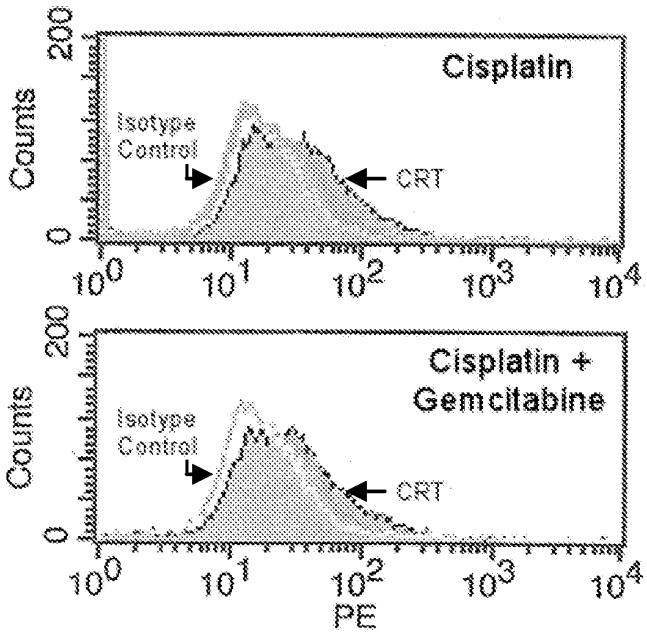

FIG. 56A

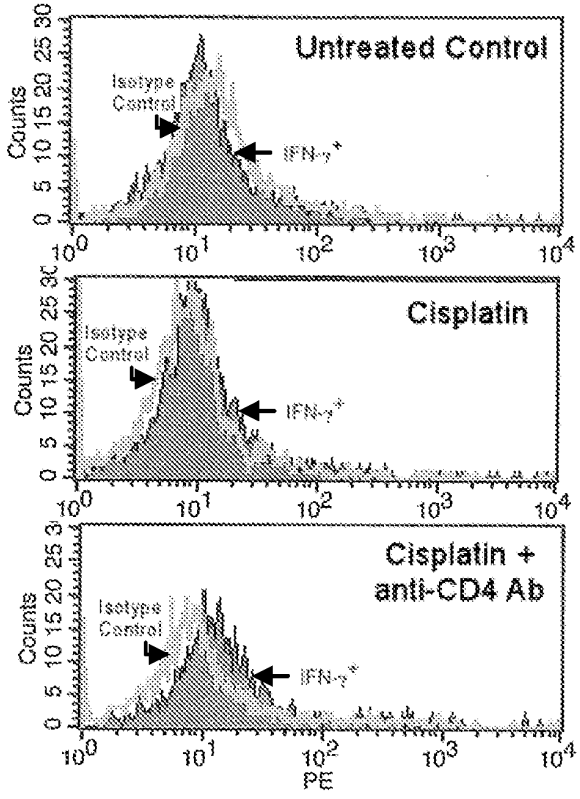

FIG. 56B

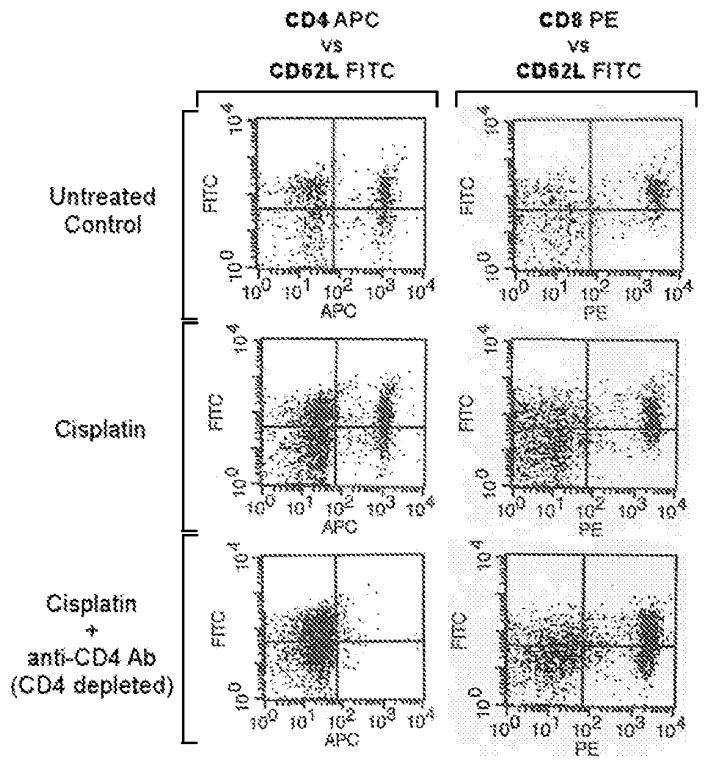

FIG. 56C

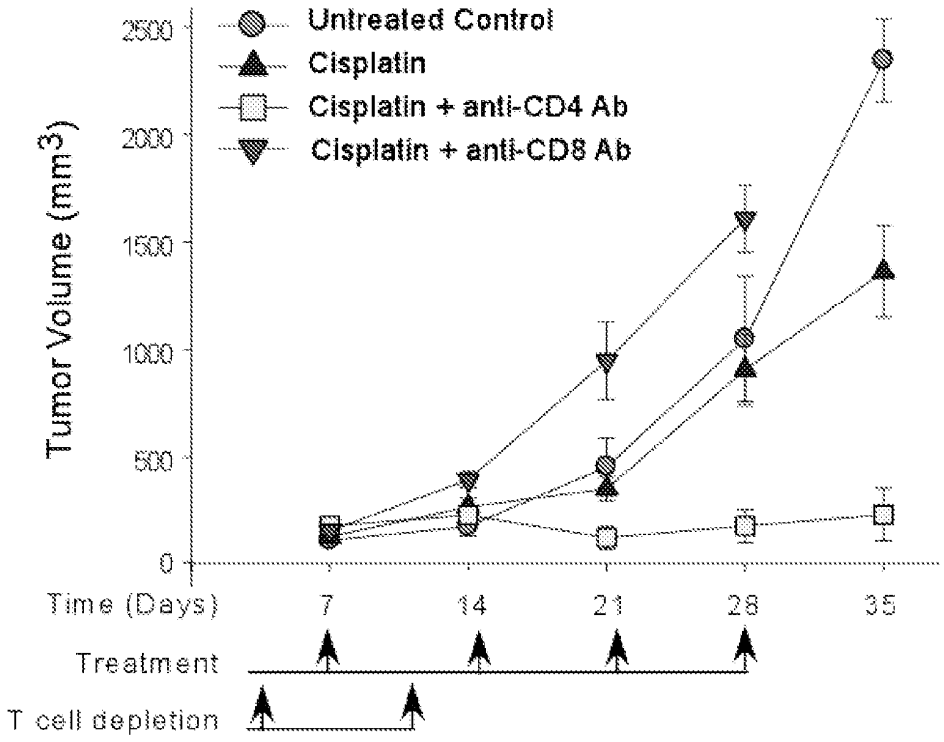

FIG. 56D

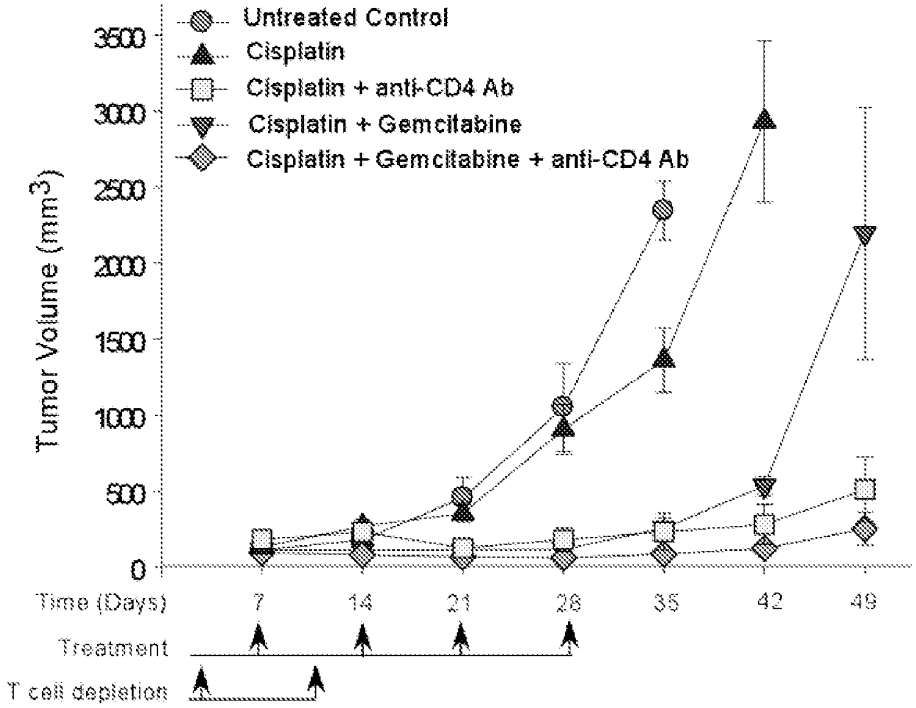

FIG. 56E

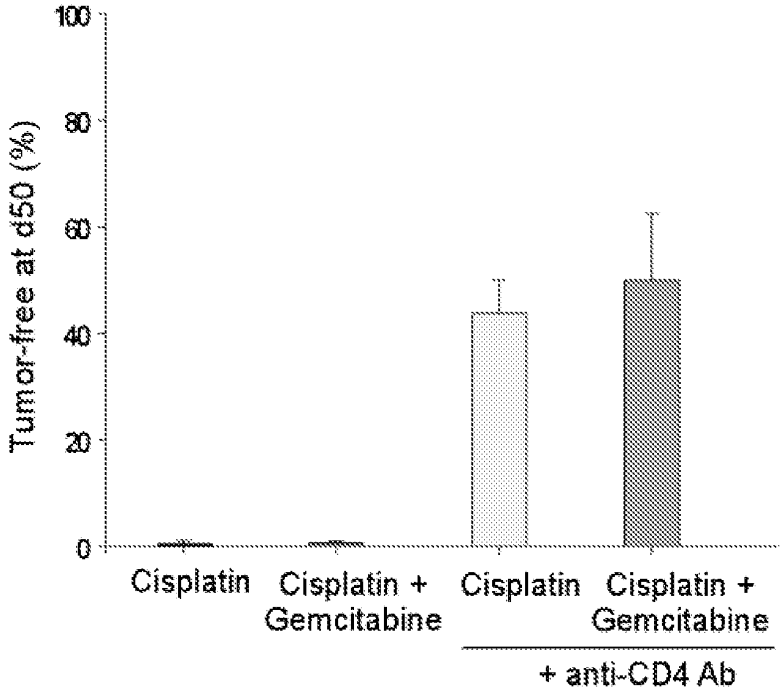

FIG. 56F

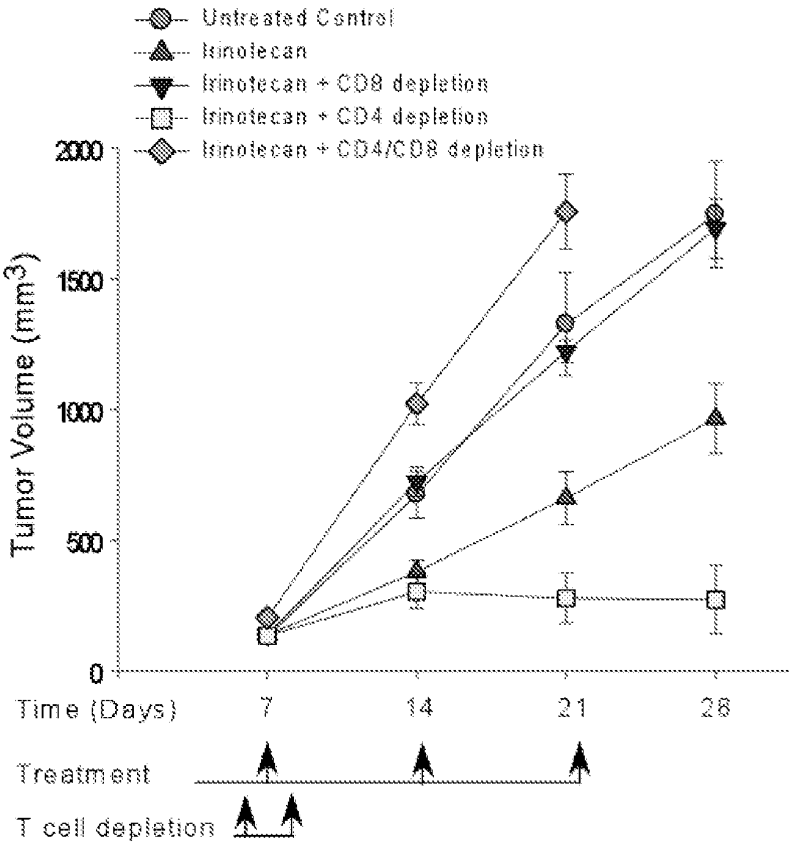

FIG. 57A

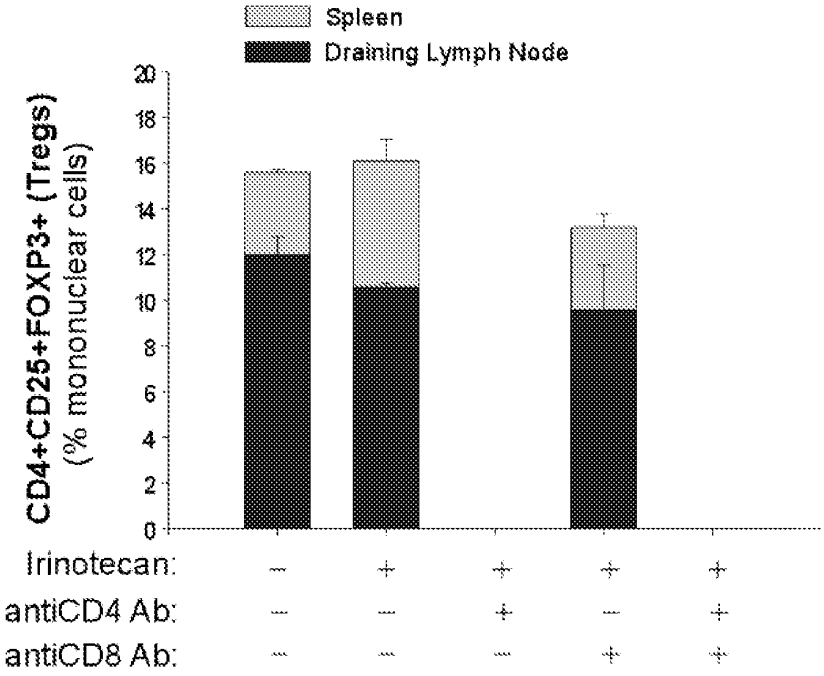

FIG. 57B

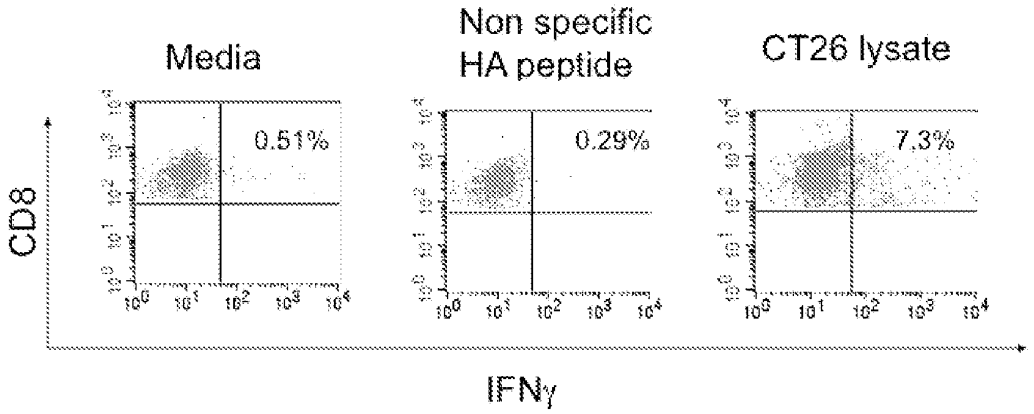

FIG. 57C

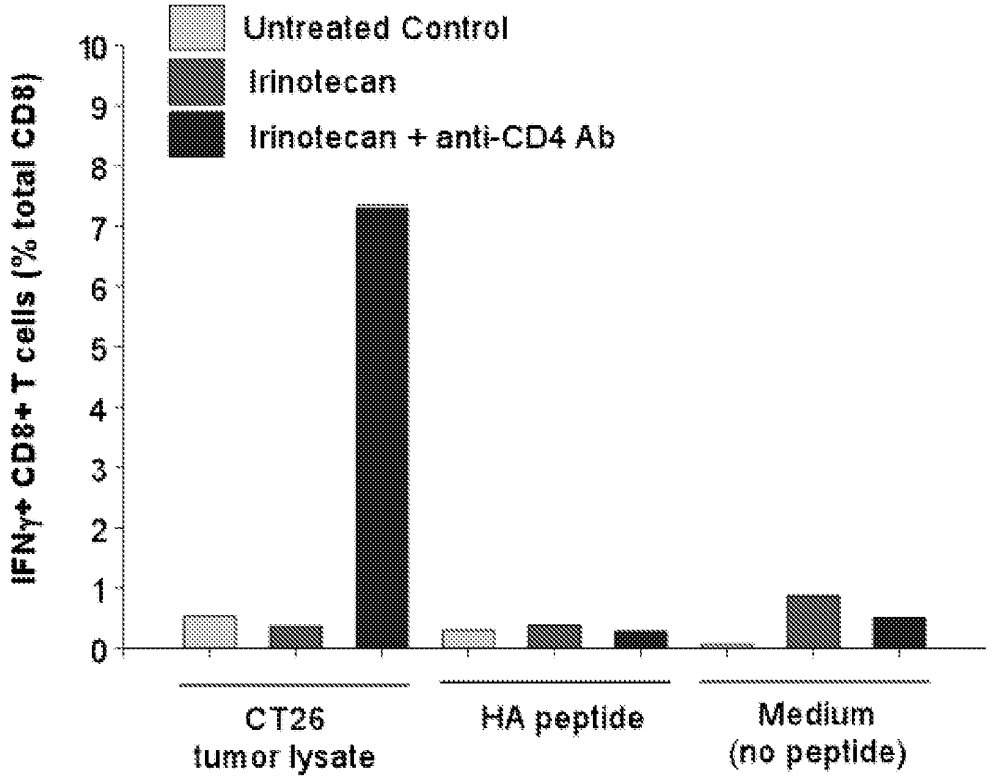

FIG. 57D

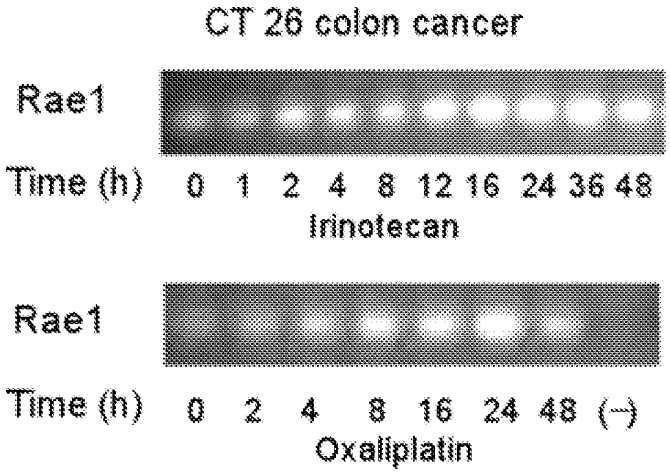

FIG. 57E

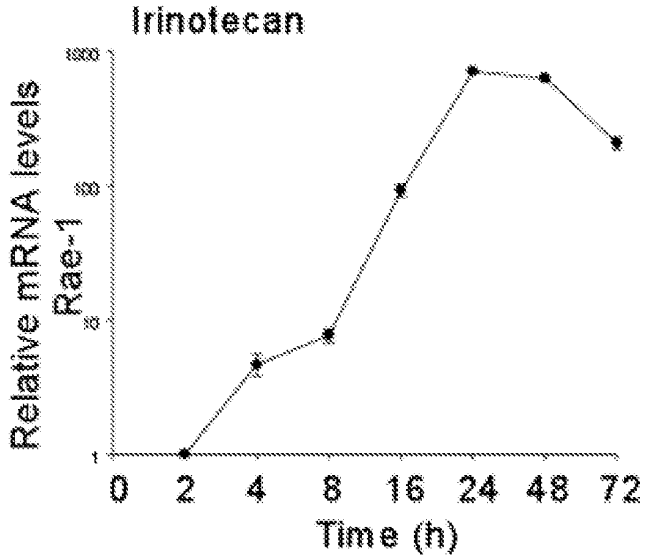

FIG. 57F

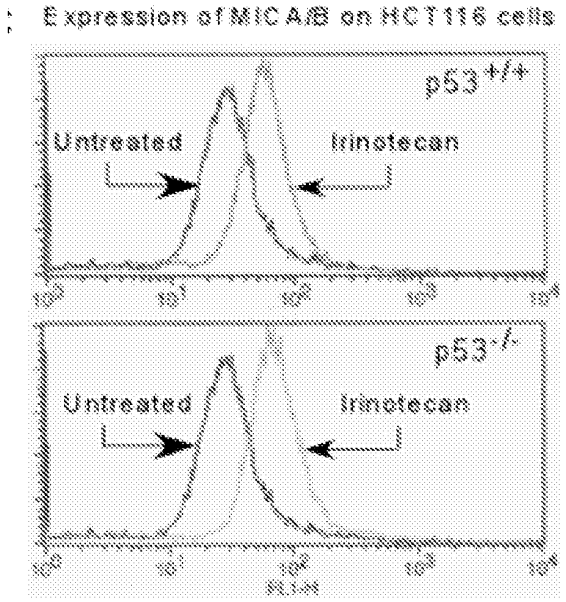

FIG. 57G

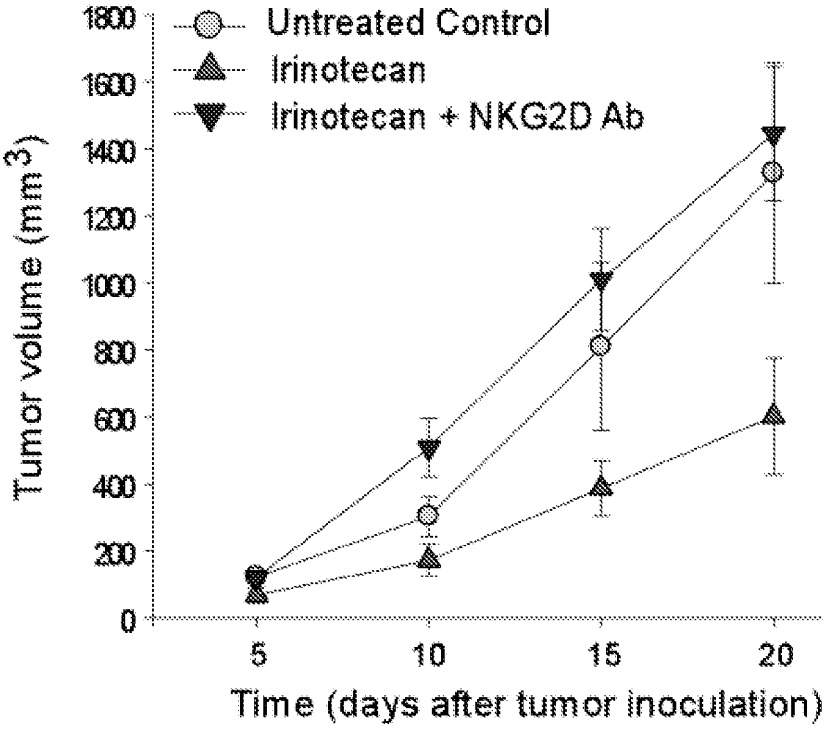

FIG. 57H

PATENT
ATTORNEY DOCKET NO.: JHU3260-8

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	Not Yet Assigned
Application No.:	16/601,347	Examiner:	Not Yet Assigned
Filed:	October 14, 2019	Conf. No.:	9807
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

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

POWER OF ATTORNEY BY ASSIGNEE

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

Statement under 37 C.F.R. §3.73(c):

THE JOHNS HOPKINS UNIVERSITY is the assignee of the entire right, title and interest in the above-identified application by virtue of an assignment recorded in the U.S. Patent and Trademark Office at Reel 041196, Frame 0155.

287948162.1
331323-001301

Direct all telephone calls to:

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

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

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

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

THE JOHNS HOPKINS UNIVERSITY

By : _____
(Signature)

Name : Steven Kousouris
(Typed or Printed Name)

Title : Executive Director, Johns Hopkins Technology Ventures

Date : 17 October, 2019

287948162.1
331323-001301

PTO/AIA/01 (08-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

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

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 15/362,632

filed on November 28, 2016.

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

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

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

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

LEGAL NAME OF INVENTOR

Inventor: Rajani Ravi Date (Optional) : _____

Signature: Rajani Ravi

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

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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

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☐ The attached application, or

☒ United States application or PCT international application number 15/362,632

filed on November 28, 2016.

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

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LEGAL NAME OF INVENTOR

Inventor: Atul Bedi Date (Optional) : _____

Signature: _____

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

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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Sequence Listing Report for Application No: 18883719

File Name: 18883719_1_1.xml (174957 B), **Created:** September 12, 2024, 05:16 PM
USPTO Receipt Date: September 12, 2024, 05:10 PM
Validation Date: September 12, 2024, 05:16 PM
Reviewed by: NKp6ZAWqxd5/YS+5kMWbeVihCo4/Q31f

DEFECTIVE: This validation report concerns the sequence listing filed 09-12-2024 17:10:37 and indicates the errors found by the USPTO during validation of the sequence listing. Any resolution of the errors identified in this validation report should not be construed as meaning that the sequence listing will comply with all of the requirements of 37 CFR §§ 1.821- 1.825, WIPO Standard ST.25, 37 CFR §§ 1.831-1.835 and 1.839, or WIPO Standard ST.26.
For any sequence listing related questions or concerns, the Applicant is invited to contact the USPTO Sequence Help Desk at 571-272-2510 or SequenceHelpDesk@USPTO.GOV

Reviewer's Comments

Variant sequence annotated with the wrong feature keys

A "variant sequence" means a nucleotide or amino acid sequence that contains one or more differences with respect to a primary sequence. These differences may include alternative residues (see paragraphs 15 and 27), modified residues (see paragraphs 3(g), 3(h), 16, and 29), deletions, insertions, and substitutions. See paragraphs 93 to 95. (WIPO ST.26 paragraph 3(m)).

Amino acid sequences with variant positions must be annotated with the feature keys "VAR_SEQ" or "VARIANT" and the qualifier "note" (see WIPO ST.26 paragraph 96). No other feature keys are permitted for the annotation of amino acid sequence variants.

SEQ ID NO: 123 is a variant sequence but is improperly annotated with the feature key "<REGION>". Please correct.

Validated By CRFValidator v 2.3.0

Application No: 18883719 Version No: 1.1

CRF Copy Request from application : Version :

Input Set: /slic/CRF_WORKFLOW/18/883/719/1.1/18883719_1_1.xml

Output Set: /slic/CRF_WORKFLOW/18/883/719/1.1/18883719_1_1.xml.pdf

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Total Warnings: 0
Total Errors: 0
No. of SeqIDs Defined: 124
Actual SeqID Count: 124

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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
18/883,719	09/12/2024		832	JHU3260-10	8	2

CONFIRMATION NO. 6410

FILING RECEIPT

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

OC00000078468939

Date Mailed: 09/27/2024

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Inventor(s)

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

Applicant(s)

The Johns Hopkins University, Baltimore, MD;

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

Domestic Priority data as claimed by applicant

This application is a CON of 17/694,541 03/14/2022
which is a CON of 16/601,347 10/14/2019 PAT 11,274,156
which is a CON of 15/362,632 11/28/2016 PAT 10,442,860
which is a DIV of 15/231,309 08/08/2016 PAT 9,850,306
which is a CON of 14/645,282 03/11/2015 PAT 9,441,044
which is a CON of 13/582,717 10/17/2012 PAT 8,993,524
which is a 371 of PCT/US2011/027317 03/04/2011
which claims benefit of 61/435,671 01/24/2011
and claims benefit of 61/311,255 03/05/2010

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

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

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

If Required, Foreign Filing License Granted: 09/25/2024

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 18/883,719**

Projected Publication Date: To Be Determined - pending completion of Sequence Disclosures.

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Preliminary Class

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

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 18/883,719

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	64		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	280		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	320		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	8 minus 20 =	* 0	x =	0	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 minus 3 =	*	x =	0			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			168			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	832		TOTAL	

APPLICATION AS AMENDED - PART II

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

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

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
18/883,719	09/12/2024	Atul Bedi	JHU3260-10

CONFIRMATION NO. 6410

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

FORMALITIES LETTER

OC000000078468942

Date Mailed: 09/27/2024

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Filing Date Granted

Applicant is given **TWO MONTHS FROM THE DATE OF THIS NOTICE** within which to comply with the sequence rules, 37 CFR §§ 1.831-1.835. Failure to comply with these requirements will result in ABANDONMENT of the application under 37 CFR §1.835(d)(1). Extension of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR §1.136.

Summary of Requirements for Patent Applications Filed On Or After July 1, 2022, That Have Sequence Disclosures

37 CFR 1.831(a) requires that patent applications which contain disclosures of nucleotide and/or amino acid sequences that fall within the definitions of 37 CFR 1.831(b) must contain a "Sequence Listing XML", *as a separate part of the disclosure*, which presents the nucleotide and/or amino acid sequences and associated information using the symbols and format in accordance with the requirements of 37 CFR 1.831-1.835. This "Sequence Listing XML" part of the disclosure may be submitted:

1. In accordance with 37 CFR 1.831(a) using the symbols and format requirements of 37 CFR 1.832 through 1.834 via the USPTO's patent electronic filing system (currently Sequence Listing XML files may **only** be properly filed electronically using **Patent Center**) (see Section I.1 of the Legal Framework for Patent Electronic System (https://www.uspto.gov/patents-application-process/filing-online/legal-framework-efs-web), hereinafter "Legal Framework") in XML format, together with an incorporation by reference statement of the material in the XML file in a separate paragraph of the specification as required by 37 CFR 1.835(a)(2) or 1.835(b)(2) identifying:
   a. the name of the XML file
   b. the date of creation; and
   c. the size of the XML file in bytes; or
2. In accordance with 37 CFR 1.831(a) using the symbols and format requirements of 37 CFR 1.832 through 1.834 on read-only optical disc(s) as permitted by 37 CFR 1.52(e)(1)(ii), labeled according to 37 CFR 1.52(e)(5), with an incorporation-by-reference statement of the material in the XML format according to 37 CFR 1.52(e)(8) and 37 CFR 1.835(a)(2) or 1.835(b)(2) in a separate paragraph of the specification identifying:
   a. the name of the XML file;
   b. the date of creation; and
   c. the size of the XML file in bytes.

SPECIFIC DEFICIENCIES AND THE REQUIRED RESPONSE TO THIS NOTICE ARE AS FOLLOWS:

- The "Sequence Listing XML" part of the disclosure filed with this application in accordance with 37 CFR 1.831-1.834 has been found to contain an error or errors as indicated on the PAIR document "Computer Readable Form (CRF) for Sequence Listing - Defective" dated 09/13/2024. Applicant must provide:
  - A replacement "Sequence Listing XML" part of the disclosure, as described above submitted in accordance with either item 1. or 2.; together with
    - A statement that identifies the location of all additions, deletions, or replacements of sequence information in the replacement "Sequence Listing XML" as required by 1.835(b)(3);
    - A statement that indicates support for the amendment in the application, as filed, as required by 37 CFR 1.835(b)(4);
    - A statement that the replacement "Sequence Listing XML" includes no new matter as required by 37 CFR 1.835(b)(5); and
    - A substitute specification in compliance with 37 CFR 1.52, 1.121(b)(3), and 1.125 inserting the required incorporation-by-reference paragraph as required by 37 CFR 1.835(b)(2), consisting of:
      - A copy of the previously-submitted specification, with deletions shown with strikethrough or brackets and insertions shown with underlining (marked-up version);
      - A copy of the amended specification without markings (clean version); and
      - A statement that the substitute specification contains no new matter.

Where to obtain assistance:

USPTO Sequence listing Resource Center at: https://www.uspto.gov/patents/apply/sequence-listing-resource-center.

For rules interpretation and filing requirements, call the Sequence Systems Service Center at (571) 272-2510, the Office of Patent Legal Administration at (571) 272-7701, or, for 35 U.S.C. 371 applications, the PCT Help Desk at (571) 272-4300.

For WIPO Standard ST.26 questions, call (571) 272-2510.

WIPO Sequence software is available at: https://www.wipo.int/standards/en/sequence/index.html.

For assistance with submission of a Sequence Listing XML file via Patent Center, call the Electronic Business Center at (866) 217-9197 or email ebc@uspto.gov.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

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- A separate document having the document description, "**Applicant response to Pre-Exam Formalities Notice**"; and
- the item(s) for which a reply period is set forth above.

For more information about electronic filing, contact the Patent Electronic Business Center (EBC) 1-866-217-9197 or visit https://www.uspto.gov/ebc

NOTE: If the reply is not being submitted electronically, a copy of this notice must be included.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/ygizaw/

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

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

Doc Code - SEQ.XML

WIPO Type Code -

SLIC Placeholder Sheet for P-ELP (CMS) Content

Application Number: 18883719 Document Date: 10/01/2024

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

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Form Revision Date: 07 January 2022

PATENT
Attorney Docket No.: JHU3260-10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	Not Yet Assigned
Application No.:	18/883,719	Examiner:	Not Yet Assigned
Filed:	September 12, 2024	Conf. No.	6410
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

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APPLICANT RESPONSE TO PRE-EXAM FORMALITIES NOTICE

Sir:

In response to the Notice to Comply with Requirements for Patent Applications Containing Nucleotide and/or Amino Acid Sequence Disclosures mailed September 27, 2024, attached are:

1. Sequence Listing in .xml format entitled "JHU3260-10.xml" (174,970 bytes);
2. Statement Accompanying Sequence Listing (1 page);
3. Statement to Support Filing and Submission in Accordance with 37 CFR §§1.831-1.835 (2 pages);
4. Preliminary Amendment in Response to Notice to Comply with Requirements for Patent Applications Containing Nucleotide and/or Amino Acid Sequence Disclosures (3 pages);
5. Marked-Up Specification (72 pages);
6. Substitute Specification (72 pages); and
7. Statement Accompanying Marked-Up/Substitute Specifications in Response to Notice to Comply with Requirements for Patent Applications Containing Nucleotide and/or Amino Acid Sequence Disclosures pursuant to 37 CFR §1.125(b) (1 page).

Applicant claims **LARGE ENTITY** status in the above-identified application.

No fee is deemed necessary in connection with the filing of this paper. However, if a fee is required, the Commissioner is hereby authorized to charge any other fees associated with the filing submitted herewith, or credit any overpayments to Deposit Account No. 07-1896.

Respectfully submitted,

Date: October 1, 2024

/Pamela E. Hwang/
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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/883,719	**10/01/2024 06:24:39 PM Z ET**	**JHU3260-10**

Title of Invention

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	6410	FILED BY	Aldon Griffis
PATENT CENTER #	67401865	FILING DATE	09/12/2024
CUSTOMER #	100899	FIRST NAMED INVENTOR	Atul Bedi
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	-

Documents

TOTAL DOCUMENTS: 8

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
JHU3260-10 Applicant Response to Pre-Exam Formalities Notice.pdf	2	Applicant Response to Pre-Exam Formalities Notice	116 KB
JHU3260-10.xml	-	Sequence Listing Supp (XML File)	171 KB
Warning: Please note that ST.23 and ST.25 sequence listings are required for applications with a filing date before July 1, 2022.			
JHU3260-10 Statement Accompanying Sequence Listing.pdf	1	Transmittal Letter	102 KB
JHU3260-10 Statement of Support for Sequence Listing.pdf	2	Transmittal Letter	119 KB

JHU3260-10 Preliminary Amendment EFS.pdf	3	Preliminary Amendment	104 KB
JHU3260-10 Specification Marked-Up-SPEC.docx	72	Specification	88 KB

Warning: One or more pages are missing page numbering. Page numbering will be automatically applied after submission. The margins of the attached document do not meet USPTO rules. This may cause processing errors or delays. Bookmarks were found and have been removed.

JHU3260-10 Specification Substitute-SPEC.docx	72	Specification	87 KB

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JHU3260-10 Statement of No New Matter Specifications.pdf	1	Transmittal Letter	118 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
JHU3260-10 Applicant Response to Pre-Exam Formalities Notice.pdf	098DBBD947F1D30E1A9BB2CFB60EBEE52D5808DA2495FD280F48FB80F4262CE9399601133E757A005EA4602CD00D8CDD788400191F13BF4A152D1299E97C6DCA
JHU3260-10.xml	3CD778873FBA84ED9C267C10E86FBA5B76CE50F64767043BAF979EB244EF939B2E70521ABEC390BB69D2509723B8D6EFD26BE706A51AAD5079E39EC8A01661EB
JHU3260-10 Statement Accompanying Sequence Listing.pdf	FB15F423438DDFB17918C108C242A18F758B60F387AD3A8C17B0223D77613558DCD2B49A216F5EAF4ABC13F3E6DA6A2BB5EB222E310163A0CA1D42A6353973C8
JHU3260-10 Statement of Support for Sequence Listing.pdf	F7972FBF41D3A85FB35E59E5869CCA0C2B31FF75F6A0D7BAA7CAB69557B7EB78289339E166C22EFB440E9017F764F65B2BD450CAE33DF13ABBA5EDA2DF5033FC
JHU3260-10 Preliminary Amendment EFS.pdf	E3CBF869F60543E6C4C269E80DF5A5E4441175232717D751AD12C57468ED9FFB987BDEA4266AEE7D3556CD38F0EC74C0F1F9C0ED98AB9841CFB0DF969A3389F1
JHU3260-10 Specification Marked-Up-SPEC.docx	06200F8D8BC4814ABD9D69C4E96786DBC84D981DDD3F34F5E2FFCDDF136AA90EBDC28692F95B4A6476C0F8B9B5F270439D53938437848238F5621B3E067C313B

JHU3260-10 Specification Substitute-SPEC.docx	432284C01DA342E58078D10EA28560247DE69C27E230492AD8E6D8830DF82032E1D7FBC7B24AA91CE00B184ED9674D292EAE8FB3D1FB590A748F457CADC0432A
JHU3260-10 Statement of No New Matter Specifications.pdf	B10D8F68D07644BF1C86FC4A5D0B1B0A6CEF2D9E9E26CE05A64D4FBDFC757DA516EBE0B1088CD11150E58A3DAACEBF8CDCD32699A73C8CAC9E1E351B67225F74

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation application of U.S. Application Serial No. 17/694,541 filed March 14, 2022, now pending; which is a continuation application of U.S. Application Serial No. 16/601,347 filed October 14, 2019, now issued as U.S. patent 11,274,156; which is a continuation application of U.S. Application Serial No. 15/362,632 filed November 28, 2016, now issued as U.S. patent 10,442,860; which is a divisional application of U.S. Application Serial No. 15/231,309 filed August 8, 2016, now issued as U.S. patent 9,850,306; which is a continuation application of U.S. Application Serial No. 14/645,282 filed March 11, 2015, now issued as U.S. patent 9,441,044; which is a continuation application of U.S. Application Serial No. 13/582,717 filed October 17, 2012, now issued as U.S. patent 8,993,524; which is a 35 USC §371 National Stage application of International Application No. PCT/US2011/027317 filed March 4, 2011, now expired; which claims the benefit under 35 USC §119(e) to U.S. Application Serial No. 61/435,671 filed January 24, 2011 and to U.S. Application Serial No. 61/311,255 filed March 5, 2010, both now expired. The disclosure of each of the prior applications is considered part of and is incorporated by reference in the disclosure of this application.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under Grant No. CA123277 awarded by the National Institutes of Health. The government has certain rights in the invention.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0003] The material in the accompanying sequence listing is hereby incorporated by reference into this application. The accompanying sequence listing text file, named JHU3260-10.xml, was created on September 23, 2024 and is 174,970 bytes in size.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

[0004] The present invention relates generally to the field of targeted immunomodulatory antibodies and fusion proteins for cancer therapy and more specifically to composition and methods for targeted immunostimulatory or immunosuppressive antibodies and fusion proteins to counteract or induce immune tolerance of cancer cells.

BACKGROUND INFORMATION

[0005] The immune system provides the human body with a means to recognize and defend itself against microorganisms and substances recognized as foreign or potentially harmful. While passive immunotherapy of cancer with monoclonal antibodies and passive transfer of T cells to attack tumor cells have demonstrated clinical efficacy, the goal of active therapeutic vaccination to induce these immune effectors and establish immunological memory against tumor cells has remained challenging. Several tumor-specific and tumor-associated antigens have been identified, yet these antigens are generally weakly immunogenic and tumors employ diverse mechanisms to create a tolerogenic environment that allows them to evade immunologic attack. Strategies to overcome such immune tolerance and activating robust levels of antibody and/or T cell responses hold the key to effective cancer immunotherapy.

SUMMARY OF THE INVENTION

[0006] The present invention is based on the seminal discovery that targeted immunomodulatory antibodies and fusion proteins can counteract or reverse immune tolerance of cancer cells. Cancer cells are able to escape elimination by chemotherapeutic agents or tumor-targeted antibodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. Such immunosuppressive mechanisms include immunosuppressive cytokines (for example, Transforming growth factor beta (TGF-β)) and regulatory T cells and/or immunosuppressive myeloid dendritic cells (DCs). By counteracting tumor-induced immune tolerance, the present invention provides effective compositions and methods for cancer treatment, optional in combination with another existing cancer treatment. The present invention provides strategies to counteract tumor-induced immune tolerance and

Bicara Ex. 1002, Page 212 of 482
Bicara v. JHU - PGR2026-00025

enhance the antitumor efficacy of chemotherapy by activating and leveraging T cell-mediated adaptive antitumor immunity against resistant or disseminated cancer cells.

[0007] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule, and the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2); (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); (iv) Transforming growth factor-beta receptor (TGF-βR); (v) Programmed death-1 (PD-1); and (vi) Receptor activator of nuclear factor-κB (RANK).

[0008] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. In one aspect, the targeting moiety specifically binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), CD20, Vascular endothelial growth factor (VEGF), insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, CD30, CD33, or CD40.

[0009] In one aspect, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: (i) CD4; (ii) CD25 (IL-2α receptor; IL-2αR); (iii) cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); (iv) Interleukin-10 (IL-10); (v) Transforming growth factor-beta receptor (TGF-βR); (vi) Transforming growth factor-beta (TGF-β); (vii) Programmed Death-1 (PD-1); (viii) Programmed death-1 ligand (PD-L1 or PD-L2); (ix) Receptor activator of nuclear factor-κB (RANK); or (x) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL).

[0010] In one aspect, the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed

death-1 ligand (PD-L1 or PD-L2); or (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL).

[0011] In one aspect, the immunomodulatory moiety includes a molecule that binds TGF-β. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Transforming growth factor-beta receptor TGF-βRII, TGF-βRIIb, or TGF-βRIII. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of TGF-β.

[0012] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

[0013] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Programmed Death-1 (PD-1), Programmed death-1 ligand 1 (PD-L1), or Programmed death-1 ligand 2 (PD-L2). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes PD-1 ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

[0014] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory

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moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

[0015] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Programmed death-1 ligand 1 (PD-L1).

[0016] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.

[0017] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Programmed Death-1 (PD-1). In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and PD-1 ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 25 or 26.

[0018] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or

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ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Receptor activator of nuclear factor-κB ligand (RANKL).

[0019] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36.

[0020] In one aspect, the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the immunomodulatory moiety increases the function of PD-1.

[0021] In one aspect, the targeting moiety specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 37. In another aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2), and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from PD-L1. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 38 or 39.

[0022] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25, or CD4, and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1

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ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 40, 41, 42, 43, 44, or 45.

[0023] In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 46 or 47.

[0024] In one aspect, the targeting moiety includes transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 48 or 49.

[0025] In one aspect, the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the immunomodulatory moiety activates the signaling function of transforming growth factor-β (TGF-β) receptor.

[0026] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from TGF-β. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 50. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 51 or 52.

[0027] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25 (IL-2α receptor; IL-2αR), or CD4, and the immunomodulatory moiety includes a sequence from transforming growth factor-β

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(TGF-β). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 53, 54, 55, 56, 57 or 58.

[0028] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 59 or 60.

[0029] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from an extracellular RANKL-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 61. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from an extracellular RANK-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 62 or 63.

[0030] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

[0031] In one aspect, the targeting moiety includes a sequence from transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

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[0032] In one aspect, the targeting moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

[0033] In various aspects, the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

[0034] In another embodiment, the present invention provides a composition including the molecule of the invention and a cell, wherein the cell is a tumor cell, immune cell, or dendritic cell.

[0035] In another embodiment, the present invention provides a method of counteracting or overcoming immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

[0036] In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof one or more molecule of the invention. In various aspects, the subject is administered one or more molecule of the invention in combination with another anticancer therapy. In one aspect, the anticancer therapy includes a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent or cytotoxic agent. In another aspect, the anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

[0037] In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof an antibody that targets and depletes CD4+ regulatory T cells (Tregs) in combination with another cytotoxic anticancer therapy. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In various aspects, the cytotoxic anticancer therapy includes a chemotherapeutic molecule, tumor-targeted antibody, small molecule kinase inhibitor, hormonal agent or tumor-targeted cytotoxic agent. In another

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

[0038] In another embodiment, the subject is administered one or more molecule of the invention in combination with any vaccine. In another aspect, the vaccine includes a tumor antigen, tumor-associated antigen, tumor epitope, tumor antigen-containing fusion protein, tumor cell, or dendritic cell. In another aspect, the vaccine includes a pathogen antigen, pathogen-associated antigen, pathogen epitope, or pathogen antigen-containing fusion protein.

[0039] In another embodiment, the present invention provides a method for treating immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treatment of a neoplastic disease. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

[0040] In another embodiment, the present invention provides a method of inducing or promoting immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

[0041] In another embodiment, the present invention provides a method of preventing or treating an autoimmune or inflammatory disease including administering to a subject in need thereof one or more molecule of the invention. In one aspect, the subject is administered one or more molecule of the invention in combination with another anti-inflammatory or immunosuppressive therapy. In another embodiment, the present invention provides a method of treatment of immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treating an autoimmune or inflammatory disease or preventing rejection of grafted cells or tissue. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] Figures 1A-1C show exemplary amino acid sequences of transforming growth factor beta receptor type II (TGF-β-RII) or TGF-β-RIIB or a fragment thereof, including (i) Transforming growth factor beta receptor type II (TGF-β-RII) (SEQ ID NO: 79); and (ii) Transforming growth factor beta receptor type IIB (TGF-β-RIIB) (SEQ ID NO: 80).

[0043] Also shown in Figures 1A-1C are exemplary truncated mutants of Transforming growth factor beta Receptor II (TGF-β-RII) or TGF-β-RIIB including the Extracellular domain (ECD) region that binds TGF-β, including (i) TGF-β R-II (ΔC terminus): TGFβ RII lacking the last 38 amino acids from the C-terminus (SEQ ID NO: 81) and TGF-β R-IIB (ΔC terminus): TGFβ RIIB lacking the last 38 aa from the C-terminus (SEQ ID NO: 82); (ii) TGF-βR-II (Δcyt): TGFβRII lacking the kinase domain & juxtamembrane region (SEQ ID NO: 83) and TGF-βR-IIB (Δcyt): TGFβRIIB lacking the kinase domain & juxtamembrane region (SEQ ID NO: 84); (iii) TGF-β R-II containing the N-terminus region including the extracellular domain (SEQ ID NO: 85) and TGF-β R-IIB containing the N-terminus region including the extracellular domain (SEQ ID NO: 86); (iv) TGF-β R-II containing the extracellular domain that binds TGF-β (SEQ ID NO: 87) and TGF-β R-IIB containing the extracellular domain that binds TGF-β (SEQ ID NO: 88); and (v) TGF-β R-II containing the region of the extracellular domain that binds TGF-β (SEQ ID NO: 89).

[0044] In addition, Figures 1A-1C also show exemplary kinase-deficient mutants, deletion mutants, or point mutants of Transforming growth factor beta Receptor II (TGFβ-RII) or TGFβ-RIIB or a fragment thereof which binds TGF-β, including (i) Transforming growth factor beta Receptor II containing point mutations - amino acid sequence of TGF-β R-II (K277R) contains a point mutation in its ATP-binding site and is inactive as a kinase (SEQ ID NO: 90); and (ii) Transforming growth factor beta Receptor II containing deletions in the amino acid sequence (deletion mutants) - Transforming growth factor beta Receptor II (Δi) - TGF-β R-II (Δi2) contains a deletion of amino acids 498 to 508 and is inactive as a kinase (SEQ ID NO: 91).

[0045] Figure 2 shows exemplary fusion proteins including anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD),

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including anti-HER2/neu heavy chain + TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 1) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

[0046] Figure 3 shows exemplary fusion proteins including anti-EGFR1 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-EGFR1 heavy chain + TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 2) and anti-EGFR1 light chain amino acid sequence (SEQ ID NO: 71).

[0047] Figure 4 shows exemplary fusion proteins including anti-CD20 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-CD20 heavy chain+ TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 3) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0048] Figure 5 shows exemplary fusion proteins including anti-VEGF antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-VEGF heavy chain+ TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 4) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

[0049] Figure 6 shows exemplary fusion proteins including anti-human CTLA-4 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including Anti-CTLA-4 heavy chain + TGFβ-RII Extracellular domain fusion amino acid sequence (SEQ ID NO: 5) and Anti-CTLA-4 light chain (SEQ ID NO: 74).

[0050] Figure 7 shows exemplary fusion proteins including IL-2, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including IL-2 + Fc + TGFβ-RII Extracellular domain (SEQ ID NO: 6) and TGFβ-RII Extracellular domain + Fc + IL-2 (SEQ ID NO: 7). The linker GGGGSGGGGSGGGGS (SEQ ID NO: 104) is optional and can be replaced with EPKSCDK (SEQ ID NO: 105) or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0051] Figures 8A-8B show exemplary fusion proteins including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD),

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including anti-CD25 (Daclizumab) heavy chain and TGFβ-RII Extracellular domain (SEQ ID NO: 8) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 8A); and anti-CD25 (Basiliximab) heavy chain and TGFβ-RII Extracellular domain (SEQ ID NO: 9) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76).

[0052] Figure 9 shows exemplary fusion proteins including anti-CD4 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-CD4 heavy chain and TGFβ-RII Extracellular domain (SEQ ID NO: 10) and anti-CD4 light chain (SEQ ID NO: 77).

[0053] Figure 10 shows exemplary fusion proteins including Programmed Death-1 (PD-1) Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ectodomain), including PD-1 ectodomain + Fc + TGFβRII ectodomain (SEQ ID NO: 11) and TGFβRII ectodomain + Fc + PD-1 ectodomain (SEQ ID NO: 12). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

[0054] Figure 11 shows exemplary fusion proteins including Receptor activator of nuclear factor-kB (RANK) Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ectodomain), including RANK ectodomain + Fc + TGFβRII ectodomain (SEQ ID NO: 13) and TGFβRII ectodomain + Fc + RANK ectodomain (SEQ ID NO: 14). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

[0055] Figure 12 shows exemplary immunomodulatory moiety that binds Programmed Death-1 ligand 1 (PD-L1 or B7-H1) or Programmed Death-1 ligand 2 (PD-L2 or B7-DC), including full-length PD-1 or fragment thereof (SEQ ID NO: 92), PD-1 extracellular domain (ectodomain) or fragment thereof (SEQ ID NO: 93), and PD-1 extracellular domain (ectodomain) ligand-binding region (SEQ ID NO: 94).

[0056] Figure 13 shows exemplary fusion proteins including anti-HER2/neu antibody and PD-1 Ectodomain, including anti-HER2/neu heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 15) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

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[0057] Figure 14 shows exemplary fusion proteins including anti-EGFR1 antibody and PD-1 Ectodomain, including anti-EGFR heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 16) and anti-EGFR light chain amino acid sequence (SEQ ID NO: 71).

[0058] Figure 15 shows exemplary fusion proteins including anti-CD20 antibody and PD-1 Ectodomain, including anti-CD20 heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 17) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0059] Figure 16 shows exemplary fusion proteins including anti-VEGF antibody and PD-1 Ectodomain, including anti-VEGF heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 18) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

[0060] Figure 17 shows exemplary fusion proteins including anti-human CTLA-4 antibody and PD-1 Ectodomain, including anti-CTLA-4 heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 19) and anti-CTLA-4 light chain (SEQ ID NO: 74).

[0061] Figures 18A-18B show exemplary fusion proteins including anti-CD25 antibody and PD-1 Ectodomain, including anti-CD25 (Daclizumab) heavy chain and PD-1 ectodomain (SEQ ID NO: 20) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 18A), and anti-CD25 (Basiliximab) heavy chain and PD-1 ectodomain (SEQ ID NO: 21) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 18B).

[0062] Figure 19 shows exemplary fusion proteins including IL-2, Fc, and PD-1 ectodomain, including IL-2 + Fc + PD-1 ectodomain (SEQ ID NO: 22) and PD-1 ectodomain + Fc + IL-2 (SEQ ID NO: 23). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0063] Figure 20 shows exemplary fusion proteins including anti-CD4 antibody and PD-1 ectodomain, including anti-CD4 heavy chain and PD-1 ectodomain (SEQ ID NO: 24) and anti-CD4 light chain (SEQ ID NO: 77).

[0064] Figure 21 shows exemplary fusion proteins including Receptor activator of nuclear factor-kB (RANK) Ectodomain, Fc, and PD-1 ectodomain, including RANK ectodomain + Fc + PD-1 ectodomain (SEQ ID NO: 25) and PD-1 ectodomain + Fc + RANK ectodomain (SEQ ID NO: 26). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

[0065] Figure 22 shows exemplary immunomodulatory moiety that binds Receptor activator of nuclear factor-kB (RANK) ligand (RANKL) including full-length RANK or fragment thereof (SEQ ID NO: 95), extracellular ligand-binding domain or ectodomain of RANK (SEQ ID NO: 96), RANKL-binding sequences or residues of RANK (SEQ ID NO: 93), or RANKL-binding sequences of Osteoprotegerin (OPG) (SEQ ID NO: 98).

[0066] Figure 23 shows exemplary fusion proteins including anti-HER2/neu antibody and RANK Ectodomain, including anti-HER2/neu heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 27) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

[0067] Figure 24 shows exemplary fusion proteins including anti-EGFR1 antibody and RANK Ectodomain, including anti-EGFR heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 28) and anti-EGFR light chain amino acid sequence (SEQ ID NO: 71).

[0068] Figure 25 shows exemplary fusion proteins including anti-CD20 antibody and RANK Ectodomain, including anti-CD20 heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 29) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0069] Figure 26 shows exemplary fusion proteins including anti-VEGF antibody and RANK Ectodomain, including anti-VEGF heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 30) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

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[0070] Figure 27 shows exemplary fusion proteins including anti-human CTLA-4 antibody and RANK Ectodomain, including anti-CTLA-4 heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 31) and anti-CTLA-4 light chain (SEQ ID NO: 74).

[0071] Figures 28A-28B show exemplary fusion proteins including anti-CD25 antibody and RANK Ectodomain, including anti-CD25 (Daclizumab) heavy chain and RANK ectodomain (SEQ ID NO: 32) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 28A), and anti-CD25 (Basiliximab) heavy chain and RANK ectodomain (SEQ ID NO: 33) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 28B).

[0072] Figure 29 shows exemplary fusion proteins including IL-2, Fc, and RANK ectodomain, including IL-2 + Fc + RANK ectodomain (SEQ ID NO: 34) and RANK ectodomain + Fc + IL-2 (SEQ ID NO: 35). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0073] Figure 30 shows exemplary fusion proteins including anti-CD4 antibody and RANK ectodomain, including anti-CD4 heavy chain and RANK ectodomain (SEQ ID NO: 36) and anti-CD4 light chain (SEQ ID NO: 77).

[0074] Figure 31 shows exemplary immunomodulatory moiety that binds Programmed Death-1 (PD-1) including a PD-1 ligand 1 (PD-L1 or B7-H1) or PD-1 ligand 2 (PD-L2 or B7-DC) or a fragment thereof (for example, SEQ ID NO: 101), full-length human PD-1 ligand 1 (B7-H1; PDCD1L1; PD-L1; or CD274) protein or a fragment thereof (SEQ ID NO: 99), and PD-L1 extracellular binding domain (ectodomain) or fragment thereof (SEQ ID NO: 100).

[0075] Figure 32 shows exemplary fusion proteins including anti-tumor necrosis factor (TNFα) antibody and PD-1 ligand, including anti-TNFα heavy chain + PD-1L (SEQ ID NO: 37) and anti-TNFα light chain (SEQ ID NO: 78). The sequence KKAE (SEQ ID NO: 107) can be replaced with KRVE (SEQ ID NO: 108) or KKVE (SEQ ID NO: 109).

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[0076] Figure 33 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand, including TNFR2 ECD + IgG Cγ1 + PD-L1 (SEQ ID NO: 38) and PD-L1 + IgG Cγ1 -TNFR2 ECD (SEQ ID NO: 39).

[0077] Figure 34 shows exemplary fusion proteins including anti-CD20 antibody and PD-1 ligand 1 (PD-L1), including anti-CD20 heavy chain + PD-L1 sequence (SEQ ID NO: 40) and anti-CD20 light chain sequence (SEQ ID NO: 72).

[0078] Figures 35A-35B show exemplary fusion proteins including anti-CD25 antibody and PD-1 ligand 1 (PD-L1), including anti-CD25 (Daclizumab) heavy chain and PD-L1 (SEQ ID NO: 41) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 35A), and anti-CD25 (Basiliximab) heavy chain and PD-1 ectodomain (SEQ ID NO: 42) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 35B).

[0079] Figure 36 shows exemplary fusion proteins including IL-2, Fc, and PD-1 ligand 1 (PD-L1), including fusion protein hPD-1 ligand 1 + Fc + IL-2 (SEQ ID NO: 43) and fusion protein IL-2 + Fc + PD-1 ligand 1 (SEQ ID NO: 44). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0080] Figure 37 shows exemplary fusion proteins including anti-CD4 antibody and PD-1 ligand 1 (PD-L1), including anti-CD4 heavy chain and PD-1 ligand 1 (PD-L1) (SEQ ID NO: 45) and anti-CD4 light chain (SEQ ID NO: 77).

[0081] Figure 38 shows exemplary fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from PD-1 ligand (PD-L1) including Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + PD-L1 (SEQ ID NO: 46) and PD-1L1 + IgG Cγ1 + CTLA-4 ECD (SEQ ID NO: 47). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

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[0082] Figure 39 shows exemplary fusion proteins including a sequence of transforming growth factor-β (TGF-β), Immunoglobulin Fc (IgG Cγ1), and a sequence of PD-1 ligand (PD-L1) including TGFβ-1 + Fc + PD-L1 (SEQ ID NO: 48), and PD-1L1 + Fc + TGFβ-1 (SEQ ID NO: 49). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0083] Figure 40 shows exemplary immunomodulatory moiety that binds Transforming growth factor-beta receptor (TGF-βR) including Transforming growth factor-beta (TGF-β1, TGF-β2, or TGF-β3 or a fragment thereof, TGF-β1 full sequence (SEQ ID NO: 102), and mature (active) TGF-β1 sequence (Ala 279 – Ser 390; 112 amino acids) (SEQ ID NO: 103).

[0084] Figure 41 shows exemplary fusion proteins including an antibody that binds TNF-α, and a sequence of transforming growth factor-β (TGF-β), including anti-TNFα heavy chain + TGF-β1 (SEQ ID NO: 50) and anti-TNFα light chain (SEQ ID NO: 78). The sequence KKAE (SEQ ID NO: 107) can be replaced with KRVE (SEQ ID NO: 108) or KKVE (SEQ ID NO: 109).

[0085] Figure 42 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain (TNFR2 ECD), immunoglobulin Fc (IgG Cγ1), and a sequence from transforming growth factor-β (TGF-β) including TNFR2 ECD + IgG Cγ1 + TGF-β1 (SEQ ID NO: 51), and TGF-β1 + IgG Cγ1 + TNFR2 ECD (SEQ ID NO: 52).

[0086] Figure 43 shows exemplary fusion proteins including anti-CD20 antibody and a sequence from transforming growth factor-β (TGF-β), including anti-CD20 heavy chain + mature TGFβ1 sequence (SEQ ID NO: 53) and anti-CD20 light chain sequence (SEQ ID NO: 72).

[0087] Figures 44A-44B show exemplary fusion proteins including anti-CD25 antibody and a sequence from transforming growth factor-β (TGF-β), including anti-CD25 (Daclizumab) heavy chain and TGF-β1 (SEQ ID NO: 54) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 44A), and anti-CD25 (Basiliximab) heavy chain and

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TGF-β1 (SEQ ID NO: 55) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 44B).

[0088] Figure 45 shows exemplary fusion proteins including IL-2, Fc, and a sequence from transforming growth factor-β (TGF-β), including TGF-β1 + Fc + IL-2 (SEQ ID NO: 56) and IL-2 + Fc + TGF-β1 (SEQ ID NO: 57). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0089] Figure 46 shows exemplary fusion proteins including anti-CD4 antibody and a sequence from transforming growth factor-β (TGF-β), including anti-CD4 heavy chain and TGF-β (SEQ ID NO: 58) and anti-CD4 light chain (SEQ ID NO: 77).

[0090] Figure 47 shows exemplary Fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from transforming growth factor-β (TGF-β) including Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + TGF-β1 (SEQ ID NO: 59), and TGF-β1 + IgG Cγ1 + CTLA-4 ECD (SEQ ID NO: 60). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

[0091] Figure 48 shows exemplary fusion proteins including an antibody that binds TNF-α, and a sequence of RANK ectodomain, including anti-TNFα heavy chain + RANK ectodomain (SEQ ID NO: 61) and anti-TNFα light chain (SEQ ID NO: 78). The sequence KKAE (SEQ ID NO: 107) can be replaced with KRVE (SEQ ID NO: 108) or KKVE (SEQ ID NO: 109).

[0092] Figure 49 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain (TNFR2 ECD), immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including TNFR2 ECD + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 62), and RANK ectodomain + IgG Cγ1 + TNFR2 ECD (SEQ ID NO: 63).

[0093] Figure 50 shows exemplary Fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 64), and RANK ectodomain + IgG Cγ1 + CTLA-4 ECD (SEQ ID NO: 65). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

[0094] Figure 51 shows exemplary fusion proteins including a sequence from transforming growth factor-β (TGF-β), immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including TGF-β + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 66), and RANK ectodomain + IgG Cγ1 + TGF-β (SEQ ID NO: 67).

[0095] Figure 52 shows exemplary fusion proteins including a sequence from PD-1 ligand (PD-L1), immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including PD-L1 + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 68), and RANK ectodomain + IgG Cγ1 + PD-L1 (SEQ ID NO: 69).

[0096] Figures 53A-53G show Regulatory T cells (Treg) accumulate in the tumor microenvironment and counteract the ability of chemotherapy to activate $CD8^+$ T cell-mediated antitumor immunity. (FIG. 53A) Surface exposure of calreticulin (CRT) in response to treatment of hum[a]n (SW780) and murine (MB49) cancer cells with doxorubicin (10 μM) for 4h. The surface exposure of CRT was determined by immunofluorescence cytometry of untreated control or doxorubicin-treated cells stained with Dylight-labeled anti-CRT antibody or an isotype control (IgG1) antibody. (FIG. 53B) Priming of tumor-reactive immune responses by MB49 tumor cells treated with doxorubicin *ex vivo* or *in vivo*. $5x10^6$ MB49 cells that were pre-treated *ex vivo* with doxorubicin (10 μM) for 4h were injected into one flank of syngeneic immunocompetent C57BL/6 mice. Alternatively, C57BL/6 mice were injected with $5x10^5$ live MB49 tumor cells and then administered intratumoral doxorubicin (10 μg) at 10d following tumor inoculatio[n]. Tumor-reactive immune responses were determined by measuring production of IFN-γ by draining lymph node (DLN) cells in response to *in vitro* re-challenge with either MB49 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone.

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(FIG. 53C) Vaccination with doxorubicin-treated tumor cells induces $CD8^+$ T cell-mediated antitumor immunity that prevents tumor formation following re-challenge with live tumor cells. MB49 cells ($5x10^6$) that were pre-treated *in vitro* with doxorubicin (10 μM) for 4h were injected subcutaneously into one flank of syngeneic immunocompetent C57BL/6 mice. Naïve or vaccinated mice were challenged with untreated live MB49 tumor cells injected into the opposite flank with or without pre-treatment with an anti-CD8 antibody (Clone GK2.43)(5 μg x 2 doses, iv) to deplete $CD8^+$ T cells. (FIG. 53D) Delayed administration of chemotherapy in mice with pre-established tumors decreases its immunogenicity and antitumor efficacy. C57BL/6 mice were injected with $5x10^5$ live syngeneic MB49 tumor cells and then administered intratumoral doxorubicin (10 μg) at d3, d7, or d10 following tumor inoculation. (FIG. 53E) Tumors foster the accumulation of $CD4^+CD25^+FoxP3^+$ cells (Tregs) in their microenvironment. Flow cytometric analyses of the percentage of $CD4^+CD25^+Fox^P3^+$ cells (Tregs) among $CD4^+$ T lymphocytes isolated from the spleen, draining lymph nodes (DLN), and tumors of immunocompetent C57BL/6 mice at d0 and d14 after subcutaneous inoculation of $5x10^5$ live MB49 tumor cells. (FIG. 53F) Tregs infiltrating the tumor microenvironment suppress priming of tumor-reactive immune responses by doxorubicin-treated tumor cells. Naïve C57BL/6 mice were vaccinated with $5x10^6$ doxorubicin-killed MB49 cells with or without intravenous adoptive transfer of $5x10^6$ $CD4^+CD25^+$ cells isolated from tumors and DLN of tumor-bearing mice via immunomagnetic separation. Tumor-reactive immune responses were determined by measuring production of IFN-γ by draining lymph node (DLN) cells in response to *in vitro* re-challenge with either MB49 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone. (FIG. 53G) Tregs infiltrating the tumor microenvironment suppress the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death. Naïve C57BL/6 mice were vaccinated with $5x10^6$ doxorubicin-killed MB49 cells (left flank) with or without pre-treatment with either an anti-CD8 antibody (Clone GK2.43)(5 μg x 2 doses, iv) to deplete $CD8^+$ T cells or adoptive transfer of $5x10^6$ $CD4^+CD25^+$ cells isolated from tumors and DLN of tumor-bearing mice. Protective antitumor immunity in vaccinated mice was determined by assessment of tumor growth upon challenge with untreated live MB49 tumor cells injected into the opposite flank.

[0097] Figures 54A-54F show inhibition of TGF-β in the tumor microenvironment reduces 'adaptive' FoxP3$^+$ regulatory T cells and enhances the antitumor efficacy chemotherapy. (FIG. 54A) Tumor growth results in a progressive increase in the level of serum TGF-β. Levels of TGF-β in serum of mice at d0, d14, and d28 following inoculation of $5x10^5$ live MB49 tumor cells were evaluated utilizing ELISA. (FIG. 54B) Tumor cell-autonomous expression of TGF-β is the dominant source of elevated TGF-β in tumor-bearing mice. Tumor cells or draining lymph node cells isolated from either tumor-bearing mice or their tumor-free counterparts were cultured *ex vivo* in serum-free medium for 24h and the amount of TGF-β/10^6 cells in supernatants was measured by ELISA. (FIG. 54C) TGFβRII:Fc sequesters TGF-β in supernatants of MB49 tumor cells in a concentration-dependent manner. MB49 tumor cells were cultured in the presence of graded concentrations of TGFβRII:Fc (0-400 ng/ml) for 24h followed by measurement of TGF-β (pg/ml/10^6 cells) in supernatants via ELISA. (FIG. 54D) TGF-β induces 'adaptive' FoxP3$^+$ regulatory T cells in the tumor microenvironment. At 5d following inoculation of MB49 tumor cells, mice were either left untreated (control) or treated with TGFβRII:Fc (1μg intratumoral; twice weekly) for 3 weeks followed by flow cytometric analyses of intracellular FoxP3 expression in CD4$^+$CD25$^+$ T cells infiltrating the tumors. (FIGS. 54E, 54F). Sequestration of intratumoral TGF-β with TGFβRII:Fc reduces CD4$^+$CD25$^+$FoxP3$^+$ Tregs in tumor tissue and improves the antitumor efficacy of doxorubicin. MB49 tumor-bearing mice were administered doxorubicin (5mg/kg i.p. weekly x 3) with or without twice weekly treatment with TGFβRII:Fc (1μg intratumoral). The percentage of CD4$^+$CD25$^+$FoxP3$^+$ cells (Tregs) among tumor cells was assessed by flow cytometry (FIG. 54E), and tumor volume was monitored to determine the effect of counteracting tumor-induced TGF-β-mediated immune tolerance on the *in vivo* antitumor efficacy of doxorubicin (FIG. 54F).

[0098] Figures 55A-55D show that anti-CD4 antibody-mediated depletion of CD4$^+$ regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive CD8$^+$ T cells and enhances the antitumor efficacy of chemotherapy. (FIG. 55A) *In vivo* depletion of tumor-infiltrating CD4$^+$CD25$^+$FoxP3$^+$ T cells by treatment of tumor-bearing mice with anti-CD4 antibody. C57BL/6 mice injected with $5 x10^5$ MB49 tumor cells s.c. were left untreated (control) or administered an anti-CD4 antibody (Clone GK1.5) i.p. at 5d and 9d

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following tumor challenge. $CD4^+CD25^+FoxP3^+$ T cells infiltrating tumors isolated from mice at d16 following tumor challenge were detected by flow cytometry. (FIG. 55B) Target-specific depletion of either $CD4^+$ T cells, $CD4^+CD25^+FoxP3^+$ T cells, or $CD8^+$ T cells by treatment of tumor-bearing mice with anti-CD4 antibody or anti-CD8 antibody. C57BL/6 mice injected s.c. with 5 $x10^5$ MB49 tumor cells were left untreated or treated with doxorubicin (5mg/kg i.p. weekly x 3) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Flow cytometric analyses of peripheral blood mononuclear cells isolated from mice at d16 following tumor challenge determined the percentage of $CD4^+$ T cells or $CD8^+$ T cells among total mononuclear cells, and the percentage of $CD4^+CD25^+FoxP3^+$ T cells among total $CD4^+$ T cells. (FIG. 55C) Depletion of $CD4^+$ regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive $CD8^+$ T cells. C57BL/6 mice injected s.c. with 5 $x10^5$ MB49 tumor cells were left untreated or treated with doxorubicin (5mg/kg i.p. weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of IFN-γ expression in $CD8^+$ T cells from the tumor and draining lymph node in response to *in vitro* stimulation with MB49 cell lysates. (FIG. 55D) Depletion of $CD4^+$ regulatory T cells augments the *in vivo* antitumor efficacy of chemotherapy via activation of tumor-reactive $CD8^+$ T cells. C57BL/6 mice injected s.c. with 5 $x10^5$ MB49 tumor cells were left untreated or treated with doxorubicin (5mg/kg i.p. weekly x 3) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting either $CD4^+$ T cells or $CD8^+$ T cells on the *in vivo* antitumor efficacy of doxorubicin.

[0099] Figures 56A-56F show anti-CD4 antibody-mediated depletion of $CD4^+$ regulatory T cells augments and sustains the antitumor effect of chemotherapy by enabling activation of adaptive antitumor immunity. (FIG. 56A) Surface exposure of calreticulin (CRT) in response to treatment of MB49 cancer cells with either cisplatin or the combination of cisplatin and gemcitabine for 4h. The surface exposure of CRT was

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determined by immunofluorescence cytometry of untreated control or chemotherapy-treated cells stained with Dylight-labeled anti-CRT antibody or an isotype control (IgG1) antibody. (FIGS. 56B, 56C) Depletion of $CD4^+$ regulatory T cells enables cisplatin-induced activation of tumor-reactive IFN-γ^+CD8^+ T cells and effector memory ($CD8^+CD62L^-$) T cells. C57BL/6 mice injected s.c. with 5 $x10^5$ MB49 tumor cells were left untreated or treated with cisplatin (0.5mg/kg i.p. weekly x 4) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of IFN-γ expression in $CD8^+$ T cells from the tumor and draining lymph node (DLN) in response to *in vitro* stimulation with MB49 cell lysates (FIG. 56B). The percentage of effector memory T_{EM} cells was determined by flow cytometric analyses of $CD8^+CD62L^-$ cells (FIG. 56C). (FIGS. 56D, 56E and 56F) Depletion of $CD4^+$ regulatory T cells augments the *in vivo* antitumor efficacy of chemotherapy via activation of tumor-reactive $CD8^+$ T cells. C57BL/6 mice injected s.c. with 5 $x10^5$ MB49 tumor cells were left untreated or treated with either cisplatin (0.5mg/kg) or the combination of cisplatin and gemcitabine (i.p. weekly x 4) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting either $CD4^+$ T cells or $CD8^+$ T cells on the *in vivo* antitumor efficacy of chemotherapy and the percentage of mice exhibiting complete tumor-regression by d50 following tumor inoculation. Establishment of adaptive antitumor immunity following regression of primary tumors was determined by re-challenging mice with live MB49 tumor cells in the opposite flank.

[0100] Figures 57A-57H show that Chemotherapy-induced expression of NKG2D ligands on tumor cells cooperates with depletion of $CD4^+$ regulatory T cells to stimulate $CD8^+$ T cell-mediated tumor regression. (FIG. 57A) Genotoxic chemotherapeutic agents induce expression of mouse NKG2D ligands (Rae-1) on cancer cells. Kinetics of the upregulation of Rae1 transcripts in mouse CT26 colon cancer cells was determined by quantitative real-time PCR following treatment with irinotecan (25 μg/ml) or oxaliplatin (10 μg/ml). Quantitative RT-PCR was carried out using Rae-1 specific primers [sense, 5'-

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CTAGTGCCACCTGGGAATTCA-3' (SEQ ID NO: 111); anti-sense, 5'-CATCATTAGCTGATCTCCAGCTCA-3'(SEQ ID NO: 112)] and probe [5'-6-FAM-CATCAGTGACAGTTACTTCTTCACCTTCTACACAGAGA-Tamra-3' (SEQ ID NO: 113)]. (FIG. 57B) Genotoxic chemotherapeutic agents induce p53-independent cell surface expression of human NKG2D ligands (MHC-I-related A and B molecules – MICA/MICB) on cancer cells. Isogenic p53-proficient ($p53^{+/+}$) or p53-deficient ($p53^{-/-}$) HCT116 cells were treated with irinotecan (25 μg/ml) for 16h or left untreated. Irinotecan-induced upregulation of cell surface expression of MICA/B was determined by flow cytometryic analysis of tumor cells labeled with an anti-human MICA/B MAb (R&D Systems). (FIG. 57C) and (FIG. 57D) Induction of NKG2D ligands contributes to the antitumor effect of chemotherapy *in vivo*. Immunocompetent Balb/C mice injected s.c. with 2 $x10^5$ syngeneic CT26 tumor cells were treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d5 following tumor inoculation, with or without pre-treatment with an NKG2D blocking antibody (CX5, eBIOscience) (200 μg i.p.) at 16h before each dose of chemotherapy. Tumor volume was monitored to determine the effect of NKG2D blockade on the *in vivo* antitumor efficacy of irinotecan. (FIG. 57E) *In vivo* depletion of $CD4^+CD25^+FoxP3^+$ T cells by treatment of tumor-bearing mice with anti-CD4 antibody. Balb/C mice injected with 2 $x10^5$ CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. $CD4^+CD25^+FoxP3^+$ T cells in spleen and draining lymph node isolated from mice at d16 following tumor challenge were detected by flow cytometry. (FIG. 57F) Depletion of $CD4^+$ regulatory T cells facilitates irinotecan-induced activation of tumor-reactive IFN-γ^+CD8^+ T cells. Balb/C mice injected with 2 $x10^5$ CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of IFN-γ expression in $CD8^+$ T cells from the tumor and draining lymph node (DLN) in response to *in vitro* stimulation with either CT26 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone. (FIG. 57G) and (FIG. 57H) Chemotherapy-induced expression of NKG2D ligands on

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tumor cells cooperates with depletion of $CD4^+$ regulatory T cells to stimulate $CD8^+$ T cell-mediated tumor regression. Balb/C mice injected with 2 $x10^5$ CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) and/or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting $CD4^+$ T cells and/or $CD8^+$ T cells on the *in vivo* antitumor efficacy of irinotecan.

DETAILED DESCRIPTION OF THE INVENTION

[0101] Targeted immunostimulatory antibodies and/or fusion proteins for prevention or treatment of cancer: Chemotherapy is a cornerstone of systemic treatment of patients with most common types of advanced cancers. The vast majority of human cancers harbor genetic alterations and signaling mechanisms that impair the direct death signaling pathways entrained by chemotherapeutic agents. Although chemotherapeutic agents employ diverse mechanisms to directly kill tumor cells, the present invention provides that these agents have immuno-adjuvant effects which activate innate and adaptive antitumor immune responses that are crucial for their antitumor efficacy *in vivo*. The present invention also provides that antitumor $CD8^+$ T cells play an instrumental role in the *in vivo* response of tumors to diverse cytotoxic chemotherapeutic agents. Although chemotherapeutic agents can induce "immunogenic" tumor cell death and facilitate cross-presentation of antigens by dendritic cells, tumors create a tolerogenic environment that allows them to suppress the activation of innate and adaptive immune responses and evade immunologic attack by immune effector cells. The present invention provides that strategies to counteract tumor-induced immune tolerance in the tumor microenvironment can enhance the antitumor efficacy of chemotherapy by activating and leveraging T cell-mediated adaptive antitumor immunity against disseminated cancer cells.

[0102] The present invention is based on the seminal discovery that targeted immunomodulatory antibodies and fusion proteins can counteract or reverse immune tolerance of cancer cells. Cancer cells are able to escape elimination by chemotherapeutic agents or tumor-targeted antibodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. By counteracting tumor-induced immune tolerance, the present invention

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provides effective compositions and methods for cancer treatment, optional in combination with another existing cancer treatment.

[0103] The present invention provides compositions and methods for targeted immunostimulatory antibodies and fusion proteins that counteract immune tolerance in the tumor microenvironment and promote T cell-mediated adaptive antitumor immunity for maintenance of durable long-term protection against recurrent or disseminated cancers. These tumor-targeted immunostimulatory molecules are designed to facilitate effective long term T cell-mediated immune responses against tumor cells by at least one of the following:

(i) promoting death of tumor cells via enhancement of antibody-dependent cellular cytotoxicity (ADCC);

(ii) facilitating effective cross-presentation of tumor antigen(s) from dying tumor cells by augmenting maturation of dendritic cells (DCs); and

(iii) increasing activation and proliferation of antitumor CD8+ T cells by negating immune suppression mediated by regulatory T cells and myeloid suppressor cells. These antitumor immune responses may be activated in tandem with the sensitization of tumor cells to immune effector-mediated cytotoxicity, thereby establishing a positive feedback loop that augments tumor cytoreduction and reinforces adaptive antitumor immunity. The tumor-targeted immunostimulatory monoclonal antibodies (mAbs) of the present invention provides the ability to generate and boost antitumor immunity to multiple cross-presented tumor antigens obtained from endogenous tumor cells during the course of therapy (as an *in situ* tumor vaccine), while simultaneously leveraging the antitumor immune response to eliminate disseminated cancer cells. Accordingly, the targeted immunostimulatory antibodies and fusion proteins of the invention can integrate the hitherto distinct fields of passive and active immunotherapy and provide a novel platform for simultaneously leveraging the synergistic benefits of these strategies to entrain effective innate and adaptive immune responses against targeted cancers.

[0104] 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

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cells has remained challenging. Several tumor-specific and tumor-associated antigens have been identified, yet tumors employ diverse mechanisms to create a tolerogenic environment that allows them to suppress the activation of a T cell-mediated antitumor immune response. The tumor-targeted immunostimulatory antibodies and/or fusion proteins of the invention are designed to overcome such immune tolerance in the tumor microenvironment and activate robust levels of T cell responses for effective cancer immunotherapy or chemo-immunotherapy. Accordingly, the tumor-targeted immunostimulatory antibodies and/or fusion proteins of the invention have broad clinical relevance for advancing the treatment of many types of human cancers.

[0105] The tumor-targeted immunostimulatory mAbs and/or fusion proteins of the invention provide their ability to generate and boost antitumor immunity to multiple cross-presented tumor antigens obtained from endogenous tumor cells during the course of therapy (as an *in situ* tumor vaccine), while simultaneously leveraging the antitumor immune response to eliminate disseminated cancer cells. Accordingly, the tumor-targeted immunostimulatory antibodies and/or fusion proteins of the invention can integrate the hitherto distinct fields of passive and active immunotherapy and provide a novel platform for simultaneously leveraging the synergistic benefits of these strategies to entrain effective innate and adaptive immune responses against targeted cancers. This approach of the present invention is distinguished from and superior to conventional tumor antigen-, allogeneic tumor cell- or DC-based vaccines in at least one of the following aspects: (i) There is no *a priori* requirement to define, clone and purify individual tumor antigens, since the patient's tumor itself is the *in vivo* source of antigens; (ii) Multivalent antitumor immune responses that are naturally tailored against antigens from the patient's own tumor are less likely to allow immune escape than a pre-selected tumor antigen; (iii) The activation of antitumor immune responses by the immuno-adjuvant effects of tumor-targeted immunostimulatory antibodies or fusion proteins occurs in tandem with the sensitization of tumor cells to immune effector-mediated cytotoxicity, thereby establishing a positive feedback loop that augments tumor cytoreduction and reinforces adaptive antitumor immunity; and (iv) The molecules of the invention have broad clinical relevance for advancing the treatment of many types of human cancers.

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[0106] In addition, the targeted immunostimulatory antibodies and/or fusion proteins of the invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) to counteract immune tolerance in the tumor microenvironment and promote T cell-mediated adaptive antitumor immunity for maintenance of long-term protection against recurrent or disseminated cancers (for prevention or treatment of diverse cancers); (ii) to produce immune cell compositions for adoptive cellular therapy of diverse cancers; and (iii) to serve as immune adjuvants or vaccines for prophylaxis of diverse cancers or infectious diseases.

[0107] The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to disrupt immunosuppressive networks in the tumor microenvironment. Tumors employ a wide array of regulatory mechanisms to avoid or suppress the immune response. Cancer cells actively promote immune tolerance in the tumor microenvironment via the expression of cytokines and molecules that inhibit the differentiation and maturation of antigen-presenting dendritic cells. The immunosuppressive cytokines and ligands produced by tumor cells include the following: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed death-1 ligand 1 (PD-L1; B7-H1); (iii) Vascular endothelial growth factor (VEGF); and (iv) Interleukin-10 (IL-10). In addition to blocking dendritic cell (DC) maturation, these molecules promote the development of specialized subsets of immunosuppressive $CD4^+$ T cells (regulatory T cells; Treg cells) and myeloid-derived suppressor cells (MDSC). Tregs are a minority sub-population of $CD4^+$ T cells that constitutively express CD25 [the interleukin-2 (IL-2) receptor α-chain] and the forkhead box P3 (FOXP3) transcription factor. Tregs (CD4+CD25+FoxP3+ cells) maintain immune tolerance by restraining the activation, proliferation, and effector functions of a wide range of immune cells, including $CD4^+$ and $CD8^+$ T cells, natural killer (NK) and NKT cells, B cells and antigen presenting cells (APCs) *in vitro* and *in vivo*. The accumulation of Treg cells in the tumor microenvironment re-inforces tumor immune tolerance and facilitates tumor progression and metastases. The increased expression of immunosuppressive cytokines (TGF-β; PD-L1) and tumor-infiltrating Tregs is correlated with a reduction of survival of patients with diverse types of cancers. The present invention provides that tumor-induced immune tolerance mediated via Tregs is a crucial determinant of the resistance of cancers to

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

[0108] The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to inhibit the development or function of Tregs and myeloid suppressor cells (DCs or MDSC) within the tumor microenvironment. Tregs (CD4+CD25+FoxP3+ cells) express an array of immunosuppressive cytokines and molecules which act in concert to induce immune tolerance and promote tumor progression and metastases. These include: (i) Cytotoxic T-lymphocyte associated protein 4 (CTLA-4; CD152), a co-inhibitory receptor that binds to the ligands CD80 (B7-1) or CD86 (B7-2) on the antigen presenting cell (APC) and inhibits co-stimulation of T cells; (ii) Programmed death-1 ligand 1 (PD-L1; B7-H1), a ligand which engages the co-inhibitory receptor Programmed death-1 (PD-1) and inhibits T cell activation and proliferation. (iii) Transforming growth factor-beta (TGF-β), a cytokine which regulates immune responses by restricting the maturation and antigen-presenting function of dendritic cells, inhibiting the proliferation and activation of naïve T cells, suppressing the expression of cytotoxic molecules (Granzyme A/B, FasL, Apo2L/TRAIL, IFN-γ) in immune effector cells, and promoting the development and function of Tregs; (iv) Receptor activator of nuclear factor-κB ligand (RANKL), a ligand which engages Receptor activator of nuclear factor-κB (RANK) and promotes osteoclast differentiation, Treg development, and tumor metastases. In addition, Tregs express other surface molecules; (v) LAG-3, a CD4-related molecule that binds MHC class II; (vi) glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); and (vii) IL-10. The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to bind a targeted molecule expressed by Tregs or myeloid suppressor cells while concurrently sequestering and inhibiting one or more immunosuppressive molecule that promotes their development, survival or function.

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In one aspect, the targeted immunostimulatory antibodies and/or fusion proteins directly deplete the number of Tregs.

[0109] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule on the tumor cell or tumor microenvironment (tumor stroma, tumor vasculature, or tumor infiltrating immune cell), and the immunomodulatory moiety specifically binds an immunosuppressive molecule expressed by the targeted tumor cell or tumor-infiltrating Treg cells and myeloid suppressor cells (DC or MDSC).

[0110] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule expressed by Treg cells, myeloid suppressor cells (MDSC), or dendritic cells (DC), and the immunomodulatory moiety specifically binds an immunosuppressive molecule that promotes their development, survival or function.

[0111] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule, and the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2); (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); (iv) Vascular endothelial growth factor (VEGF); (v) Transforming growth factor-beta receptor (TGF-βR); (vi) Programmed death-1 (PD-1); and (vii) Receptor activator of nuclear factor-κB (RANK).

[0112] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. In one aspect, the targeting moiety specifically binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), CD20, Vascular endothelial growth factor (VEGF), insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial

cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, CD30, CD33, or CD40.

[0113] In one aspect, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: (i) CD4; (ii) CD25 (IL-2α receptor; IL-2αR); (iii) cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); (iv) Interleukin-10 (IL-10); (v) Transforming growth factor-beta receptor (TGF-βR); (vi) Transforming growth factor-beta (TGF-β); (vii) Programmed Death-1 (PD-1); (viii) Programmed death-1 ligand (PD-L1 or PD-L2); (ix) Receptor activator of nuclear factor-κB (RANK); (x) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); (xi) LAG-3; or (xii) glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18).

[0114] In one aspect, the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed death-1 ligand (PD-L1 or PD-L2); (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); or (iv) vascular endothelial growth factor (VEGF).

[0115] In one aspect, the immunomodulatory moiety includes a molecule that binds TGF-β. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Transforming growth factor-beta receptor TGF-βRII, TGF-βRIIb, or TGF-βRIII. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes a TGF-β-binding amino acid sequence corresponding to SEQ ID NOs: 79-91. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of TGF-β.

[0116] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the

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molecule includes an amino acid sequence corresponding to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

[0117] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Programmed Death-1 (PD-1), Programmed death-1 ligand 1 (PD-L1), or Programmed death-1 ligand 2 (PD-L2). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes PD-1 ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

[0118] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

[0119] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes a PD-L1-binding amino acid sequence corresponding to SEQ ID NO: 92, 93, or 94. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Programmed death-1 ligand 1 (PD-L1).

[0120] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial

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growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.

[0121] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Programmed Death-1 (PD-1). In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and PD-1 ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 25 or 26.

[0122] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes a RANKL-binding amino acid sequence corresponding to SEQ ID NO: 95, 96, 97, or 98. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Receptor activator of nuclear factor-κB ligand (RANKL).

[0123] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36.

[0124] The present invention provides novel targeted immunosuppressive antibodies and fusion proteins that induce or promote immune tolerance by at least one of the following:

(i) inhibiting the activation of dendritic cells, T cells, and/or B cells; and

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

[0125] Targeted immunosuppressive antibodies and/or fusion proteins: The aberrant activation of self-reactive T cells and/or breakdown of the mechanisms of immune tolerance promotes the development of autoimmunity that results in various diseases including type I diabetes mellitus, multiple sclerosis, systemic lupus erythematosus, inflammatory bowel disease, and rheumatoid arthritis. The targeted immunosuppressive antibodies and/or fusion proteins of the invention are designed to suppress unwanted or excessive immune or inflammatory responses and restore or promote immune tolerance. Accordingly, the compositions and methods of the invention have broad clinical relevance for the treatment of diverse autoimmune or inflammatory diseases and preventing the rejection of a transplanted cell, tissue, or organ grafts.

[0126] The targeted immunosuppressive antibodies and/or fusion proteins of the invention provides their ability to inhibit the activity of targeted pro-inflammatory cytokines or immune cells while simultaneously promoting immune tolerance via the targeted delivery of immunosuppressive molecules that facilitate the development and/or function of regulatory T cells. These molecules of the present invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) The molecules of the invention enable targeted delivery of immunosuppressive molecules to immune cells or pro-inflammatory molecules in the milieu of the affected cell, tissue or organ; (ii) The molecules of the invention can couple the inhibition of the targeted pro-inflammatory molecule or immune cell with the

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

[0127] Further, the targeted immunosuppressive antibodies and/or fusion proteins of the invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) To suppress unwanted or excessive immune or inflammatory responses in order to treat autoimmune or inflammatory diseases; and (ii) To prevent the rejection of a transplanted cell, tissue, or organ grafts.

[0128] In one aspect, the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes a PD-1-binding amino acid sequence corresponding to SEQ ID NO: 99, 100, or 101. In an additional aspect, the immunomodulatory moiety increases the function of PD-1.

[0129] In one aspect, the targeting moiety specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 37. In another aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2), and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from PD-L1. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 38 or 39.

[0130] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25, or CD4, and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1

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ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 40, 41, 42, 43, 44, or 45.

[0131] In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 46 or 47.

[0132] In one aspect, the targeting moiety includes transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 48 or 49.

[0133] In one aspect, the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 99, 100, or 101. In an additional aspect, the immunomodulatory moiety activates the signaling function of transforming growth factor-β (TGF-β) receptor.

[0134] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from TGF-β. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 50. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 51 or 52.

[0135] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25 (IL-2α receptor; IL-2αR), or CD4, and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 53, 54, 55, 56, 57 or 58.

[0136] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 59 or 60.

[0137] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) or Osteoprotegerin (OPG). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from an extracellular RANKL-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 61. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from an extracellular RANK-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 62 or 63.

[0138] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) or Osteoprotegerin (OPG). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

[0139] In one aspect, the targeting moiety includes a sequence from transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), and the

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immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

[0140] In one aspect, the targeting moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

[0141] In various aspects, the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

[0142] In another embodiment, the present invention provides a composition including the molecule of the invention and a cell, wherein the cell is a tumor cell, immune cell, or dendritic cell.

[0143] In another embodiment, the present invention provides a method of counteracting or overcoming immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

[0144] In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. In one aspect, the neoplastic disease is a non-T cell malignancy which does not express CD4 on the tumor cell. In one embodiment, the method includes administration to a subject in need thereof an antibody that targets and depletes CD4+ regulatory T cells (Tregs) in combination with a cytotoxic anticancer therapy. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In various aspects, the cytotoxic anticancer therapy includes a chemotherapeutic molecule, tumor-targeted antibody, small molecule kinase inhibitor, hormonal agent, or tumor-targeted cytotoxic agent, anti-angiogenic agent or any combination thereof. In another aspect, the cytotoxic anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

[0145] In another embodiment, the method includes administration to a subject in need thereof an antibody or molecule that targets and depletes CD4+ regulatory T cells (Tregs) in combination with an immunostimulatory antibody, fusion protein, peptide or ligand that targets CTLA-4, PD1, PD-1L, RANKL, TGF- β, GITR, 4-1BB, OX-40, or Toll-like receptors (TLR 1-10). In one aspect, the TLR-agonist comprises an activator of TLR-8 or TLR-9. In one aspect, the TLR agonist comprises an immunostimulatory nucleic acid sequence containing CpG nucleotides. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody.

[0146] In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof one or more molecule of the invention. In various aspects, the subject is administered one or more molecule of the invention in combination with another anticancer therapy. In one aspect, the anticancer therapy includes a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent, cytotoxic agent, targeted therapeutic agent, or anti-angiogenic agent. In another aspect, the anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation. In another aspect, the subject is administered one or more molecule of the invention in combination with an antibody or molecule that inhibits the production or function of regulatory T cells (Tregs) or depletes the number of Tregs. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In another aspect, the molecule that counteracts the function of Tregs is an antibody, fusion protein, peptide or ligand that targets CTLA-4, PD1, PD-1L, RANKL, TGF- β, GITR. In another aspect, the molecule that counteracts the function of Tregs is an antibody or fusion protein or ligand that targets 4-1BB or OX-40. In another aspect, the molecule that counteracts the function of Tregs is an agonist of Toll-like receptors (TLR 1-10). In one aspect, the TLR-agonist comprises an activator of TLR-8 or TLR-9. In one aspect, the TLR agonist is an immunostimulatory nucleic acid sequence containing CpG nucleotides.

[0147] In one aspect the chemotherapeutic agent is a topoisomerase-interacting agent, anthracycline, doxorubicin, mitoxantrone, camptothecin, camptothecin analogue, irinotecan, epipodophyilotoxin, etoposide, alkylating agent, cyclophosphamide, cisplatin,

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cisplatin analogue, oxaliplatin, antimetabolite, fluoropyrimidine analogue, 5-fluorouracil, gemcitabine, azacytidine, antimicrotubule agent, taxane, paclitaxel, or docetaxel.

[0148] In another embodiment, the subject is administered one or more molecule of the invention in combination with any vaccine. In another aspect, the vaccine includes a tumor antigen, tumor-associated antigen, tumor epitope, tumor antigen-containing fusion protein, tumor cell, or dendritic cell. In another aspect, the vaccine includes a pathogen antigen, pathogen-associated antigen, pathogen epitope, or pathogen antigen-containing fusion protein. In one aspect, the vaccine includes a surrogate CD4+ T cell helper epitope from tetanus toxin. In one aspect, the CD4+ T helper sequence contains a domain of tetanus toxin fragment C (pDOM1). In one aspect, the pDOM sequence is fused to a cell-permeabilizing cationic polypeptide (*e.g.,* Arginine-9). In another aspect, the Arg9-pDOM sequence is fused to a specific antigen comprising the vaccine.

[0149] In another embodiment, the present invention provides a method for treating immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treatment of a neoplastic disease. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

[0150] In another embodiment, the present invention provides a method of inducing or promoting immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

[0151] In another embodiment, the present invention provides a method of preventing or treating an autoimmune or inflammatory disease including administering to a subject in need thereof one or more molecule of the invention. In one aspect, the subject is administered one or more molecule of the invention in combination with another anti-inflammatory or immunosuppressive therapy. In another embodiment, the present invention provides a method of treatment of immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treating an autoimmune or inflammatory disease or preventing rejection of grafted cells or tissue. The method includes administering to a

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subject in need thereof a composition of immune cells contacted with a molecule of the invention.

[0152] 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.

[0153] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, references to "the method" includes one or more methods, and/or steps of the type described herein which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0154] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described.

[0155] As used herein "immune cells" or "immune effector cells" include T lymphocytes, B lymphocytes, natural killer (NK) cells, NKT cells, monocytes, macrophages, dendritic cells (DC), antigen presenting cells (APC).

[0156] As used herein, "neoplasm" or "tumor" including grammatical variations thereof, means new and abnormal growth of tissue, which may be benign or cancerous. In a related aspect, the neoplasm is indicative of a neoplastic disease or disorder, including but not limited, to various cancers. For example, such cancers can include prostate, pancreatic, biliary, colon, rectal, liver, kidney, lung, testicular, breast, ovarian, pancreatic, brain, and head and neck cancers, melanoma, sarcoma, multiple myeloma, leukemia, lymphoma, and the like.

[0157] A used herein, "subject," including grammatical variations thereof, means a human or vertebrate animal including a dog, cat, horse, cow, pig, sheep, goat, chicken, monkey, rat, and mouse.

[0158] As used herein, "targeting moiety" refers to a molecule that has the ability to localize and bind to a specific molecule or cellular component. The targeting moiety can be an antibody, antibody fragment, scFv, Fc-containing polypeptide, fusion antibody, polypeptide, peptide, aptamer, ligand, nucleic acid, or any combination thereof. In one embodiment, a targeting moiety can bind to a molecule present in a cell or tissue. In one aspect, the targeting moiety can bind a molecule in a diseased cell or tissue, such as a cancer cell or tumor. In, another aspect, the targeting molecule can bind a normal cell or tissue, such as an immune cell. In another aspect, the targeting moiety can bind a cellular or extracellular molecule that modulates the immune response. In another aspect, the targeting moiety binds a growth factor receptor, growth factor, cytokine receptor, cytokine, or cell surface molecule.

[0159] In another embodiment, the targeting moiety is a tumor-targeting moiety. The tumor-targeting moiety can bind a component of a tumor cell or bind in the vicinity of a tumor cell (*e.g.*, tumor vasculature or tumor microenvironment). In one embodiment, the tumor targeting moiety binds to a component of a tumor cell, tumor microenvironment, tumor vasculature, tumor-associated lymphocyte, tumor antigen, tumor-associated antigen, tumor cell surface molecule, tumor antigenic determinant, tumor antigen-containing fusion protein, tumor-associated cell, tumor-associated immune cell, or tumor vaccine.

[0160] For example, in various embodiments, a targeting moiety is specific for or binds to a molecule or component, which includes but is not limited to, epidermal growth factor receptor (EGFR, EGFR1, ErbB-1, HER1), ErbB-2 (HER2/neu), ErbB-3/HER3, ErbB-4/HER4, EGFR ligand family; insulin-like growth factor receptor (IGFR) family, IGF-binding proteins (IGFBPs), IGFR ligand family (IGF-1R); platelet derived growth factor receptor (PDGFR) family, PDGFR ligand family; fibroblast growth factor receptor (FGFR) family, FGFR ligand family, vascular endothelial growth factor receptor (VEGFR) family, VEGF family; HGF receptor family: TRK receptor family; ephrin (EPH) receptor family; AXL receptor family; leukocyte tyrosine kinase (LTK) receptor

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family; TIE receptor family, angiopoietin 1, 2; receptor tyrosine kinase-like orphan receptor (ROR) receptor family; discoidin domain receptor (DDR) family; RET receptor family; KLG receptor family; RYK receptor family; MuSK receptor family; Transforming growth factor alpha (TGF-α), TGF-α receptor; Transforming growth factor-beta (TGF-β), TGF-β receptor; Interleukin 13 receptor alpha2 chain (lL13Ralpha2), Interleukin-6 (IL-6), 1L-6 receptor, Interleukin-4, IL-4 receptor, Cytokine receptors, Class I (hematopoietin family) and Class II (interferon/lL-10 family) receptors, tumor necrosis factor (TNF) family, TNF-α, tumor necrosis factor (TNF) receptor superfamily (TNTRSF), death receptor family, TRAIL-receptor; cancer-testis (CT) antigens, lineage-specific antigens, differentiation antigens, alpha-actinin-4, ARTC1, breakpoint cluster region-Abelson (Bcr-abl) fusion products, B-RAF, caspase-5 (CASP-5), caspase-8 (CASP-8), beta-catenin (CTNNB1), cell division cycle 27 (CDC27), cyclin-dependent kinase 4 (CDK4), CDKN2A, COA-1, dek-can fusion protein, EFTUD-2, Elongation factor 2 (ELF2), Ets variant gene 6/acute myeloid leukemia 1 gene ETS (ETC6-AML1) fusion protein, fibronectin (FN), GPNMB, low density lipid receptor/GDP-L fucose: beta-Dgalactose 2-alpha-Lfucosyltraosferase (LDLR/FUT) fusion protein, HLA-A2, arginine to isoleucine exchange at residue 170 of the alpha-helix of the alpha2-domain in the HLA-A2 gene (HLA-A*201-R170I), MLA-A11, heat shock protein 70-2 mutated (HSP70-2M), KIAA0205, MART2, melanoma ubiquitous mutated 1,2, 3 (MUM-1, 2,3), prostatic acid phosphatase (PAP), neo-PAP, Myosin class 1, NFYC, OGT, OS-9, pml-RARalpha fusion protein, PRDX5, PTPRK, K-ras (KRAS2), N-ras (NRAS), HRAS, RBAF600, SIRT2, SNRPD1, SYT-SSX1 or -SSX2 fusion protein, Triosephosphate Isomerase, BAGE, BAGE-1, BAGE-2,3,4,5, GAGE-1,2,3,4,5,6,7,8, GnT-V (aberrant N-acetyl giucosaminyl transferase V, MGAT5), HERV-K-MEL, KK-LC, KM-HN-1, LAGE, LAGE-1, CTL-recognized antigen on melanoma (CAMEL), MAGE-Al (MAGE-1), MAGE-A2, MAGE-A3, MAGE-A4, MAGE-AS, MAGE-A6, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-3, MAGE-B1, MAGE-B2, MAGE-B5, MAGE-B6, MAGE-C1, MAGE-C2, mucin 1 (MUCl), MART-1/Melan-A (MLANA), gp100, gp100/Pme117 (S1LV), tyrosinase (TYR), TRP-1, HAGE, NA-88, NY-ESO-1, NY-ESO-l/LAGE-2, SAGE, Sp17, SSX-1,2,3,4, TRP2-1NT2, carcino-embryonic antigen (CEA), Kallikfein 4, mammaglobm-A, OA1, prostate specific antigen (PSA), prostate specific membrane

antigen, TRP-l/gp75, TRP-2, adipophilin, interferon inducible protein absent in nielanorna 2 (AIM-2), BING-4, CPSF, cyclin D1, epithelial cell adhesion molecule (Ep-CAM), EpbA3, fibroblast growth factor-5 (FGF-5), glycoprotein 250 (gp250intestinal carboxyl esterase (iCE), alpha-feto protein (AFP), M-CSF, mdm-2, MUCI, p53 (TP53), PBF, FRAME, PSMA, RAGE-1, RNF43, RU2AS, SOX10, STEAP1, survivin (BIRCS), human telomerase reverse transcriptase (hTERT), telomerase, Wilms' tumor gene (WT1), SYCP1, BRDT, SPANX, XAGE, ADAM2, PAGE-5, LIP1, CTAGE-1, CSAGE, MMA1, CAGE, BORIS, HOM-TES-85, AF15q14, HCA66I, LDHC, MORC, SGY-1, SPO11, TPX1, NY-SAR-35, FTHLI7, NXF2 TDRD1, TEX 15, FATE, TPTE, immunoglobulin idiotypes, Bence-Jones protein, estrogen receptors (ER), androgen receptors (AR), CD40, CD30, CD20, CD19, CD33, CD4, CD25, CD3, cancer antigen 72-4 (CA 72-4), cancer antigen 15-3 (CA 15-3), cancer antigen 27-29 (CA 27-29), cancer antigen 125 (CA 125), cancer antigen 19-9 (CA 19-9), beta-human chorionic gonadotropin, 1-2 microglobulin, squamous cell carcinoma antigen, neuron-specific enoJase, heat shock protein gp96, GM2, sargramostim, CTLA-4, 707 alanine proline (707-AP), adenocarcinoma antigen recognized by T cells 4 (ART-4), carcinoembryogenic antigen peptide-1 (CAP-1), calcium-activated chloride channel-2 (CLCA2), cyclophilin B (Cyp-B), human signet ring tumor-2 (HST-2), Human papilloma virus (HPV) proteins (HPV-E6, HPV-E7, major or minor capsid antigens, others), Epstein-Barr vims (EBV) proteins (EBV latent membrane proteins—LMP1, LMP2; others), Hepatitis B or C virus proteins, and HIV proteins. A composition of the invention can further include the foregoing as a peptide/polypeptide and/or encoding the same.

[0161] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. In one aspect, the targeting moiety specifically binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), CD20, Vascular endothelial growth factor (VEGF), insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, CD30, CD33, CD40, or a combination thereof.

[0162] Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as trastuzumab (anti-HER2/neu antibody); Pertuzumab (anti-HER2 mAb); cetuximab (chimeric monoclonal antibody to epidermal growth factor receptor EGFR); panitumumab (anti-EGFR antibody); nimotuzumab (anti-EGFR antibody); Zalutumumab (anti-EGFR mAb); Necitumumab (anti-EGFR mAb); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-447 (humanized anti-EGF receptor bispecific antibody); Rituximab (chimeric murine/human anti-CD20 mAb); Obinutuzumab (anti-CD20 mAb); Ofatumumab (anti-CD20 mAb); Tositumumab-I131 (anti-CD20 mAb); Ibritumomab tiuxetan (anti-CD20 mAb); Bevacizumab (anti-VEGF mAb); Ramucirumab (anti-VEGFR2 mAb); Ranibizumab (anti-VEGF mAb); Aflibercept (extracellular domains of VEGFR1 and VEGFR2 fused to IgG1 Fc); AMG386 (angiopoietin-1 and -2 binding peptide fused to IgG1 Fc); Dalotuzumab (anti-IGF-1R mAb); Gemtuzumab ozogamicin (anti-CD33 mAb); Alemtuzumab (anti-Campath-1/CD52 mAb); Brentuximab vedotin (anti-CD30 mAb); Catumaxomab (bispecific mAb that targets epithelial cell adhesion molecule and CD3); Naptumomab (anti-5T4 mAb); Girentuximab (anti-Carbonic anhydrase ix); or Farletuzumab (anti-folate receptor). Other examples include antibodies such as Panorex™ (17-1A) (murine monoclonal antibody); Panorex (@ (17-1A) (chimeric murine monoclonal antibody); BEC2 (ami-idiotypic mAb, mimics the GD epitope) (with BCG); Oncolym (Lym-1 monoclonal antibody); SMART M195 Ab, humanized 13' 1 LYM-1 (Oncolym), Ovarex (B43.13, anti-idiotypic mouse mAb); 3622W94 mAb that binds to EGP40 (17-1A) pancarcinoma antigen on adenocarcinomas; Zenapax (SMART Anti-Tac (IL-2 receptor); SMART M195 Ab, humanized Ab, humanized); NovoMAb-G2 (pancarcinoma specific Ab); TNT (chimeric mAb to histone antigens); TNT (chimeric mAb to histone antigens); Gliomab-H (Monoclonals—Humanized Abs); GNI-250 Mab; EMD-72000 (chimeric-EGF antagonist); LymphoCide (humanized IL.L.2 antibody); and MDX-260 bispecific, targets GD-2, ANA Ab, SMART IDIO Ab, SMART ABL 364 Ab or ImmuRAIT-CEA. Examples of antibodies include those disclosed in US5736167, US7060808, and US5821337.

[0163] In one embodiment, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: CD4; CD25 (IL-2α receptor; IL-2αR); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF-βR); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); Programmed death-1 ligand (PD-L1 or PD-L2); Receptor activator of nuclear factor-κB (RANK); Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); or Interleukin-4 receptor (IL-4R). In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

[0164] In one aspect, the targeting moiety binds a specific cytokine, cytokine receptor, co-stimulatory molecule, co-inhibitory molecule, or immunomodulatory receptor that modulates the immune system. In another aspect, the targeting moiety specifically binds one of the following molecules: tumor necrosis factor (TNF) superfamily; tumor necrosis factor-α (TNF-α); tumor necrosis factor receptor (TNFR) superfamily; Interleukin-12 (IL-12); IL-12 receptor; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNR4); OX40 ligand (OX40L; CD40; CD40 ligand (CD40L); CTLA-4; Programmed death-1 (PD-1); PD-1 ligand 1 (PD-L1; B7-H1); or PD-1 ligand 2 (PD-L2; B7-DC); B7 family; B7-1 (CD80); B7-2 (CD86); B7-H3; B7-H4; GITR/AITR; GITRL/AITRL; BTLA; CD70; CD27; LIGHT; HVEM; Toll-like receptor (TLR)(TLR 1,2,3,4,5,6,7,8,9, 10). In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

[0165] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide, or peptide that specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a cytokine, cytokine receptor, co-stimulatory molecule,

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or co-inhibitory molecule that modulates the immune system. In another aspect, the targeting moiety specifically binds one of the following molecules: CD4; CD25 (IL-2α receptor; IL-2αR); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF-βR); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); PD-1 ligand 1 (PD-L1; B7-H1); PD-1 ligand 2 (PD-L2; B7-DC); Receptor activator of nuclear factor-κB (RANK); Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); Interleukin-4 receptor (IL-4R); tumor necrosis factor (TNF) superfamily; tumor necrosis factor-α (TNF-α); tumor necrosis factor receptor (TNFR) superfamily; Interleukin-12 (IL-12); IL-12 receptor; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNR4); OX40 ligand (OX40L; CD40; CD40 ligand (CD40L); CTLA-4; B7 family; B7-1 (CD80); B7-2 (CD86); B7-H3; B7-H4; GITR/AITR; GITRL/AITRL; BTLA; CD70; CD27; LIGHT; or HVEM. In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

[0166] Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as Zanulimumab (anti-CD4 mAb), Keliximab (anti-CD4 mAb); Ipilimumab (MDX-101; anti-CTLA-4 mAb); Tremilimumab (anti-CTLA-4 mAb); (Daclizumab (anti-CD25/IL-2R mAb); Basiliximab (anti-CD25/IL-2R mAb); MDX-1106 (anti-PD1 mAb); antibody to GITR; GC1008 (anti-TGF-β antibody); metelimumab/CAT-192 (anti- TGF-β antibody); lerdelimumab/CAT-152 (anti- TGF-β antibody); ID11 (anti- TGF-β antibody); Denosumab (anti-RANKL mAb); BMS-663513 (humanized anti-4-1BB mAb); SGN-40 (humanized anti-CD40 mAb); CP870,893 (human anti-CD40 mAb); Infliximab (chimeric anti-TNF mAb; Adalimumab (human anti-TNF mAb); Certolizumab (humanized Fab anti-TNF); Golimumab (anti-TNF); Etanercept (Extracellular domain of TNFR fused to IgG1 Fc); Belatacept (Extracellular domain of CTLA-4 fused to Fc); Abatacept (Extracellular domain of CTLA-4 fused to Fc); Belimumab (anti-B Lymphocyte stimulator); Muromonab-CD3 (anti-CD3 mAb); Otelixizumab (anti-CD3 mAb); Teplizumab (anti-CD3 mAb); Tocilizumab (anti-IL6R mAb); REGN88 (anti-IL6R mAb); Ustekinumab

(anti-IL-12/23 mAb); Briakinumab (anti-IL-12/23 mAb); Natalizumab (anti-α4 integrin); Vedolizumab (anti-α4 β7 integrin mAb); T1h (anti-CD6 mAb); Epratuzumab (anti-CD22 mAb); Efalizumab (anti-CD11a mAb); and Atacicept (extracellular domain of transmembrane activator and calcium-modulating ligand interactor fused with Fc).

[0167] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an "immunomodulatory moiety". As used herein, "immunomodulatory moiety" refers to a ligand, peptide, polypeptide, or Fc-containing polypeptide that binds a specific component of a regulatory T cell, myeloid suppressor cell, or dendritic cell and modulates the number or function of Tregs or myeloid suppressor cells. In an additional aspect, the "immunomodulatory moiety" specifically binds a cytokine, cytokine receptor, co-stimulatory molecule, or co-inhibitory molecule that modulates the immune system. In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: Transforming growth factor-beta receptor (TGF-βR); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); PD-1 ligand 1 (PD-L1; B7-H1); PD-1 ligand 2 (PD-L2; B7-DC); Receptor activator of nuclear factor-κB (RANK); or Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); or vascular endothelial growth factor (VEGF). In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR; TNFRSF18); GITRL/AITRL; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNR4); OX40 ligand (OX40L); B7-H3; B7-H4; BTLA; CD40; CD40 ligand (CD40L); CD70; CD27; LIGHT; or HVEM. In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: tumor necrosis factor-α (TNF-α); Interleukin-12 (IL-12); IL-12R; Interleukin-10 (IL-10); IL-10R. In another aspect, the immunoodulatory moiety comprises an extracellular domain of CTLA-4. In one aspect, the immunomodulatory moiety is an agonist that increases the function of the bound molecule. In another aspect, the immunomodulatory moiety is an antagonist that inhibits the function of the targeted molecule.

[0168] In another aspect, the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming

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

[0169] In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: Transforming growth factor-beta (TGF-β); PD-1 ligand 1 (PD-L1); PD-1 ligand 2 (PD-L2); or IL-10. In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL.

[0170] In another aspect, the immunomodulatory moiety is fused to the C-terminus of the targeting moiety. In another aspect, the immunomodulatory moiety is fused to the N-terminus of the targeting moiety. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is the targeting moiety, Fc is an immunoglobulin Fc region, and Y is the immunomodulatory moiety. In another aspect, the fusion molecule is represented by Y-Fc-X, wherein X is the targeting moiety, and Y is the immunomodulatory moiety. In one aspect, the targeting moiety may additionally be an immunomodulatory moiety.

[0171] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell, and the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF-βRII, TGF-βRIIb, or TGF-βRIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor-κB (RANK); vascular endothelial growth factor receptor (VEGFR1 or VEGFR2); or IL-10R.

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[0172] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell, and the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL.

[0173] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds a specific component of a regulatory T cell, myeloid suppressor cell, or dendritic cell, and the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF-βRII, TGF-βRIIb, or TGF-βRIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor-κB (RANK); or IL-10R. In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL. In another aspect, the specific targeted component of a regulatory T cell, myeloid suppressor cell, or dendritic cell is one of the following molecules: CD4; CD25 (IL-2α receptor; IL-2αR); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); Programmed death-1 ligand (PD-L1 or PD-L2); Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); or Interleukin-4 receptor (IL-4R).

[0174] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds one of the following: CTLA-4; 4-1BB (CD137); OX40 (CD134; TNR4); CD40; IL-12R; or glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR; TNFRSF18); and the immunomodulatory moiety comprises a different molecule selected from the following: (i) an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF-βRII, TGF-βRIIb, or TGF-βRIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor-κB (RANK); or IL-

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10R; or (ii) Fc-containing polypeptide or ligand that binds one of the following: CTLA-4; 4-1BB (CD137); OX40 (CD134; TNR4); CD40; IL-12R; or GITR (AITR; TNFRSF18).

[0175] In another aspect, the targeting moiety and immunomodulatory moiety are two different molecules selected from any of the following: an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds TGF-β, CTLA-4, PD-1, 4-1BB (CD137), OX40 (CD134; TNR4), CD40; IL-12R, or GITR/AITR (TNFRSF18), or Toll-like receptor (TLR); an extracellular domain or ligand-binding sequence of Transforming growth factor-beta receptor (TGF-βRII, TGF-βRIIb, or TGF-βRIII), Programmed Death-1 (PD-1), Receptor activator of nuclear factor-κB (RANK), or IL-10R. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is an immunomodulatory targeting moiety and Y is a different immunomodulatory moiety.

[0176] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds one of the following molecules: CD4; CD25 (IL-2α receptor; IL-2αR); or CD20; and the immunomodulatory moiety comprises one of the following ligands or active ligand fragments: Transforming growth factor-beta (TGF-β); PD-1 ligand 1 (PD-L1); PD-1 ligand 2 (PD-L2); or IL-10.

[0177] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds tumor necrosis factor-α (TNF-α), Interleukin-12 (IL-12), IL-6R, B-lymphocyte stimulator, CD11a, CD6, or CD22; and the immunomodulatory moiety comprises one of the following: (i) ligands or active ligand fragments of Transforming growth factor-beta (TGF-β), PD-1 ligand 1 (PD-L1), or IL-10; or (ii) an extracellular domain or ligand-binding fragment of RANK, 4-1BB (CD137), OX40 (CD134; TNR4), CD40, IL-12R or GITR/AITR (TNFRSF18).

[0178] In another aspect, the targeting moiety comprises the extracellular domain of CTLA-4 fused to immunoglobulin Fc, and the immunomodulatory moiety comprises one of the following: (i) ligands or active ligand fragments of Transforming growth factor-beta (TGF-β), PD-1 ligand 1 (PD-L1), or IL-10; or (ii) ligand-binding fragment of TNFR2, RANK, 4-1BB (CD137), OX40 (CD134; TNR4), CD40, IL-12R or GITR/AITR (TNFRSF18).

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[0179] In another aspect, the targeting moiety and immunomodulatory moiety are two different molecules selected from any of the following: an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds tumor necrosis factor-α (TNF-α), Interleukin-12 (IL-12), IL-6R, B-lymphocyte stimulator, CD11a, CD6, or CD22; a ligand-binding fragment of TNFR2, RANK, 4-1BB (CD137), OX40 (CD134; TNR4), CD40, IL-12R or GITR/AITR (TNFRSF18); ligands or active ligand fragments of Transforming growth factor-beta (TGF-β), PD-1 ligand 1 (PD-L1), or IL-10; or CTLA-4-Fc. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is the immunomodulatory targeting moiety and Y is a different immunomodulatory moiety.

[0180] Antibodies: In one embodiment, the targeting moiety or fusion protein is an immunoglobulin. As used herein, the term "immunoglobulin" includes natural or artificial mono- or polyvalent antibodies including, but not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments. F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin ion can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgGl, IgG2, IgG3, IgG4, IgAl, and IgA2) or subclass of immunoglobulin molecule.

[0181] An antibody as disclosed herein includes an antibody fragment, such as, but not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdfv) and fragments including either a VL or VH domain. In one embodiment, the targeting moiety is an antibody or scFv.

[0182] An antigen-binding antibody fragment, including single-chain antibody, may include the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. An antigen-binding fragment can also include any combination of variable region(s) with a hinge region, CHI, CH2, and CH3 domains. Also includes is a Fc fragment, antigen-Fc fusion proteins, and Fc-

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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.

[0183] The antibody herein specifically include a "chimeric" antibody in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al. (1984) Proc. Natl. Acad. Sci. USA. 81:6851-6855). A chimeric antibody of interest herein includes "primatized" antibodies including variable domain antigen-binding sequences derived from a non-human primate (*e.g.*, Old World Monkey, Ape etc.) and human constant region sequences.

[0184] 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.

[0185] In one embodiment, a hybridoma can produce a targeted fusion protein comprising a targeting moiety and an immunomodulatory moiety. In one embodiment, a targeting moiety comprising an antibody, antibody fragment, or polypeptide is linked or fused to an immunomodulatory moiety consisting of a polypeptide, with a linker or without a linker. The linker can be an amino acid linker. In one embodiment, a linker is (GGGGS)n (SEQ ID NO: 123) wherein n is 1, 2, 3, 4, 5, 6, 7, or 8. For example, GGGGSGGGGSGGGGS (SEQ ID NO: 104). In another embodiment, a linker is EPKSCDK (SEQ ID NO: 105). In another embodiment, a linker is IEGRDMD (SEQ. ID. NO: 106). In various aspects, the length of the linker may be modified to optimize binding of the target moiety or the function of the immunomodulatory moiety. In various aspects, the immunomodulatory moiety is a polypeptide that is fused to the C-terminus of the Fc region of the heavy chain of a targeting antibody or Fc-containing fusion protein. In another aspect, the immunomodulatory moiety is a polypeptide that is fused to the C-terminus of the light chain of a targeting antibody. In another aspect, the fusion protein comprises an X-Fc-Y sequence, wherein X is a targeting polypeptide and Y is an immunomodulatory polypeptide.

[0186] For example, a hybridoma can produce the polypeptides corresponding to SEQ. ID. NO: 1-69.

[0187] An antibody fragment can include a portion of an intact, antibody, *e.g.,* including the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')2, and Fv fragments; Fc fragments or Fc-fusion products; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragment(s).

[0188] An intact antibody is one which includes an antigen-binding variable region as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variant thereof tor any other modified Fc (*e.g.,* glycosylation or other engineered Fc).

[0189] The intact antibody may have one or more "effector functions" which refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region or any other modified Fc region) of an antibody. Examples of antibody effector functions include Clq binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor (BCR); and cross-presentation of antigens by antigen presenting cells or dendritic cells. In one embodiment, the targeting antibody or Fc-containing fusion protein facilitates focused or preferential delivery of a immunomodulatory moiety to a target cell. In another aspect, a targeting antibody can induce death of the targeted cell or sensitize it to immune cell-mediated cytotoxicity. In another aspect, the Fc-fusion protein or antibody can facilitate delivery of the immunomodulatory moiety or immunogenic apoptotic material from antibody-bound tumor targets, or both, to an antigen presenting cells (APC) via interactions between their Fc and Fc receptors (on APC).

[0190] Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different "classes." There arc five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" (isotypes), *e.g.*, IgG1, IgG2, IgC3, IgG4, IgA, and IgA2. The heavy-chain constant domains (hat correspond to the different classes of antibodies are called alpha (α), delta (δ), epsilon (ε), gamma (γ), and mu (μ) respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0191] Peptides: In some aspects of the invention the targeting moiety or immunomodulatory moiety is a peptide or polypeptide. A peptide includes any analog, fragment or chemical derivative of a peptide whose amino acid residue sequence is shown herein. Therefore, a present peptide can be subject to various changes, substitutions, insertions, and deletions where such changes provide for certain advantages in its use. In this regard, a peptide of this invention corresponds to, rather than is identical to, the sequence of a recited peptide where one or more changes are made and it retains the ability to function as the unmodified peptide in one or more of the assays.

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[0192] The term "analog" includes any peptide having an amino acid residue sequence substantially identical to a sequence specifically shown herein in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the activity as described herein. Examples of conservative substitutions include the substitution of one non-polar (hydrophobic) residue such as isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another.

[0193] 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.

[0194] 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.

[0195] 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.

[0196] Aptamers: In one aspect of the invention, the targeting moiety is an aptamer. In various embodiments, an aptamer is specific for a molecule on a tumor cell, tumor vasculature, and/or a tumor microenvironment. The term "aptamer" includes DNA, RNA or peptides that are selected based on specific binding properties to a particular molecule. For example, an aptamer(s) can be selected for binding a particular gene product in a tumor cell, tumor vasculature, tumor microenvironment, and/or an immune cell, as disclosed herein, where selection is made by methods known in the art and familiar to one of skill in the art. Subsequently, said aptamer(s) can be administered to a subject to modulate or regulate an immune response.

[0197] Some aptamers having affinity to a specific protein, DNA, amino acid and nucleotides have been described (*e.g.*, K. Y. Wang, et al., Biochemistry 32:1899-1904 (1993); Pitner et al., U.S. Pat. No. 5,691,145: Gold, et al., Ann. Rev. Biochem. 64:763-797 (1995); Szostak et al., U.S. Pat. No. 5,631,146). High affinity and high specificity binding aptamers have been derived from combinatorial libraries (supra, Gold, et al.). Aptamers may have high affinities, with equilibrium dissociation constants ranging from micromolar to sub-nanomolar depending on the selection used, aptamers may also exhibit high selectivity, for example, showing a thousand fold, discrimination between 7-methyl G and G (Haller and Sarnow, Proc. Natl. Acad. Sci. USA 94:8521-8526 (1997)) or between D and L-tryptophan (supra, Gold et al.). An aptamer can be selected based on the particular molecule targeted (*e.g.*, aptamer targeting EGFR or other cancer markers). Standard procedures for in vitro selection are known, such as SELEX experiments, described at Science 249 (4968) 505-510 (1990), and Nature (London), 346 (6287) 818-822 (1990) which can be followed throughout, or with modifications and improvements known in the art.

[0198] 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.

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[0199] 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.

[0200] 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.

[0201] 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.

[0202] 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.

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[0203] The following examples are provided to further illustrate the embodiments of the present invention, but are not intended to limit the scope of the invention. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

EXAMPLE 1

[0204] Counteracting Tumor Immune Tolerance via Antibody-mediated Depletion of $CD4^+$ Regulatory T Cells Facilitates the Activation of Tumor-Reactive $CD8^+$ T Cells and Enhances the *In Vivo* Antitumor Efficacy of Cytotoxic Anticancer Agents.

[0205] Immunogenic death of tumor cells by chemotherapeutic agents can induce $CD8^+$ T cell-mediated antitumor immunity. In response to specific chemotherapeutic agents, tumor cells exhibit the rapid translocation of intracellular calreticulin (CRT) to the cell surface where its aggregation provides a signal for the recognition and engulfment of dying tumor cells by antigen presenting dendritic cells (DCs). Treatment of mouse MB49 or human SW780 bladder cancer cells with doxorubicin, an anthracycline chemotherapeutic agent, induced rapid surface exposure of CRT that was detected by immunofluorescence cytometry of cells stained with Dylight 488-labeled anti-CRT antibody (FIG. 53A). To determine whether *ex vivo* treatment with doxorubicin induced an immunogenic death of tumor cells, either untreated live MB49 cells or an equivalent number of MB49 cells that were pre-treated *in vitro* with doxorubicin were injected into one flank of syngeneic immunocompetent C57BL/6 mice. Unlike mice injected with live tumor cells, mice injected with doxorubicin-treated tumor cells exhibited increased production of IFN-γ by draining lymph node (DLN) cells in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 53B). Vaccination with doxorubicin-killed MB49 cells generated a tumor-specific immune response since no corresponding increase in IFN-γ secretion by DLN cells was observed following *in vitro* exposure to an irrelevant peptide (Hemagglutinin-HA). Injection of doxorubicin-treated MB49 tumor cells protected mice against tumor growth upon challenge with untreated live MB49 tumor cells injected into the opposite flank. (FIG. 1C). The protection against tumor growth by vaccination with doxorubicin-treated tumor cells was not observed in mice that were depleted of $CD8^+$ T cells with an anti-CD8 antibody before challenge with live tumor cells (FIG. 53C). These

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observations indicate that *ex vivo* treatment with chemotherapeutic agents can induce an immunogenic death of tumor cells that generates $CD8^+$ T cell-mediated adaptive antitumor immunity.

[0206] Tumor-induced immune tolerance inhibits activation of $CD8^+$ T cells in response to chemotherapy. To examine whether *in vivo* treatment with chemotherapeutic agents can activate $CD8^+$ T cell-mediated immune responses in mice with pre-established tumors, C57BL/6 mice were injected with live syngeneic MB49 tumor cells and then administered intratumoral doxorubicin at various time points following tumor inoculation. In contrast to vaccination of naïve mice with doxorubicin-killed MB49 cells, *in vivo* treatment of mice with established MB49 tumors at d10 following tumor inoculation failed to induce a corresponding increase in IFN-γ secretion by DLN cells in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 53B). Whereas treatment with doxorubicin on d3 following tumor inoculation was able to arrest tumor growth, delayed administration of the same dose of doxorubicin on d10 failed to inhibit the progressive growth of established MB49 tumors (FIG. 53D). These results indicate that tumor-induced immune tolerance in the microenvironment of established cancers counteracts the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death.

[0207] Regulatory T cells (Treg) accumulate in the tumor microenvironment and counteract the ability of chemotherapy to activate $CD8^+$ T cell mediated antitumor immunity. To investigate whether $FoxP3^+$ Tregs are involved in enforcing immune tolerance in the tumor microenvironment, we examined the percentage of $CD4^+CD25^+FoxP3^+$ cells (Tregs) among $CD4^+$ T lymphocytes in the spleen, draining lymph nodes (DLN), and tumors of immunocompetent C57BL/6 mice at d0 and d14 after tumor inoculation. Whereas tumor-bearing mice exhibited only a minor increase in the percentage of Tregs among $CD4^+$ T cells in the spleen and DLN at d14 following tumor inoculation, a majority of tumor-infiltrating $CD4^+$ T cells at this time were $CD4^+CD25^+FoxP3^+$ cells (FIG. 53E). To investigate whether Tregs infiltrating the tumor microenvironment can suppress the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death, $CD4^+CD25^+$ cells isolated from tumors and DLN of tumor-bearing mice were adoptively transferred into syngeneic C57BL/6 naïve

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mice before vaccination with doxorubicin-killed MB49 cells. The adoptive transfer of tumor-infiltrating $CD4^+CD25^+$ cells into naïve mice inhibited the ability of subsequent *in vivo* vaccination with doxorubicin-treated MB49 tumor cells to increase production of IFN-γ by draining lymph node (DLN) cells in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 53F). Consistent with the ability of tumor-infiltrating $CD4^+CD25^+$ cells to suppress the tumor-specific immune response, the adoptive transfer of these cells counteracted the protection conferred by vaccination with doxorubicin-treated MB49 cells against tumor growth upon challenge with untreated live MB49 tumor cells (FIG. 53G). These results indicate that the tumor microenvironment fosters the accumulation of $FoxP3^+$ Tregs which counteract the activation of $CD8^+$ T cell mediated antitumor immunity in response to chemotherapy-induced tumor cell death.

[0208] Inhibition of TGF-β in the tumor microenvironment reduces tumor-infiltrating $FoxP3^+$ regulatory T cells and enhances the antitumor efficacy chemotherapy. TGF-β induces FoxP3 expression in naïve peripheral $CD4^+CD25^-FoxP3^-$ T cells and facilitates their conversion into 'adaptive' $FoxP3^+$ Tregs that share the immunosuppressive ability of natural $FoxP3^+$ Tregs generated in the thymus. Since human cancers frequently become refractory to the growth-inhibitory effect of TGF-β and acquire an ability to increase expression and secretion of TGF-β, we investigated whether this switch enables tumor cells to increase the number of adaptive Tregs in the tumor microenvironment. Examination of serum levels of TGF-β in mice at d0, d14, and d28 following inoculation of live MB49 tumor cells demonstrated that tumor growth resulted in a progressive increase in the level of serum TGF-β (FIG. 54A). To assess the precise source of TGF-β in tumor-bearing mice, the total amount of TGF-β in supernatants of tumor cells or draining lymph node cells isolated from tumor-bearing mice were measured following *ex vivo* culture in serum-free medium for 24h. Measurement of the level of TGF-β/10^6 cells showed that tumor cells were the dominant source of the increased level of TGF-β in tumor-bearing mice (FIG. 54B). In addition to tumor cell-autonomous expression of TGF-β, T cells from tumor-bearing mice also expressed higher levels of TGF-β compared to their counterparts from tumor-free mice (FIG. 54B). To determine whether the elevation of TGF-β is responsible for the upregulation of Tregs in the tumor microenvironment, tumor-bearing mice were treated with a soluble chimeric protein comprising the

extracellular domain of TGFβRII and the Fc portion of the murine IgG1 heavy chain (TGFβRII:Fc). This fusion protein interferes with the binding of TGF-β to endogenous TGFβRII and functions as a stable TGF-β antagonist. ELISA assays confirmed the ability of TGFβRII:Fc to sequester TGF-β in supernatants of MB49 tumor cells in a concentration-dependent manner (FIG. 54C). At 5d following inoculation of MB49 tumor cells, mice were either left untreated or treated with TGFβRII:Fc (1μg intratumoral; twice weekly) for 3 weeks followed by flow cytometric analyses of intracellular FoxP3 expression in $CD4^+CD25^+$ T cells infiltrating the tumors. *In vivo* treatment of tumors with TGFβRII:Fc resulted in a significant decline in FoxP3 expression in tumor-infiltrating $CD4^+$ T cells (FIG. 54D) and a dramatic reduction of $CD4^+CD25^+FoxP3^+$ Tregs in tumor tissue (FIG. 54E). To determine whether inhibition of TGF-β in the tumor microenvironment can improve the antitumor efficacy of chemotherapy, MB49 tumor-bearing mice were administered doxorubicin (5mg/kg i.p. weekly x 3) with or without twice weekly treatment with TGFβRII:Fc (1μg intratumoral). In contrast to treatment with either doxorubicin or TGFβRII:Fc alone, combined treatment with both agents was able to arrest the growth of MB49 tumors. These results indicate that tumor cell autonomous expression of TGF-β in the tumor microenvironment induces 'adaptive' $FoxP3^+$ Tregs and that counteracting tumor-induced TGF-β-mediated immune tolerance enhances the antitumor efficacy of chemotherapy.

[0209] Anti-CD4 antibody-mediated depletion of $CD4^+$ regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive $CD8^+$ T cells and enhances the antitumor efficacy of chemotherapy. To determine whether depletion of $CD4^+$ regulatory T cells can improve the antitumor efficacy of chemotherapy by enhancing the activity of $CD8^+$ T cells in the tumor microenvironment, immunocompetent mice bearing syngeneic tumors were administered an anti-CD4 antibody (Clone GK1.5) to deplete $CD4^+$ T cells or an anti-CD8 antibody (Clone GK2.43) to deplete $CD8^+$ T cells and then treated with specific chemotherapeutic agents. Flow cytometric analyses of peripheral blood mononuclear cells from MB49 tumor-bearing mice at d7 following administration of anti-CD4 antibody or anti-CD8 antibody confirmed the target-specific depletion of either $CD4^+$ T cells or $CD8^+$ T cells, respectively (FIG. 55A). Mice treated with anti-CD4 antibody showed loss of $CD4^+CD25^+FoxP3^+$ T cells in the peripheral blood as well as among

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tumor-infiltrating cells (FIGS. 55A, 55B). To determine whether antibody-mediated depletion of $CD4^+CD25^+FoxP3^+$ cells facilitates chemotherapy-induced activation of tumor-reactive $CD8^+$ T cells in the tumor microenvironment, we evaluated the expression of IFN-γ in $CD8^+$ T cells extracted from the tumor and draining lymph node of MB49 tumor-bearing mice that were left untreated or treated with doxorubicin (with or without anti-CD4 antibody). Flow cytometric analyses showed that $CD8^+$ T cells from untreated mice did not express IFN-γ in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 55C). Whereas IFN-γ^+CD8^+ T cells became evident in mice treated with doxorubicin alone, antibody-mediated depletion of $CD4^+$ T cells further enhanced the percentage of tumor-reactive $CD8^+$ T cells that expressed IFN-γ in doxorubicin-treated animals (FIG. 55C). To directly evaluate whether the activation of tumor-reactive $CD8^+$ T cells determines the *in vivo* antitumor efficacy of chemotherapy, we examined the effect of antibody-mediated depletion of $CD8^+$ T cells or $CD4^+$ T cells on the response of MB49 tumor-bearing mice to systemic treatment with doxorubicin (5mg/kg). Treatment with doxorubicin alone inhibited the growth of MB49 tumors but failed to arrest tumor progression. Whereas depletion of $CD8^+$ T cells completely impaired the *in vivo* antitumor efficacy of doxorubicin, depletion of $CD4^+$ T cells enhanced the response to doxorubicin and resulted in tumor regression (FIG. 55D).

[0210] Anti-CD4 antibody-mediated depletion of $CD4^+$ regulatory T cells augments and sustains the antitumor effect of chemotherapy by enabling activation of adaptive antitumor immunity. Whereas tumor cells treated with anthracyclins, such as doxorubicin, are particularly effective in eliciting an antitumor immune response, other chemotherapeutic agents are less effective in inducing immunogenic tumor cell death. The surface exposure of calreticulin is a key determinant of the immunogenicity of tumor cell death in response to chemotherapeutic agents. Compared to the efficient translocation of CRT to the cell surface in response to treatment with doxorubicin (FIG. 1A), treatment of MB49 tumor cells with equitoxic doses of either cisplatin or the combination of cisplatin and gemcitabine was less effective in increasing CRT exposure (FIG. 56A). Whereas tumor-reactive IFN-γ^+CD8^+ T cells were evident in tumors of MB49 tumor-bearing mice treated with doxorubicin (FIG. 55C), treatment with cisplatin was unable to induce a corresponding elevation of IFN-γ expression in $CD8^+$ T cells in response to *in*

vitro re-challenge with MB49 cell lysates (FIG. 56B). To examine whether counteraction of Treg-mediated immune tolerance enables the activation of antitumor immunity by cisplatin, immunocompetent tumor-bearing mice were treated with cisplatin following depletion of Tregs with anti-CD4 antibody. Antibody-mediated depletion of $CD4^+$ T cells enhanced the percentage of tumor-reactive IFN-γ^+CD8^+ T cells as well as $CD8^+CD62L^-$ T cells in cisplatin-treated animals (FIGS. 56B, 56C). Treatment of MB49 tumor-bearing mice with cisplatin partially inhibited tumor growth but failed to arrest tumor progression. Whereas depletion of $CD8^+$ T cells completely negated the *in vivo* antitumor effect of cisplatin, depletion of $CD4^+$ T cells enhanced the response to cisplatin and arrested tumor growth (FIG. 56D). Although treatment of tumor-bearing mice with the combination of cisplatin and gemcitabine was also able to arrest tumor growth, tumor growth rapidly resumed following termination of therapy with none of the animals (0/8) being tumor-free at d50 following tumor inoculation (FIG. 56E). In contrast, mice depleted of $CD4^+$ T cells exhibited a more sustained response to either single agent or combination chemotherapy, with 7/16 mice exhibiting complete tumor regression. The complete regression of tumors was attended with establishment of adaptive antitumor immunity since none of the cured mice (7/7) developed tumors when re-challenged with live MB49 tumor cells in the opposite flank.

[0211] Chemotherapy-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD^{4+} regulatory T cells to stimulate CD^{8+} T cell-mediated tumor regression. NKG2D (NK group 2, member D) is a lectin-like type II transmembrane stimulatory receptor used by NK cells, $\gamma\delta$-TC^{R+} T cells and $\alpha\beta$-TC^{R+} T cells for immune surveillance of tumors. Expression of mouse and human ligands for NKG2D is upregulated in transformed epithelial cell lines in response to genotoxic stress or stalled DNA replication, via activation of a DNA damage checkpoint pathway initiated by ATM (ataxia telengiectasia, mutated) or ATR (ATM- and Rad3-related) protein kinases. Treatment of CT26 mouse colon cancer cells with genotoxic chemotherapeutic agents resulted in upregulation of mouse NKG2D ligands of the retinoic acid inducible gene family (Rae1) (FIG. 57A). RT-PCR showed that Rae1 mRNA was induced in CT26 cells by 2-4h, reached a plateau after 16-24h, and began to decline after 48h of treatment with either the irinotecan or oxaliplatin (FIG. 57A). Flow cytometric analysis

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demonstrated that cell surface expression of human NKG2D ligands (MHC-I-related A and B molecules – MICA, MICB) was also upregulated on human colorectal cancer cells (HCT116) in response to treatment with irinotecan (FIG. 53B). Isogenic HCT116 cells that differ only in their p53 status demonstrated that p53 is not required for irinotecan-induced upregulation of MICA/B (FIG. 53B). To examine whether the induction of NKG2D ligands contributes to the antitumor effect of chemotherapy *in vivo*, immunocompetent Balb/C mice inoculated with syngeneic CT26 tumor cells were treated with irinotecan (50 mg/kg i.p) with or without pre-treatment with an NKG2D blocking antibody (200 μg i.p.). Whereas treatment with irinotecan alone inhibited the growth of CT26 tumors, the antitumor effect of irinotecan was negated by pre-treatment with the NKG2D blocking antibody (FIG. 1C). Since engagement of NKG2D by its ligands provides a costimulatory signal for the activation of CD^{8+} T cells, we investigated whether DNA damage-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD^{4+} regulatory T cells to stimulate CD^{8+} T cell-mediated tumor regression. Balb/C mice bearing CT26 tumors were administered an anti-CD4 antibody (Clone GK1.5) to deplete CD^{4+} T cells and/or an anti-CD8 antibody (Clone GK2.43) to deplete CD^{8+} T cells and then treated with irinotecan. Flow cytometric analyses confirmed the loss of $CD^{4+}CD2^{5+}FoxP^{3+}$ T cells in the spleen and draining lymph node of mice treated with anti-CD4 antibody (FIG. 57D). Antibody-mediated depletion of CD^{4+} T cells enhanced the percentage of tumor-reactive IFN-$\gamma^{+}CD^{8+}$ T cells in irinotecan-treated animals (FIG. 57E). Whereas treatment of CT26 tumor-bearing mice with irinotecan only slowed tumor growth, depletion of CD^{4+} T cells enhanced the response to irinotecan and arrested tumor growth (FIG. 57F). The ability of CD^{4+} T cell depletion to augment the antitumor efficacy of irinotecan was mediated by CD^{8+} T cells since antibody-mediated depletion of CD^{8+} T cells completely negated the *in vivo* antitumor effect of chemotherapy in CD^{4+} T cell-depleted mice (FIG. 57F).

[0212] These data provide the following insights: (i) activation of tumor-reactive $CD8^{+}$ T cells in response to immunogenic tumor cell death is a crucial determinant of the antitumor efficacy of chemotherapy *in vivo;* (ii) tumor-induced Tregs impair the antitumor efficacy of chemotherapy by inhibiting the activation of $CD8^{+}$ T cells in the tumor microenvironment; and (iii) Counteracting tumor-induced immune tolerance via antibody-

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

EXAMPLE 2

[0213] Exemplary Targeted Immunomodulatory Antibodies and Fusion Proteins

[0214] A targeting moiety, including an antibody, can be coupled to an immunomodulatory moiety including a polypeptide derived from the extracellular domain of TGFBR2. Crosslinkers or activating agents for such coupling or conjugation are well known in the art. Alternatively, the fusion proteins of the invention can be synthesized using recombination DNA technology well known in the art where the coding sequences of various portions of the fusion proteins can be linked together at the nucleic acid level. Subsequently the fusion proteins of the invention can be produced using a host cell well known in the art. Examples of targeted immunomodulatory antibodies and fusion proteins are shown in Figures 1-33 and briefly described below.

[0215] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety specifically binds to Transforming growth factor-beta (TGF-β). SEQ ID NO: 1 provides a fusion protein including anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 2). SEQ ID NO: 2 provides a fusion protein including anti-EGFR1 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 3). SEQ ID NO: 3 provides a fusion protein including anti-CD20 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 4). SEQ ID NO: 4 provides a fusion protein including anti-VEGF antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 5). SEQ ID NO: 5 provides a fusion

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

[0216] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety is a molecule that specifically binds to Programmed Death-1 ligand 1 (PD-L1 or B7-H1) or Programmed Death-1 ligand 2 (PD-L2 or B7-DC). SEQ ID NO: 15 provides a fusion protein including anti-HER2/neu antibody and PD-1 Ectodomain (FIG. 13). SEQ ID NO: 16 provides a fusion protein including anti-EGFR1 antibody and PD-1 Ectodomain (FIG. 14). SEQ ID NO: 17 provides a fusion protein including anti-CD20 antibody and PD-1 Ectodomain

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(FIG. 15). SEQ ID NO: 18 provides a fusion protein including anti-VEGF antibody and PD-1 Ectodomain (FIG. 16). SEQ ID NO: 19 provides a fusion protein including anti-human CTLA-4 antibody and PD-1 Ectodomain (FIG. 17). SEQ ID NO: 20 provides a fusion protein including anti-CD25 antibody and PD-1 Ectodomain (FIG. 18A). SEQ ID NO: 21 provides a fusion protein including anti-CD25 antibody and PD-1 Ectodomain (FIG. 18B). SEQ ID NO: 22 provides a fusion protein including IL-2, Fc, and PD-1 ectodomain (IL-2 + Fc + PD-1 ectodomain; FIG. 19). SEQ ID NO: 23 provides a fusion protein including PD-1 ectodomain, Fc, and IL-2 (PD-1 ectodomain + Fc + IL-2; FIG. 19). SEQ ID NO: 24 provides a fusion protein including anti-CD4 antibody and PD-1 Ectodomain (FIG. 20). SEQ ID NO: 25 provides a fusion protein including RANK Ectodomain, Fc, and PD-1 ectodomain (RANK ectodomain + Fc + PD-1 ectodomain; FIG. 21). SEQ ID NO: 26 provides a fusion protein including PD-1 ectodomain, Fc, and RANK Ectodomain (PD-1 ectodomain + Fc + RANK ectodomain; FIG. 21).

[0217] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety is a molecule that specifically binds to Receptor activator of NF-kB ligand (RANKL). SEQ ID NO: 27 provides a fusion protein including anti-HER2/neu antibody and RANK Ectodomain (FIG. 23). SEQ ID NO: 28 provides a fusion protein including anti-EGFR1 antibody and RANK Ectodomain (FIG. 24). SEQ ID NO: 29 provides a fusion protein including anti-CD20 antibody and RANK Ectodomain (FIG. 25). SEQ ID NO: 30 provides a fusion protein including anti-VEGF antibody and RANK Ectodomain (FIG. 26). SEQ ID NO: 31 provides a fusion protein including anti-human CTLA-4 antibody and RANK Ectodomain (FIG. 27). SEQ ID NO: 32 provides a fusion protein including anti-CD25 antibody and RANK Ectodomain (FIG. 28A). SEQ ID NO: 33 provides a fusion protein including anti-CD25 antibody and RANK Ectodomain (FIG. 28B). SEQ ID NO: 34 provides a fusion protein including IL-2, Fc, and RANK ectodomain (IL-2 + Fc + RANK ectodomain; FIG. 29). SEQ ID NO: 35 provides a fusion protein including RANK ectodomain, Fc, and IL-2 (RANK ectodomain + Fc + IL-2; FIG. 29). SEQ ID NO: 36 provides a fusion protein including anti-CD4 antibody and RANK Ectodomain (FIG. 30).

[0218] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety includes a molecule that specifically binds to Programmed death-1 (PD-1). SEQ ID NO: 37 provides a fusion protein including anti-tumor necrosis factor (TNFα) antibody and PD-1 ligand 1 (FIG. 32). SEQ ID NO: 38 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand: (TNFR2 ECD + IgG Cγ1 + PD-L1; FIG. 33). SEQ ID NO: 39 provides a fusion protein including PD-1 ligand, Fc, and TNFR2 Extracellular ligand binding domain: (PD-L1 + IgG Cγ1 -TNFR2 ECD; FIG. 33). SEQ ID NO: 40 provides a fusion protein including anti-CD20 antibody and PD-1 ligand 1 (PD-L1) (FIG. 34). SEQ ID NO: 41 provides a fusion protein including anti-CD25 antibody and PD-1 ligand 1 (PD-L1) (FIG. 35A). SEQ ID NO: 42 provides a fusion protein including anti-CD25 antibody and PD-1 ligand 1 (PD-L1) (FIG. 35B). SEQ ID NO: 43 provides a fusion protein including PD-1 ligand 1 (PD-L1), Fc, and IL-2 (PD-L1 – Fc – IL2; FIG. 36). SEQ ID NO: 44 provides a fusion protein including IL-2, Fc, and PD-1 ligand 1 (PD-L1) (IL-2 – Fc – PD-L1; FIG. 36). SEQ ID NO: 45 provides a fusion protein including anti-CD4 antibody and PD-1 ligand 1 (PD-L1) (FIG. 37). SEQ ID NO: 46 provides a fusion protein including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from PD-1 ligand (PD-L1) (Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + PD-L1; FIG. 38). SEQ ID NO: 47 provides a fusion protein including the extracellular domain of PD-1 ligand (PD-L1), immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4: (PD-L1 + IgG Cγ1 + CTLA-4 ECD; FIG. 38). SEQ ID NO: 48 provides a fusion protein including Transforming growth factor-β (TGF-β), immunoglobulin Fc (IgG Cγ1), and a sequence from PD-1 ligand 1 (PD-L1) (TGFβ-1 + Fc + PD-L1; FIG. 39). SEQ ID NO: 49 provides a fusion protein including a sequence from PD-1 ligand 1 (PD-L1), immunoglobulin Fc (IgG Cγ1), and Transforming growth factor beta (TGF-□β) (PD-L1 + Fc + TGFβ-1; FIG. 39).

[0219] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety includes a

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molecule that specifically binds to Transforming growth factor-beta receptor (TGF-βR). SEQ ID NO: 50 provides a fusion protein including an antibody that binds TNF-α, and a sequence from Transforming growth factor-β (TGF-β) (FIG. 41). SEQ ID NO: 51 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and a sequence from Transforming growth factor-β (TGF-β) (TNFR2 ECD + IgG Cγ1 + TGF-β; FIG. 42). SEQ ID NO: 52 provides a fusion protein including a sequence from Transforming growth factor-β (TGF-β), Fc, and TNFR2 Extracellular ligand binding domain: (TGF-β + IgG Cγ1 + TNFR2 ECD; FIG. 42). SEQ ID NO: 53 provides a fusion protein including anti-CD20 antibody and a sequence from Transforming growth factor-β (TGF-β) (FIG. 43). SEQ ID NO: 54 provides a fusion protein including anti-CD25 antibody and a sequence from transforming growth factor-β (TGF-β) (FIG. 44A). SEQ ID NO: 55 provides a fusion protein including anti-CD25 antibody and a sequence from transforming growth factor-β (TGF-β) (FIG. 44B). SEQ ID NO: 56 provides a fusion protein including a sequence from Transforming growth factor-β (TGF-β), Fc, and IL-2 (TGF-β + Fc + IL-2; FIG. 45). SEQ ID NO: 57 provides a fusion protein including IL-2, Fc, and Transforming growth factor-β (TGF-β)(IL-2 + Fc + TGF-β; FIG. 45). SEQ ID NO: 58 provides a fusion protein including anti-CD4 antibody and a sequence from transforming growth factor-β (TGF-β) (FIG. 46). SEQ ID NO: 59 provides a fusion protein including the extracellular domain of CTLA-4, immunoglobulin Fc (IgG Cγ1), and a sequence from a sequence from transforming growth factor-β (TGF-β) (Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + TGF-β1; FIG. 47). SEQ ID NO: 60 provides a fusion protein including a sequence from Transforming growth factor-β (TGF-β), immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4: (TGF-β1 + IgG Cγ1 + CTLA-4 ECD) (FIG. 47).

[0220] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety is a molecule that specifically binds to Receptor activator of NF-kB ligand (RANKL). SEQ ID NO: 61 provides a fusion protein including an antibody that binds TNF-α, and a sequence from RANK Ectodomain (FIG. 48). SEQ ID NO: 62 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and a sequence from RANK Ectodomain

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(TNFR2 ECD + IgG Cγ1 + RANK Ectodomain; FIG. 49). SEQ ID NO: 63 provides a fusion protein including a sequence from RANK Ectodomain, Fc, and TNFR2 Extracellular ligand binding domain: (RANK Ectodomain + IgG Cγ1 + TNFR2 ECD; FIG. 49). SEQ ID NO: 64 provides a fusion protein including the extracellular domain of CTLA-4, immunoglobulin Fc (IgG Cγ1), and a sequence from a sequence from RANK Ectodomain (Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + RANK Ectodomain; FIG. 50). SEQ ID NO: 65 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4: (RANK Ectodomain + IgG Cγ1 + CTLA-4 ECD) (FIG. 50). SEQ ID NO: 66 provides a fusion protein including a sequence from transforming growth factor-β (TGF-β), immunoglobulin Fc region (IgG Cγ1), and an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) (TGF-β + IgG Cγ1 + RANK Ectodomain; FIG. 51). SEQ ID NO: 67 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG Cγ1), and a sequence from transforming growth factor-β (TGF-β): (RANK Ectodomain + IgG Cγ1 + TGF-β) (FIG. 51). SEQ ID NO: 68 provides a fusion protein including a sequence from Programmed death-1 ligand 1 (PD-L1), immunoglobulin Fc region (IgG Cγ1), and an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) (PD-L1 + IgG Cγ1 + RANK Ectodomain; FIG. 52). SEQ ID NO: 69 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG Cγ1), and a sequence from Programmed death-1 ligand 1 (PD-L1): (RANK Ectodomain + IgG Cγ1 + PD-L1) (FIG. 52).

[0221] Although the invention has been described with reference to the above example, it will be understood that modifications and variations are encompassed within the spirit and scope of the invention. Accordingly, the invention is limited only by the following claims.

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation application of U.S. Application Serial No. 17/694,541 filed March 14, 2022, now pending; which is a continuation application of U.S. Application Serial No. 16/601,347 filed October 14, 2019, now issued as U.S. patent 11,274,156; which is a continuation application of U.S. Application Serial No. 15/362,632 filed November 28, 2016, now issued as U.S. patent 10,442,860; which is a divisional application of U.S. Application Serial No. 15/231,309 filed August 8, 2016, now issued as U.S. patent 9,850,306; which is a continuation application of U.S. Application Serial No. 14/645,282 filed March 11, 2015, now issued as U.S. patent 9,441,044; which is a continuation application of U.S. Application Serial No. 13/582,717 filed October 17, 2012, now issued as U.S. patent 8,993,524; which is a 35 USC §371 National Stage application of International Application No. PCT/US2011/027317 filed March 4, 2011, now expired; which claims the benefit under 35 USC §119(e) to U.S. Application Serial No. 61/435,671 filed January 24, 2011 and to U.S. Application Serial No. 61/311,255 filed March 5, 2010, both now expired. The disclosure of each of the prior applications is considered part of and is incorporated by reference in the disclosure of this application.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under Grant No. CA123277 awarded by the National Institutes of Health. The government has certain rights in the invention.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0003] The material in the accompanying sequence listing is hereby incorporated by reference into this application. The accompanying sequence listing text file, named JHU3260-10.xml, was created on September 23, 2024 and is 174,970 bytes in size.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

[0004] The present invention relates generally to the field of targeted immunomodulatory antibodies and fusion proteins for cancer therapy and more specifically to composition and methods for targeted immunostimulatory or immunosuppressive antibodies and fusion proteins to counteract or induce immune tolerance of cancer cells.

BACKGROUND INFORMATION

[0005] The immune system provides the human body with a means to recognize and defend itself against microorganisms and substances recognized as foreign or potentially harmful. While passive immunotherapy of cancer with monoclonal antibodies and passive transfer of T cells to attack tumor cells have demonstrated clinical efficacy, the goal of active therapeutic vaccination to induce these immune effectors and establish immunological memory against tumor cells has remained challenging. Several tumor-specific and tumor-associated antigens have been identified, yet these antigens are generally weakly immunogenic and tumors employ diverse mechanisms to create a tolerogenic environment that allows them to evade immunologic attack. Strategies to overcome such immune tolerance and activating robust levels of antibody and/or T cell responses hold the key to effective cancer immunotherapy.

SUMMARY OF THE INVENTION

[0006] The present invention is based on the seminal discovery that targeted immunomodulatory antibodies and fusion proteins can counteract or reverse immune tolerance of cancer cells. Cancer cells are able to escape elimination by chemotherapeutic agents or tumor-targeted antibodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. Such immunosuppressive mechanisms include immunosuppressive cytokines (for example, Transforming growth factor beta (TGF-β)) and regulatory T cells and/or immunosuppressive myeloid dendritic cells (DCs). By counteracting tumor-induced immune tolerance, the present invention provides effective compositions and methods for cancer treatment, optional in combination with another existing cancer treatment. The present invention provides strategies to counteract tumor-induced immune tolerance and

enhance the antitumor efficacy of chemotherapy by activating and leveraging T cell-mediated adaptive antitumor immunity against resistant or disseminated cancer cells.

[0007] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule, and the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2); (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); (iv) Transforming growth factor-beta receptor (TGF-βR); (v) Programmed death-1 (PD-1); and (vi) Receptor activator of nuclear factor-κB (RANK).

[0008] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. In one aspect, the targeting moiety specifically binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), CD20, Vascular endothelial growth factor (VEGF), insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, CD30, CD33, or CD40.

[0009] In one aspect, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: (i) CD4; (ii) CD25 (IL-2α receptor; IL-2αR); (iii) cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); (iv) Interleukin-10 (IL-10); (v) Transforming growth factor-beta receptor (TGF-βR); (vi) Transforming growth factor-beta (TGF-β); (vii) Programmed Death-1 (PD-1); (viii) Programmed death-1 ligand (PD-L1 or PD-L2); (ix) Receptor activator of nuclear factor-κB (RANK); or (x) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL).

[0010] In one aspect, the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed

death-1 ligand (PD-L1 or PD-L2); or (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL).

[0011] In one aspect, the immunomodulatory moiety includes a molecule that binds TGF-β. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Transforming growth factor-beta receptor TGF-βRII, TGF-βRIIb, or TGF-βRIII. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of TGF-β.

[0012] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

[0013] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Programmed Death-1 (PD-1), Programmed death-1 ligand 1 (PD-L1), or Programmed death-1 ligand 2 (PD-L2). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes PD-1 ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

[0014] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory

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moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

[0015] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Programmed death-1 ligand 1 (PD-L1).

[0016] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.

[0017] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Programmed Death-1 (PD-1). In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and PD-1 ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 25 or 26.

[0018] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or

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ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Receptor activator of nuclear factor-κB ligand (RANKL).

[0019] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36.

[0020] In one aspect, the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the immunomodulatory moiety increases the function of PD-1.

[0021] In one aspect, the targeting moiety specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 37. In another aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2), and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from PD-L1. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 38 or 39.

[0022] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25, or CD4, and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1

ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 40, 41, 42, 43, 44, or 45.

[0023] In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 46 or 47.

[0024] In one aspect, the targeting moiety includes transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 48 or 49.

[0025] In one aspect, the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the immunomodulatory moiety activates the signaling function of transforming growth factor-β (TGF-β) receptor.

[0026] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from TGF-β. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 50. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 51 or 52.

[0027] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25 (IL-2α receptor; IL-2αR), or CD4, and the immunomodulatory moiety includes a sequence from transforming growth factor-β

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(TGF-β). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 53, 54, 55, 56, 57 or 58.

[0028] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 59 or 60.

[0029] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from an extracellular RANKL-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 61. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from an extracellular RANK-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 62 or 63.

[0030] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

[0031] In one aspect, the targeting moiety includes a sequence from transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

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[0032] In one aspect, the targeting moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

[0033] In various aspects, the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

[0034] In another embodiment, the present invention provides a composition including the molecule of the invention and a cell, wherein the cell is a tumor cell, immune cell, or dendritic cell.

[0035] In another embodiment, the present invention provides a method of counteracting or overcoming immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

[0036] In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof one or more molecule of the invention. In various aspects, the subject is administered one or more molecule of the invention in combination with another anticancer therapy. In one aspect, the anticancer therapy includes a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent or cytotoxic agent. In another aspect, the anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

[0037] In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof an antibody that targets and depletes CD4+ regulatory T cells (Tregs) in combination with another cytotoxic anticancer therapy. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In various aspects, the cytotoxic anticancer therapy includes a chemotherapeutic molecule, tumor-targeted antibody, small molecule kinase inhibitor, hormonal agent or tumor-targeted cytotoxic agent. In another

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

[0038] In another embodiment, the subject is administered one or more molecule of the invention in combination with any vaccine. In another aspect, the vaccine includes a tumor antigen, tumor-associated antigen, tumor epitope, tumor antigen-containing fusion protein, tumor cell, or dendritic cell. In another aspect, the vaccine includes a pathogen antigen, pathogen-associated antigen, pathogen epitope, or pathogen antigen-containing fusion protein.

[0039] In another embodiment, the present invention provides a method for treating immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treatment of a neoplastic disease. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

[0040] In another embodiment, the present invention provides a method of inducing or promoting immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

[0041] In another embodiment, the present invention provides a method of preventing or treating an autoimmune or inflammatory disease including administering to a subject in need thereof one or more molecule of the invention. In one aspect, the subject is administered one or more molecule of the invention in combination with another anti-inflammatory or immunosuppressive therapy. In another embodiment, the present invention provides a method of treatment of immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treating an autoimmune or inflammatory disease or preventing rejection of grafted cells or tissue. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] Figures 1A-1C show exemplary amino acid sequences of transforming growth factor beta receptor type II (TGF-β-RII) or TGF-β-RIIB or a fragment thereof, including (i) Transforming growth factor beta receptor type II (TGF-β-RII) (SEQ ID NO: 79); and (ii) Transforming growth factor beta receptor type IIB (TGF-β-RIIB) (SEQ ID NO: 80).

[0043] Also shown in Figures 1A-1C are exemplary truncated mutants of Transforming growth factor beta Receptor II (TGF-β-RII) or TGF-β-RIIB including the Extracellular domain (ECD) region that binds TGF-β, including (i) TGF-β R-II (ΔC terminus): TGFβ RII lacking the last 38 amino acids from the C-terminus (SEQ ID NO: 81) and TGF-β R-IIB (ΔC terminus): TGFβ RIIB lacking the last 38 aa from the C-terminus (SEQ ID NO: 82); (ii) TGF-βR-II (Δcyt): TGFβRII lacking the kinase domain & juxtamembrane region (SEQ ID NO: 83) and TGF-βR-IIB (Δcyt): TGFβRIIB lacking the kinase domain & juxtamembrane region (SEQ ID NO: 84); (iii) TGF-β R-II containing the N-terminus region including the extracellular domain (SEQ ID NO: 85) and TGF-β R-IIB containing the N-terminus region including the extracellular domain (SEQ ID NO: 86); (iv) TGF-β R-II containing the extracellular domain that binds TGF-β (SEQ ID NO: 87) and TGF-β R-IIB containing the extracellular domain that binds TGF-β (SEQ ID NO: 88); and (v) TGF-β R-II containing the region of the extracellular domain that binds TGF-β (SEQ ID NO: 89).

[0044] In addition, Figures 1A-1C also show exemplary kinase-deficient mutants, deletion mutants, or point mutants of Transforming growth factor beta Receptor II (TGFβ-RII) or TGFβ-RIIB or a fragment thereof which binds TGF-β, including (i) Transforming growth factor beta Receptor II containing point mutations - amino acid sequence of TGF-β R-II (K277R) contains a point mutation in its ATP-binding site and is inactive as a kinase (SEQ ID NO: 90); and (ii) Transforming growth factor beta Receptor II containing deletions in the amino acid sequence (deletion mutants) - Transforming growth factor beta Receptor II (Δi) - TGF-β R-II (Δi2) contains a deletion of amino acids 498 to 508 and is inactive as a kinase (SEQ ID NO: 91).

[0045] Figure 2 shows exemplary fusion proteins including anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD),

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including anti-HER2/neu heavy chain + TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 1) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

[0046] Figure 3 shows exemplary fusion proteins including anti-EGFR1 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-EGFR1 heavy chain + TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 2) and anti-EGFR1 light chain amino acid sequence (SEQ ID NO: 71).

[0047] Figure 4 shows exemplary fusion proteins including anti-CD20 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-CD20 heavy chain+ TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 3) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0048] Figure 5 shows exemplary fusion proteins including anti-VEGF antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-VEGF heavy chain+ TGFβ-RII ECD fusion amino acid sequence (SEQ ID NO: 4) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

[0049] Figure 6 shows exemplary fusion proteins including anti-human CTLA-4 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including Anti-CTLA-4 heavy chain + TGFβ-RII Extracellular domain fusion amino acid sequence (SEQ ID NO: 5) and Anti-CTLA-4 light chain (SEQ ID NO: 74).

[0050] Figure 7 shows exemplary fusion proteins including IL-2, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including IL-2 + Fc + TGFβ-RII Extracellular domain (SEQ ID NO: 6) and TGFβ-RII Extracellular domain + Fc + IL-2 (SEQ ID NO: 7). The linker GGGGSGGGGSGGGGS (SEQ ID NO: 104) is optional and can be replaced with EPKSCDK (SEQ ID NO: 105) or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0051] Figures 8A-8B show exemplary fusion proteins including anti-CD25 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD),

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including anti-CD25 (Daclizumab) heavy chain and TGFβ-RII Extracellular domain (SEQ ID NO: 8) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 8A); and anti-CD25 (Basiliximab) heavy chain and TGFβ-RII Extracellular domain (SEQ ID NO: 9) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76).

[0052] Figure 9 shows exemplary fusion proteins including anti-CD4 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD), including anti-CD4 heavy chain and TGFβ-RII Extracellular domain (SEQ ID NO: 10) and anti-CD4 light chain (SEQ ID NO: 77).

[0053] Figure 10 shows exemplary fusion proteins including Programmed Death-1 (PD-1) Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ectodomain), including PD-1 ectodomain + Fc + TGFβRII ectodomain (SEQ ID NO: 11) and TGFβRII ectodomain + Fc + PD-1 ectodomain (SEQ ID NO: 12). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

[0054] Figure 11 shows exemplary fusion proteins including Receptor activator of nuclear factor-kB (RANK) Ectodomain, Fc, and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ectodomain), including RANK ectodomain + Fc + TGFβRII ectodomain (SEQ ID NO: 13) and TGFβRII ectodomain + Fc + RANK ectodomain (SEQ ID NO: 14). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

[0055] Figure 12 shows exemplary immunomodulatory moiety that binds Programmed Death-1 ligand 1 (PD-L1 or B7-H1) or Programmed Death-1 ligand 2 (PD-L2 or B7-DC), including full-length PD-1 or fragment thereof (SEQ ID NO: 92), PD-1 extracellular domain (ectodomain) or fragment thereof (SEQ ID NO: 93), and PD-1 extracellular domain (ectodomain) ligand-binding region (SEQ ID NO: 94).

[0056] Figure 13 shows exemplary fusion proteins including anti-HER2/neu antibody and PD-1 Ectodomain, including anti-HER2/neu heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 15) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

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[0057] Figure 14 shows exemplary fusion proteins including anti-EGFR1 antibody and PD-1 Ectodomain, including anti-EGFR heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 16) and anti-EGFR light chain amino acid sequence (SEQ ID NO: 71).

[0058] Figure 15 shows exemplary fusion proteins including anti-CD20 antibody and PD-1 Ectodomain, including anti-CD20 heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 17) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0059] Figure 16 shows exemplary fusion proteins including anti-VEGF antibody and PD-1 Ectodomain, including anti-VEGF heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 18) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

[0060] Figure 17 shows exemplary fusion proteins including anti-human CTLA-4 antibody and PD-1 Ectodomain, including anti-CTLA-4 heavy chain + PD-1 ectodomain fusion amino acid sequence (SEQ ID NO: 19) and anti-CTLA-4 light chain (SEQ ID NO: 74).

[0061] Figures 18A-18B show exemplary fusion proteins including anti-CD25 antibody and PD-1 Ectodomain, including anti-CD25 (Daclizumab) heavy chain and PD-1 ectodomain (SEQ ID NO: 20) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 18A), and anti-CD25 (Basiliximab) heavy chain and PD-1 ectodomain (SEQ ID NO: 21) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 18B).

[0062] Figure 19 shows exemplary fusion proteins including IL-2, Fc, and PD-1 ectodomain, including IL-2 + Fc + PD-1 ectodomain (SEQ ID NO: 22) and PD-1 ectodomain + Fc + IL-2 (SEQ ID NO: 23). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0063] Figure 20 shows exemplary fusion proteins including anti-CD4 antibody and PD-1 ectodomain, including anti-CD4 heavy chain and PD-1 ectodomain (SEQ ID NO: 24) and anti-CD4 light chain (SEQ ID NO: 77).

[0064] Figure 21 shows exemplary fusion proteins including Receptor activator of nuclear factor-kB (RANK) Ectodomain, Fc, and PD-1 ectodomain, including RANK ectodomain + Fc + PD-1 ectodomain (SEQ ID NO: 25) and PD-1 ectodomain + Fc + RANK ectodomain (SEQ ID NO: 26). The linker sequence EPKSCDK (SEQ ID NO: 105) is optional and can be deleted or replaced with another linker.

[0065] Figure 22 shows exemplary immunomodulatory moiety that binds Receptor activator of nuclear factor-kB (RANK) ligand (RANKL) including full-length RANK or fragment thereof (SEQ ID NO: 95), extracellular ligand-binding domain or ectodomain of RANK (SEQ ID NO: 96), RANKL-binding sequences or residues of RANK (SEQ ID NO: 93), or RANKL-binding sequences of Osteoprotegerin (OPG) (SEQ ID NO: 98).

[0066] Figure 23 shows exemplary fusion proteins including anti-HER2/neu antibody and RANK Ectodomain, including anti-HER2/neu heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 27) and anti-HER2/neu light chain amino acid sequence (SEQ ID NO: 70).

[0067] Figure 24 shows exemplary fusion proteins including anti-EGFR1 antibody and RANK Ectodomain, including anti-EGFR heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 28) and anti-EGFR light chain amino acid sequence (SEQ ID NO: 71).

[0068] Figure 25 shows exemplary fusion proteins including anti-CD20 antibody and RANK Ectodomain, including anti-CD20 heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 29) and anti-CD20 light chain amino acid sequence (SEQ ID NO: 72).

[0069] Figure 26 shows exemplary fusion proteins including anti-VEGF antibody and RANK Ectodomain, including anti-VEGF heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 30) and anti-VEGF Light chain sequence (SEQ ID NO: 73).

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[0070] Figure 27 shows exemplary fusion proteins including anti-human CTLA-4 antibody and RANK Ectodomain, including anti-CTLA-4 heavy chain + RANK ectodomain fusion amino acid sequence (SEQ ID NO: 31) and anti-CTLA-4 light chain (SEQ ID NO: 74).

[0071] Figures 28A-28B show exemplary fusion proteins including anti-CD25 antibody and RANK Ectodomain, including anti-CD25 (Daclizumab) heavy chain and RANK ectodomain (SEQ ID NO: 32) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 28A), and anti-CD25 (Basiliximab) heavy chain and RANK ectodomain (SEQ ID NO: 33) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 28B).

[0072] Figure 29 shows exemplary fusion proteins including IL-2, Fc, and RANK ectodomain, including IL-2 + Fc + RANK ectodomain (SEQ ID NO: 34) and RANK ectodomain + Fc + IL-2 (SEQ ID NO: 35). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0073] Figure 30 shows exemplary fusion proteins including anti-CD4 antibody and RANK ectodomain, including anti-CD4 heavy chain and RANK ectodomain (SEQ ID NO: 36) and anti-CD4 light chain (SEQ ID NO: 77).

[0074] Figure 31 shows exemplary immunomodulatory moiety that binds Programmed Death-1 (PD-1) including a PD-1 ligand 1 (PD-L1 or B7-H1) or PD-1 ligand 2 (PD-L2 or B7-DC) or a fragment thereof (for example, SEQ ID NO: 101), full-length human PD-1 ligand 1 (B7-H1; PDCD1L1; PD-L1; or CD274) protein or a fragment thereof (SEQ ID NO: 99), and PD-L1 extracellular binding domain (ectodomain) or fragment thereof (SEQ ID NO: 100).

[0075] Figure 32 shows exemplary fusion proteins including anti-tumor necrosis factor (TNFα) antibody and PD-1 ligand, including anti-TNFα heavy chain + PD-1L (SEQ ID NO: 37) and anti-TNFα light chain (SEQ ID NO: 78). The sequence KKAE (SEQ ID NO: 107) can be replaced with KRVE (SEQ ID NO: 108) or KKVE (SEQ ID NO: 109).

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[0076] Figure 33 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand, including TNFR2 ECD + IgG Cγ1 + PD-L1 (SEQ ID NO: 38) and PD-L1 + IgG Cγ1 -TNFR2 ECD (SEQ ID NO: 39).

[0077] Figure 34 shows exemplary fusion proteins including anti-CD20 antibody and PD-1 ligand 1 (PD-L1), including anti-CD20 heavy chain + PD-L1 sequence (SEQ ID NO: 40) and anti-CD20 light chain sequence (SEQ ID NO: 72).

[0078] Figures 35A-35B show exemplary fusion proteins including anti-CD25 antibody and PD-1 ligand 1 (PD-L1), including anti-CD25 (Daclizumab) heavy chain and PD-L1 (SEQ ID NO: 41) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 35A), and anti-CD25 (Basiliximab) heavy chain and PD-1 ectodomain (SEQ ID NO: 42) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 35B).

[0079] Figure 36 shows exemplary fusion proteins including IL-2, Fc, and PD-1 ligand 1 (PD-L1), including fusion protein hPD-1 ligand 1 + Fc + IL-2 (SEQ ID NO: 43) and fusion protein IL-2 + Fc + PD-1 ligand 1 (SEQ ID NO: 44). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0080] Figure 37 shows exemplary fusion proteins including anti-CD4 antibody and PD-1 ligand 1 (PD-L1), including anti-CD4 heavy chain and PD-1 ligand 1 (PD-L1) (SEQ ID NO: 45) and anti-CD4 light chain (SEQ ID NO: 77).

[0081] Figure 38 shows exemplary fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from PD-1 ligand (PD-L1) including Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + PD-L1 (SEQ ID NO: 46) and PD-1L1 + IgG Cγ1 + CTLA-4 ECD (SEQ ID NO: 47). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

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[0082] Figure 39 shows exemplary fusion proteins including a sequence of transforming growth factor-β (TGF-β), Immunoglobulin Fc (IgG Cγ1), and a sequence of PD-1 ligand (PD-L1) including TGFβ-1 + Fc + PD-L1 (SEQ ID NO: 48), and PD-1L1 + Fc + TGFβ-1 (SEQ ID NO: 49). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0083] Figure 40 shows exemplary immunomodulatory moiety that binds Transforming growth factor-beta receptor (TGF-βR) including Transforming growth factor-beta (TGF-β1, TGF-β2, or TGF-β3 or a fragment thereof, TGF-β1 full sequence (SEQ ID NO: 102), and mature (active) TGF-β1 sequence (Ala 279 – Ser 390; 112 amino acids) (SEQ ID NO: 103).

[0084] Figure 41 shows exemplary fusion proteins including an antibody that binds TNF-α, and a sequence of transforming growth factor-β (TGF-β), including anti-TNFα heavy chain + TGF-β1 (SEQ ID NO: 50) and anti-TNFα light chain (SEQ ID NO: 78). The sequence KKAE (SEQ ID NO: 107) can be replaced with KRVE (SEQ ID NO: 108) or KKVE (SEQ ID NO: 109).

[0085] Figure 42 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain (TNFR2 ECD), immunoglobulin Fc (IgG Cγ1), and a sequence from transforming growth factor-β (TGF-β) including TNFR2 ECD + IgG Cγ1 + TGF-β1 (SEQ ID NO: 51), and TGF-β1 + IgG Cγ1 + TNFR2 ECD (SEQ ID NO: 52).

[0086] Figure 43 shows exemplary fusion proteins including anti-CD20 antibody and a sequence from transforming growth factor-β (TGF-β), including anti-CD20 heavy chain + mature TGFβ1 sequence (SEQ ID NO: 53) and anti-CD20 light chain sequence (SEQ ID NO: 72).

[0087] Figures 44A-44B show exemplary fusion proteins including anti-CD25 antibody and a sequence from transforming growth factor-β (TGF-β), including anti-CD25 (Daclizumab) heavy chain and TGF-β1 (SEQ ID NO: 54) and anti-CD25 (Daclizumab) light chain (SEQ ID NO: 75) (FIG. 44A), and anti-CD25 (Basiliximab) heavy chain and

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TGF-β1 (SEQ ID NO: 55) and anti-CD25 (Basiliximab) light chain (SEQ ID NO: 76) (FIG. 44B).

[0088] Figure 45 shows exemplary fusion proteins including IL-2, Fc, and a sequence from transforming growth factor-β (TGF-β), including TGF-β1 + Fc + IL-2 (SEQ ID NO: 56) and IL-2 + Fc + TGF-β1 (SEQ ID NO: 57). The linker GGGGSGGGGSGGGGS SEQ ID NO: 104 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence well known in the art. Certain amino acid sequences can be replaced in Fc, including underlined E with D and underlined M with L.

[0089] Figure 46 shows exemplary fusion proteins including anti-CD4 antibody and a sequence from transforming growth factor-β (TGF-β), including anti-CD4 heavy chain and TGF-β (SEQ ID NO: 58) and anti-CD4 light chain (SEQ ID NO: 77).

[0090] Figure 47 shows exemplary Fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from transforming growth factor-β (TGF-β) including Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + TGF-β1 (SEQ ID NO: 59), and TGF-β1 + IgG Cγ1 + CTLA-4 ECD (SEQ ID NO: 60). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

[0091] Figure 48 shows exemplary fusion proteins including an antibody that binds TNF-α, and a sequence of RANK ectodomain, including anti-TNFα heavy chain + RANK ectodomain (SEQ ID NO: 61) and anti-TNFα light chain (SEQ ID NO: 78). The sequence KKAE (SEQ ID NO: 107) can be replaced with KRVE (SEQ ID NO: 108) or KKVE (SEQ ID NO: 109).

[0092] Figure 49 shows exemplary fusion proteins including TNFR2 Extracellular ligand binding domain (TNFR2 ECD), immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including TNFR2 ECD + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 62), and RANK ectodomain + IgG Cγ1 + TNFR2 ECD (SEQ ID NO: 63).

[0093] Figure 50 shows exemplary Fusion proteins including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 64), and RANK ectodomain + IgG Cγ1 + CTLA-4 ECD (SEQ ID NO: 65). The IgG sequence shown can have optional C to S conversion in (bold underlined). The linker QEPKSCDK SEQ ID NO: 110 is optional and can be replaced with EPKSCDK SEQ ID NO: 105 or another linker sequence.

[0094] Figure 51 shows exemplary fusion proteins including a sequence from transforming growth factor-β (TGF-β), immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including TGF-β + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 66), and RANK ectodomain + IgG Cγ1 + TGF-β (SEQ ID NO: 67).

[0095] Figure 52 shows exemplary fusion proteins including a sequence from PD-1 ligand (PD-L1), immunoglobulin Fc (IgG Cγ1), and a sequence from RANK ectodomain including PD-L1 + IgG Cγ1 + RANK ectodomain (SEQ ID NO: 68), and RANK ectodomain + IgG Cγ1 + PD-L1 (SEQ ID NO: 69).

[0096] Figures 53A-53G show Regulatory T cells (Treg) accumulate in the tumor microenvironment and counteract the ability of chemotherapy to activate $CD8^+$ T cell-mediated antitumor immunity. (FIG. 53A) Surface exposure of calreticulin (CRT) in response to treatment of huma n (SW780) and murine (MB49) cancer cells with doxorubicin (10 μM) for 4h. The surface exposure of CRT was determined by immunofluorescence cytometry of untreated control or doxorubicin-treated cells stained with Dylight-labeled anti-CRT antibody or an isotype control (IgG1) antibody. (FIG. 53B) Priming of tumor-reactive immune responses by MB49 tumor cells treated with doxorubicin *ex vivo* or *in vivo*. $5x10^6$ MB49 cells that were pre-treated *ex vivo* with doxorubicin (10 μM) for 4h were injected into one flank of syngeneic immunocompetent C57BL/6 mice. Alternatively, C57BL/6 mice were injected with $5x10^5$ live MB49 tumor cells and then administered intratumoral doxorubicin (10 μg) at 10d following tumor inoculation. Tumor-reactive immune responses were determined by measuring production of IFN-γ by draining lymph node (DLN) cells in response to *in vitro* re-challenge with either MB49 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone.

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(FIG. 53C) Vaccination with doxorubicin-treated tumor cells induces $CD8^+$ T cell-mediated antitumor immunity that prevents tumor formation following re-challenge with live tumor cells. MB49 cells ($5x10^6$) that were pre-treated *in vitro* with doxorubicin (10 μM) for 4h were injected subcutaneously into one flank of syngeneic immunocompetent C57BL/6 mice. Naïve or vaccinated mice were challenged with untreated live MB49 tumor cells injected into the opposite flank with or without pre-treatment with an anti-CD8 antibody (Clone GK2.43)(5 μg x 2 doses, iv) to deplete $CD8^+$ T cells. (FIG. 53D) Delayed administration of chemotherapy in mice with pre-established tumors decreases its immunogenicity and antitumor efficacy. C57BL/6 mice were injected with $5x10^5$ live syngeneic MB49 tumor cells and then administered intratumoral doxorubicin (10 μg) at d3, d7, or d10 following tumor inoculation. (FIG. 53E) Tumors foster the accumulation of $CD4^+CD25^+FoxP3^+$ cells (Tregs) in their microenvironment. Flow cytometric analyses of the percentage of $CD4^+CD25^+Fox^P3^+$ cells (Tregs) among $CD4^+$ T lymphocytes isolated from the spleen, draining lymph nodes (DLN), and tumors of immunocompetent C57BL/6 mice at d0 and d14 after subcutaneous inoculation of $5x10^5$ live MB49 tumor cells. (FIG. 53F) Tregs infiltrating the tumor microenvironment suppress priming of tumor-reactive immune responses by doxorubicin-treated tumor cells. Naïve C57BL/6 mice were vaccinated with $5x10^6$ doxorubicin-killed MB49 cells with or without intravenous adoptive transfer of $5x10^6$ $CD4^+CD25^+$ cells isolated from tumors and DLN of tumor-bearing mice via immunomagnetic separation. Tumor-reactive immune responses were determined by measuring production of IFN-γ by draining lymph node (DLN) cells in response to *in vitro* re-challenge with either MB49 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone. (FIG. 53G) Tregs infiltrating the tumor microenvironment suppress the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death. Naïve C57BL/6 mice were vaccinated with $5x10^6$ doxorubicin-killed MB49 cells (left flank) with or without pre-treatment with either an anti-CD8 antibody (Clone GK2.43)(5 μg x 2 doses, iv) to deplete $CD8^+$ T cells or adoptive transfer of $5x10^6$ $CD4^+CD25^+$ cells isolated from tumors and DLN of tumor-bearing mice. Protective antitumor immunity in vaccinated mice was determined by assessment of tumor growth upon challenge with untreated live MB49 tumor cells injected into the opposite flank.

[0097] Figures 54A-54F show inhibition of TGF-β in the tumor microenvironment reduces 'adaptive' FoxP3$^+$ regulatory T cells and enhances the antitumor efficacy chemotherapy. (FIG. 54A) Tumor growth results in a progressive increase in the level of serum TGF-β. Levels of TGF-β in serum of mice at d0, d14, and d28 following inoculation of $5x10^5$ live MB49 tumor cells were evaluated utilizing ELISA. (FIG. 54B) Tumor cell-autonomous expression of TGF-β is the dominant source of elevated TGF-β in tumor-bearing mice. Tumor cells or draining lymph node cells isolated from either tumor-bearing mice or their tumor-free counterparts were cultured *ex vivo* in serum-free medium for 24h and the amount of TGF-β/10^6 cells in supernatants was measured by ELISA. (FIG. 54C) TGFβRII:Fc sequesters TGF-β in supernatants of MB49 tumor cells in a concentration-dependent manner. MB49 tumor cells were cultured in the presence of graded concentrations of TGFβRII:Fc (0-400 ng/ml) for 24h followed by measurement of TGF-β (pg/ml/10^6 cells) in supernatants via ELISA. (FIG. 54D) TGF-β induces 'adaptive' FoxP3$^+$ regulatory T cells in the tumor microenvironment. At 5d following inoculation of MB49 tumor cells, mice were either left untreated (control) or treated with TGFβRII:Fc (1μg intratumoral; twice weekly) for 3 weeks followed by flow cytometric analyses of intracellular FoxP3 expression in CD4$^+$CD25$^+$ T cells infiltrating the tumors. (FIGS. 54E, 54F). Sequestration of intratumoral TGF-β with TGFβRII:Fc reduces CD4$^+$CD25$^+$FoxP3$^+$ Tregs in tumor tissue and improves the antitumor efficacy of doxorubicin. MB49 tumor-bearing mice were administered doxorubicin (5mg/kg i.p. weekly x 3) with or without twice weekly treatment with TGFβRII:Fc (1μg intratumoral). The percentage of CD4$^+$CD25$^+$FoxP3$^+$ cells (Tregs) among tumor cells was assessed by flow cytometry (FIG. 54E), and tumor volume was monitored to determine the effect of counteracting tumor-induced TGF-β-mediated immune tolerance on the *in vivo* antitumor efficacy of doxorubicin (FIG. 54F).

[0098] Figures 55A-55D show that anti-CD4 antibody-mediated depletion of CD4$^+$ regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive CD8$^+$ T cells and enhances the antitumor efficacy of chemotherapy. (FIG. 55A) *In vivo* depletion of tumor-infiltrating CD4$^+$CD25$^+$FoxP3$^+$ T cells by treatment of tumor-bearing mice with anti-CD4 antibody. C57BL/6 mice injected with 5 $x10^5$ MB49 tumor cells s.c. were left untreated (control) or administered an anti-CD4 antibody (Clone GK1.5) i.p. at 5d and 9d

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following tumor challenge. $CD4^+CD25^+FoxP3^+$ T cells infiltrating tumors isolated from mice at d16 following tumor challenge were detected by flow cytometry. (FIG. 55B) Target-specific depletion of either $CD4^+$ T cells, $CD4^+CD25^+FoxP3^+$ T cells, or $CD8^+$ T cells by treatment of tumor-bearing mice with anti-CD4 antibody or anti-CD8 antibody. C57BL/6 mice injected s.c. with 5 $x10^5$ MB49 tumor cells were left untreated or treated with doxorubicin (5mg/kg i.p. weekly x 3) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Flow cytometric analyses of peripheral blood mononuclear cells isolated from mice at d16 following tumor challenge determined the percentage of $CD4^+$ T cells or $CD8^+$ T cells among total mononuclear cells, and the percentage of $CD4^+CD25^+FoxP3^+$ T cells among total $CD4^+$ T cells. (FIG. 55C) Depletion of $CD4^+$ regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive $CD8^+$ T cells. C57BL/6 mice injected s.c. with 5 $x10^5$ MB49 tumor cells were left untreated or treated with doxorubicin (5mg/kg i.p. weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of IFN-γ expression in $CD8^+$ T cells from the tumor and draining lymph node in response to *in vitro* stimulation with MB49 cell lysates. (FIG. 55D) Depletion of $CD4^+$ regulatory T cells augments the *in vivo* antitumor efficacy of chemotherapy via activation of tumor-reactive $CD8^+$ T cells. C57BL/6 mice injected s.c. with 5 $x10^5$ MB49 tumor cells were left untreated or treated with doxorubicin (5mg/kg i.p. weekly x 3) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting either $CD4^+$ T cells or $CD8^+$ T cells on the *in vivo* antitumor efficacy of doxorubicin.

[0099] Figures 56A-56F show anti-CD4 antibody-mediated depletion of $CD4^+$ regulatory T cells augments and sustains the antitumor effect of chemotherapy by enabling activation of adaptive antitumor immunity. (FIG. 56A) Surface exposure of calreticulin (CRT) in response to treatment of MB49 cancer cells with either cisplatin or the combination of cisplatin and gemcitabine for 4h. The surface exposure of CRT was

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determined by immunofluorescence cytometry of untreated control or chemotherapy-treated cells stained with Dylight-labeled anti-CRT antibody or an isotype control (IgG1) antibody. (FIGS. 56B, 56C) Depletion of $CD4^+$ regulatory T cells enables cisplatin-induced activation of tumor-reactive IFN-γ^+CD8^+ T cells and effector memory ($CD8^+CD62L^-$) T cells. C57BL/6 mice injected s.c. with 5 $x10^5$ MB49 tumor cells were left untreated or treated with cisplatin (0.5mg/kg i.p. weekly x 4) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of IFN-γ expression in $CD8^+$ T cells from the tumor and draining lymph node (DLN) in response to *in vitro* stimulation with MB49 cell lysates (FIG. 56B). The percentage of effector memory T_{EM} cells was determined by flow cytometric analyses of $CD8^+CD62L^-$ cells (FIG. 56C). (FIGS. 56D, 56E and 56F) Depletion of $CD4^+$ regulatory T cells augments the *in vivo* antitumor efficacy of chemotherapy via activation of tumor-reactive $CD8^+$ T cells. C57BL/6 mice injected s.c. with 5 $x10^5$ MB49 tumor cells were left untreated or treated with either cisplatin (0.5mg/kg) or the combination of cisplatin and gemcitabine (i.p. weekly x 4) beginning at d7 following tumor inoculation, with or without administration of either anti-CD4 antibody (Clone GK1.5) or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting either $CD4^+$ T cells or $CD8^+$ T cells on the *in vivo* antitumor efficacy of chemotherapy and the percentage of mice exhibiting complete tumor-regression by d50 following tumor inoculation. Establishment of adaptive antitumor immunity following regression of primary tumors was determined by re-challenging mice with live MB49 tumor cells in the opposite flank.

[0100] Figures 57A-57H show that Chemotherapy-induced expression of NKG2D ligands on tumor cells cooperates with depletion of $CD4^+$ regulatory T cells to stimulate $CD8^+$ T cell-mediated tumor regression. (FIG. 57A) Genotoxic chemotherapeutic agents induce expression of mouse NKG2D ligands (Rae-1) on cancer cells. Kinetics of the upregulation of Rae1 transcripts in mouse CT26 colon cancer cells was determined by quantitative real-time PCR following treatment with irinotecan (25 μg/ml) or oxaliplatin (10 μg/ml). Quantitative RT-PCR was carried out using Rae-1 specific primers [sense, 5'-

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CTAGTGCCACCTGGGAATTCA-3' (SEQ ID NO: 111); anti-sense, 5'-CATCATTAGCTGATCTCCAGCTCA-3'(SEQ ID NO: 112)] and probe [5'-6-FAM-CATCAGTGACAGTTACTTCTTCACCTTCTACACAGAGA-Tamra-3' (SEQ ID NO: 113)]. (FIG. 57B) Genotoxic chemotherapeutic agents induce p53-independent cell surface expression of human NKG2D ligands (MHC-I-related A and B molecules – MICA/MICB) on cancer cells. Isogenic p53-proficient ($p53^{+/+}$) or p53-deficient ($p53^{-/-}$) HCT116 cells were treated with irinotecan (25 μg/ml) for 16h or left untreated. Irinotecan-induced upregulation of cell surface expression of MICA/B was determined by flow cytometryic analysis of tumor cells labeled with an anti-human MICA/B MAb (R&D Systems). (FIG. 57C) and (FIG. 57D) Induction of NKG2D ligands contributes to the antitumor effect of chemotherapy *in vivo*. Immunocompetent Balb/C mice injected s.c. with 2 $x10^5$ syngeneic CT26 tumor cells were treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d5 following tumor inoculation, with or without pre-treatment with an NKG2D blocking antibody (CX5, eBIOscience) (200 μg i.p.) at 16h before each dose of chemotherapy. Tumor volume was monitored to determine the effect of NKG2D blockade on the *in vivo* antitumor efficacy of irinotecan. (FIG. 57E) *In vivo* depletion of $CD4^+CD25^+FoxP3^+$ T cells by treatment of tumor-bearing mice with anti-CD4 antibody. Balb/C mice injected with 2 $x10^5$ CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. $CD4^+CD25^+FoxP3^+$ T cells in spleen and draining lymph node isolated from mice at d16 following tumor challenge were detected by flow cytometry. (FIG. 57F) Depletion of $CD4^+$ regulatory T cells facilitates irinotecan-induced activation of tumor-reactive IFN-γ^+CD8^+ T cells. Balb/C mice injected with 2 $x10^5$ CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) at d5 and d9 following tumor inoculation. Tumor-reactive immune responses were determined by flow cytometric analyses of IFN-γ expression in $CD8^+$ T cells from the tumor and draining lymph node (DLN) in response to *in vitro* stimulation with either CT26 cell lysates, an irrelevant peptide (Hemagglutinin-HA), or medium alone. (FIG. 57G) and (FIG. 57H) Chemotherapy-induced expression of NKG2D ligands on

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tumor cells cooperates with depletion of $CD4^+$ regulatory T cells to stimulate $CD8^+$ T cell-mediated tumor regression. Balb/C mice injected with 2 $x10^5$ CT26 tumor cells s.c. were left untreated or treated with irinotecan (50 mg/kg i.p weekly x 3) beginning at d7 following tumor inoculation, with or without administration of anti-CD4 antibody (Clone GK1.5) and/or anti-CD8 antibody (Clone GK2.43) at d5 and d9 following tumor inoculation. Tumor volume was monitored to determine the effect of depleting $CD4^+$ T cells and/or $CD8^+$ T cells on the *in vivo* antitumor efficacy of irinotecan.

DETAILED DESCRIPTION OF THE INVENTION

[0101] Targeted immunostimulatory antibodies and/or fusion proteins for prevention or treatment of cancer: Chemotherapy is a cornerstone of systemic treatment of patients with most common types of advanced cancers. The vast majority of human cancers harbor genetic alterations and signaling mechanisms that impair the direct death signaling pathways entrained by chemotherapeutic agents. Although chemotherapeutic agents employ diverse mechanisms to directly kill tumor cells, the present invention provides that these agents have immuno-adjuvant effects which activate innate and adaptive antitumor immune responses that are crucial for their antitumor efficacy *in vivo*. The present invention also provides that antitumor $CD8^+$ T cells play an instrumental role in the *in vivo* response of tumors to diverse cytotoxic chemotherapeutic agents. Although chemotherapeutic agents can induce "immunogenic" tumor cell death and facilitate cross-presentation of antigens by dendritic cells, tumors create a tolerogenic environment that allows them to suppress the activation of innate and adaptive immune responses and evade immunologic attack by immune effector cells. The present invention provides that strategies to counteract tumor-induced immune tolerance in the tumor microenvironment can enhance the antitumor efficacy of chemotherapy by activating and leveraging T cell-mediated adaptive antitumor immunity against disseminated cancer cells.

[0102] The present invention is based on the seminal discovery that targeted immunomodulatory antibodies and fusion proteins can counteract or reverse immune tolerance of cancer cells. Cancer cells are able to escape elimination by chemotherapeutic agents or tumor-targeted antibodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. By counteracting tumor-induced immune tolerance, the present invention

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provides effective compositions and methods for cancer treatment, optional in combination with another existing cancer treatment.

[0103] The present invention provides compositions and methods for targeted immunostimulatory antibodies and fusion proteins that counteract immune tolerance in the tumor microenvironment and promote T cell-mediated adaptive antitumor immunity for maintenance of durable long-term protection against recurrent or disseminated cancers. These tumor-targeted immunostimulatory molecules are designed to facilitate effective long term T cell-mediated immune responses against tumor cells by at least one of the following:

(i) promoting death of tumor cells via enhancement of antibody-dependent cellular cytotoxicity (ADCC);

(ii) facilitating effective cross-presentation of tumor antigen(s) from dying tumor cells by augmenting maturation of dendritic cells (DCs); and

(iii) increasing activation and proliferation of antitumor CD8+ T cells by negating immune suppression mediated by regulatory T cells and myeloid suppressor cells. These antitumor immune responses may be activated in tandem with the sensitization of tumor cells to immune effector-mediated cytotoxicity, thereby establishing a positive feedback loop that augments tumor cytoreduction and reinforces adaptive antitumor immunity. The tumor-targeted immunostimulatory monoclonal antibodies (mAbs) of the present invention provides the ability to generate and boost antitumor immunity to multiple cross-presented tumor antigens obtained from endogenous tumor cells during the course of therapy (as an *in situ* tumor vaccine), while simultaneously leveraging the antitumor immune response to eliminate disseminated cancer cells. Accordingly, the targeted immunostimulatory antibodies and fusion proteins of the invention can integrate the hitherto distinct fields of passive and active immunotherapy and provide a novel platform for simultaneously leveraging the synergistic benefits of these strategies to entrain effective innate and adaptive immune responses against targeted cancers.

[0104] 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

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cells has remained challenging. Several tumor-specific and tumor-associated antigens have been identified, yet tumors employ diverse mechanisms to create a tolerogenic environment that allows them to suppress the activation of a T cell-mediated antitumor immune response. The tumor-targeted immunostimulatory antibodies and/or fusion proteins of the invention are designed to overcome such immune tolerance in the tumor microenvironment and activate robust levels of T cell responses for effective cancer immunotherapy or chemo-immunotherapy. Accordingly, the tumor-targeted immunostimulatory antibodies and/or fusion proteins of the invention have broad clinical relevance for advancing the treatment of many types of human cancers.

[0105] The tumor-targeted immunostimulatory mAbs and/or fusion proteins of the invention provide their ability to generate and boost antitumor immunity to multiple cross-presented tumor antigens obtained from endogenous tumor cells during the course of therapy (as an *in situ* tumor vaccine), while simultaneously leveraging the antitumor immune response to eliminate disseminated cancer cells. Accordingly, the tumor-targeted immunostimulatory antibodies and/or fusion proteins of the invention can integrate the hitherto distinct fields of passive and active immunotherapy and provide a novel platform for simultaneously leveraging the synergistic benefits of these strategies to entrain effective innate and adaptive immune responses against targeted cancers. This approach of the present invention is distinguished from and superior to conventional tumor antigen-, allogeneic tumor cell- or DC-based vaccines in at least one of the following aspects: (i) There is no *a priori* requirement to define, clone and purify individual tumor antigens, since the patient's tumor itself is the *in vivo* source of antigens; (ii) Multivalent antitumor immune responses that are naturally tailored against antigens from the patient's own tumor are less likely to allow immune escape than a pre-selected tumor antigen; (iii) The activation of antitumor immune responses by the immuno-adjuvant effects of tumor-targeted immunostimulatory antibodies or fusion proteins occurs in tandem with the sensitization of tumor cells to immune effector-mediated cytotoxicity, thereby establishing a positive feedback loop that augments tumor cytoreduction and reinforces adaptive antitumor immunity; and (iv) The molecules of the invention have broad clinical relevance for advancing the treatment of many types of human cancers.

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[0106] In addition, the targeted immunostimulatory antibodies and/or fusion proteins of the invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) to counteract immune tolerance in the tumor microenvironment and promote T cell-mediated adaptive antitumor immunity for maintenance of long-term protection against recurrent or disseminated cancers (for prevention or treatment of diverse cancers); (ii) to produce immune cell compositions for adoptive cellular therapy of diverse cancers; and (iii) to serve as immune adjuvants or vaccines for prophylaxis of diverse cancers or infectious diseases.

[0107] The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to disrupt immunosuppressive networks in the tumor microenvironment. Tumors employ a wide array of regulatory mechanisms to avoid or suppress the immune response. Cancer cells actively promote immune tolerance in the tumor microenvironment via the expression of cytokines and molecules that inhibit the differentiation and maturation of antigen-presenting dendritic cells. The immunosuppressive cytokines and ligands produced by tumor cells include the following: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed death-1 ligand 1 (PD-L1; B7-H1); (iii) Vascular endothelial growth factor (VEGF); and (iv) Interleukin-10 (IL-10). In addition to blocking dendritic cell (DC) maturation, these molecules promote the development of specialized subsets of immunosuppressive $CD4^+$ T cells (regulatory T cells; Treg cells) and myeloid-derived suppressor cells (MDSC). Tregs are a minority sub-population of $CD4^+$ T cells that constitutively express CD25 [the interleukin-2 (IL-2) receptor α-chain] and the forkhead box P3 (FOXP3) transcription factor. Tregs (CD4+CD25+FoxP3+ cells) maintain immune tolerance by restraining the activation, proliferation, and effector functions of a wide range of immune cells, including $CD4^+$ and $CD8^+$ T cells, natural killer (NK) and NKT cells, B cells and antigen presenting cells (APCs) *in vitro* and *in vivo*. The accumulation of Treg cells in the tumor microenvironment re-inforces tumor immune tolerance and facilitates tumor progression and metastases. The increased expression of immunosuppressive cytokines (TGF-β; PD-L1) and tumor-infiltrating Tregs is correlated with a reduction of survival of patients with diverse types of cancers. The present invention provides that tumor-induced immune tolerance mediated via Tregs is a crucial determinant of the resistance of cancers to

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

[0108] The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to inhibit the development or function of Tregs and myeloid suppressor cells (DCs or MDSC) within the tumor microenvironment. Tregs (CD4+CD25+FoxP3+ cells) express an array of immunosuppressive cytokines and molecules which act in concert to induce immune tolerance and promote tumor progression and metastases. These include: (i) Cytotoxic T-lymphocyte associated protein 4 (CTLA-4; CD152), a co-inhibitory receptor that binds to the ligands CD80 (B7-1) or CD86 (B7-2) on the antigen presenting cell (APC) and inhibits co-stimulation of T cells; (ii) Programmed death-1 ligand 1 (PD-L1; B7-H1), a ligand which engages the co-inhibitory receptor Programmed death-1 (PD-1) and inhibits T cell activation and proliferation. (iii) Transforming growth factor-beta (TGF-β), a cytokine which regulates immune responses by restricting the maturation and antigen-presenting function of dendritic cells, inhibiting the proliferation and activation of naïve T cells, suppressing the expression of cytotoxic molecules (Granzyme A/B, FasL, Apo2L/TRAIL, IFN-γ) in immune effector cells, and promoting the development and function of Tregs; (iv) Receptor activator of nuclear factor-κB ligand (RANKL), a ligand which engages Receptor activator of nuclear factor-κB (RANK) and promotes osteoclast differentiation, Treg development, and tumor metastases. In addition, Tregs express other surface molecules; (v) LAG-3, a CD4-related molecule that binds MHC class II; (vi) glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); and (vii) IL-10. The targeted immunostimulatory antibodies and/or fusion proteins of the invention provide the ability to bind a targeted molecule expressed by Tregs or myeloid suppressor cells while concurrently sequestering and inhibiting one or more immunosuppressive molecule that promotes their development, survival or function.

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In one aspect, the targeted immunostimulatory antibodies and/or fusion proteins directly deplete the number of Tregs.

[0109] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule on the tumor cell or tumor microenvironment (tumor stroma, tumor vasculature, or tumor infiltrating immune cell), and the immunomodulatory moiety specifically binds an immunosuppressive molecule expressed by the targeted tumor cell or tumor-infiltrating Treg cells and myeloid suppressor cells (DC or MDSC).

[0110] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule expressed by Treg cells, myeloid suppressor cells (MDSC), or dendritic cells (DC), and the immunomodulatory moiety specifically binds an immunosuppressive molecule that promotes their development, survival or function.

[0111] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety. The targeting moiety specifically binds a target molecule, and the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2); (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); (iv) Vascular endothelial growth factor (VEGF); (v) Transforming growth factor-beta receptor (TGF-βR); (vi) Programmed death-1 (PD-1); and (vii) Receptor activator of nuclear factor-κB (RANK).

[0112] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. In one aspect, the targeting moiety specifically binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), CD20, Vascular endothelial growth factor (VEGF), insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial

cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, CD30, CD33, or CD40.

[0113] In one aspect, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: (i) CD4; (ii) CD25 (IL-2α receptor; IL-2αR); (iii) cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); (iv) Interleukin-10 (IL-10); (v) Transforming growth factor-beta receptor (TGF-βR); (vi) Transforming growth factor-beta (TGF-β); (vii) Programmed Death-1 (PD-1); (viii) Programmed death-1 ligand (PD-L1 or PD-L2); (ix) Receptor activator of nuclear factor-κB (RANK); (x) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); (xi) LAG-3; or (xii) glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18).

[0114] In one aspect, the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β); (ii) Programmed death-1 ligand (PD-L1 or PD-L2); (iii) Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); or (iv) vascular endothelial growth factor (VEGF).

[0115] In one aspect, the immunomodulatory moiety includes a molecule that binds TGF-β. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Transforming growth factor-beta receptor TGF-βRII, TGF-βRIIb, or TGF-βRIII. In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes a TGF-β-binding amino acid sequence corresponding to SEQ ID NOs: 79-91. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of TGF-β.

[0116] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the

molecule includes an amino acid sequence corresponding to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

[0117] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Programmed Death-1 (PD-1), Programmed death-1 ligand 1 (PD-L1), or Programmed death-1 ligand 2 (PD-L2). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes PD-1 ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 11 or 12.

[0118] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF-βRII. In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and TGFβRII ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 13 or 14.

[0119] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes a PD-L1-binding amino acid sequence corresponding to SEQ ID NO: 92, 93, or 94. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Programmed death-1 ligand 1 (PD-L1).

[0120] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial

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growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death-1 (PD-1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.

[0121] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Receptor activator of nuclear factor-κB (RANK) or Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the targeting moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain of Programmed Death-1 (PD-1). In another aspect, the molecule includes RANK ectodomain, immunoglobulin Fc region, and PD-1 ectodomain. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 25 or 26.

[0122] In one aspect, the immunomodulatory moiety includes a molecule that specifically binds to Receptor activator of nuclear factor-κB ligand (RANKL). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes a RANKL-binding amino acid sequence corresponding to SEQ ID NO: 95, 96, 97, or 98. In an additional aspect, the immunomodulatory moiety inhibits the activity or function of Receptor activator of nuclear factor-κB ligand (RANKL).

[0123] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (IL-2α receptor; IL-2αR), or CD4. In an additional aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36.

[0124] The present invention provides novel targeted immunosuppressive antibodies and fusion proteins that induce or promote immune tolerance by at least one of the following:

(i) inhibiting the activation of dendritic cells, T cells, and/or B cells; and

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

[0125] Targeted immunosuppressive antibodies and/or fusion proteins: The aberrant activation of self-reactive T cells and/or breakdown of the mechanisms of immune tolerance promotes the development of autoimmunity that results in various diseases including type I diabetes mellitus, multiple sclerosis, systemic lupus erythematosus, inflammatory bowel disease, and rheumatoid arthritis. The targeted immunosuppressive antibodies and/or fusion proteins of the invention are designed to suppress unwanted or excessive immune or inflammatory responses and restore or promote immune tolerance. Accordingly, the compositions and methods of the invention have broad clinical relevance for the treatment of diverse autoimmune or inflammatory diseases and preventing the rejection of a transplanted cell, tissue, or organ grafts.

[0126] The targeted immunosuppressive antibodies and/or fusion proteins of the invention provides their ability to inhibit the activity of targeted pro-inflammatory cytokines or immune cells while simultaneously promoting immune tolerance via the targeted delivery of immunosuppressive molecules that facilitate the development and/or function of regulatory T cells. These molecules of the present invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) The molecules of the invention enable targeted delivery of immunosuppressive molecules to immune cells or pro-inflammatory molecules in the milieu of the affected cell, tissue or organ; (ii) The molecules of the invention can couple the inhibition of the targeted pro-inflammatory molecule or immune cell with the

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

[0127] Further, the targeted immunosuppressive antibodies and/or fusion proteins of the invention are distinguished from and superior to existing therapeutic molecules in at least one of the following aspects: (i) To suppress unwanted or excessive immune or inflammatory responses in order to treat autoimmune or inflammatory diseases; and (ii) To prevent the rejection of a transplanted cell, tissue, or organ grafts.

[0128] In one aspect, the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes a PD-1-binding amino acid sequence corresponding to SEQ ID NO: 99, 100, or 101. In an additional aspect, the immunomodulatory moiety increases the function of PD-1.

[0129] In one aspect, the targeting moiety specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 37. In another aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2), and the immunomodulatory moiety includes a sequence from PD-1 ligand 1 (PD-L1 or B7-H1). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from PD-L1. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 38 or 39.

[0130] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25, or CD4, and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1

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ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 40, 41, 42, 43, 44, or 45.

[0131] In one aspect, the targeting moiety includes the extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 46 or 47.

[0132] In one aspect, the targeting moiety includes transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 48 or 49.

[0133] In one aspect, the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 99, 100, or 101. In an additional aspect, the immunomodulatory moiety activates the signaling function of transforming growth factor-β (TGF-β) receptor.

[0134] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from TGF-β. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 50. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 51 or 52.

[0135] In one aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, CD25 (IL-2α receptor; IL-2αR), or CD4, and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In an additional aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 53, 54, 55, 56, 57 or 58.

[0136] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 59 or 60.

[0137] In one aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to Tumor Necrosis Factor-α (TNF-α), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) or Osteoprotegerin (OPG). In an additional aspect, the targeting moiety includes an antibody that binds TNF-α, and the immunomodulatory moiety includes a sequence from an extracellular RANKL-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 61. In one aspect, the targeting moiety includes an extracellular ligand-binding domain of tumor necrosis factor receptor 2 (TNFR2). In another aspect, the molecule includes TNFR2 Extracellular ligand binding domain, immunoglobulin Fc region, and a sequence from an extracellular RANK-binding domain or ectodomain of RANK. In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 62 or 63.

[0138] In one aspect, the targeting moiety includes an extracellular domain of CTLA-4 and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) or Osteoprotegerin (OPG). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 64 or 65.

[0139] In one aspect, the targeting moiety includes a sequence from transforming growth factor-β (TGF-β) and immunoglobulin Fc region (IgG Cγ1), and the

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immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 66 or 67.

[0140] In one aspect, the targeting moiety includes a sequence from Programmed death-1 ligand 1 (PD-L1) and immunoglobulin Fc region (IgG Cγ1), and the immunomodulatory moiety includes an extracellular RANKL-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK). In another aspect, the molecule includes an amino acid sequence corresponding to SEQ ID NO: 68 or 69.

[0141] In various aspects, the molecule is fused or directly linked to one or more antigen, antigenic determinant, or epitope.

[0142] In another embodiment, the present invention provides a composition including the molecule of the invention and a cell, wherein the cell is a tumor cell, immune cell, or dendritic cell.

[0143] In another embodiment, the present invention provides a method of counteracting or overcoming immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

[0144] In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. In one aspect, the neoplastic disease is a non-T cell malignancy which does not express CD4 on the tumor cell. In one embodiment, the method includes administration to a subject in need thereof an antibody that targets and depletes CD4+ regulatory T cells (Tregs) in combination with a cytotoxic anticancer therapy. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In various aspects, the cytotoxic anticancer therapy includes a chemotherapeutic molecule, tumor-targeted antibody, small molecule kinase inhibitor, hormonal agent, or tumor-targeted cytotoxic agent, anti-angiogenic agent or any combination thereof. In another aspect, the cytotoxic anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

[0145] In another embodiment, the method includes administration to a subject in need thereof an antibody or molecule that targets and depletes CD4+ regulatory T cells (Tregs) in combination with an immunostimulatory antibody, fusion protein, peptide or ligand that targets CTLA-4, PD1, PD-1L, RANKL, TGF- β, GITR, 4-1BB, OX-40, or Toll-like receptors (TLR 1-10). In one aspect, the TLR-agonist comprises an activator of TLR-8 or TLR-9. In one aspect, the TLR agonist comprises an immunostimulatory nucleic acid sequence containing CpG nucleotides. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody.

[0146] In another embodiment, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof one or more molecule of the invention. In various aspects, the subject is administered one or more molecule of the invention in combination with another anticancer therapy. In one aspect, the anticancer therapy includes a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent, cytotoxic agent, targeted therapeutic agent, or anti-angiogenic agent. In another aspect, the anticancer therapy includes ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation. In another aspect, the subject is administered one or more molecule of the invention in combination with an antibody or molecule that inhibits the production or function of regulatory T cells (Tregs) or depletes the number of Tregs. In one aspect, the antibody that targets and depletes Tregs is an anti-CD4 antibody. In another aspect, the molecule that counteracts the function of Tregs is an antibody, fusion protein, peptide or ligand that targets CTLA-4, PD1, PD-1L, RANKL, TGF- β, GITR. In another aspect, the molecule that counteracts the function of Tregs is an antibody or fusion protein or ligand that targets 4-1BB or OX-40. In another aspect, the molecule that counteracts the function of Tregs is an agonist of Toll-like receptors (TLR 1-10). In one aspect, the TLR-agonist comprises an activator of TLR-8 or TLR-9. In one aspect, the TLR agonist is an immunostimulatory nucleic acid sequence containing CpG nucleotides.

[0147] In one aspect the chemotherapeutic agent is a topoisomerase-interacting agent, anthracycline, doxorubicin, mitoxantrone, camptothecin, camptothecin analogue, irinotecan, epipodophyilotoxin, etoposide, alkylating agent, cyclophosphamide, cisplatin,

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cisplatin analogue, oxaliplatin, antimetabolite, fluoropyrimidine analogue, 5-fluorouracil, gemcitabine, azacytidine, antimicrotubule agent, taxane, paclitaxel, or docetaxel.

[0148] In another embodiment, the subject is administered one or more molecule of the invention in combination with any vaccine. In another aspect, the vaccine includes a tumor antigen, tumor-associated antigen, tumor epitope, tumor antigen-containing fusion protein, tumor cell, or dendritic cell. In another aspect, the vaccine includes a pathogen antigen, pathogen-associated antigen, pathogen epitope, or pathogen antigen-containing fusion protein. In one aspect, the vaccine includes a surrogate CD4+ T cell helper epitope from tetanus toxin. In one aspect, the CD4+ T helper sequence contains a domain of tetanus toxin fragment C (pDOM1). In one aspect, the pDOM sequence is fused to a cell-permeabilizing cationic polypeptide (*e.g.,* Arginine-9). In another aspect, the Arg9-pDOM sequence is fused to a specific antigen comprising the vaccine.

[0149] In another embodiment, the present invention provides a method for treating immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treatment of a neoplastic disease. The method includes administering to a subject in need thereof a composition of immune cells contacted with a molecule of the invention.

[0150] In another embodiment, the present invention provides a method of inducing or promoting immune tolerance. The method includes administering to a subject in need thereof one or more molecule of the invention.

[0151] In another embodiment, the present invention provides a method of preventing or treating an autoimmune or inflammatory disease including administering to a subject in need thereof one or more molecule of the invention. In one aspect, the subject is administered one or more molecule of the invention in combination with another anti-inflammatory or immunosuppressive therapy. In another embodiment, the present invention provides a method of treatment of immune cells wherein the cells are contacted *ex vivo* or *in vitro* with a molecule of the invention. In another embodiment, the present invention provides a method of treating an autoimmune or inflammatory disease or preventing rejection of grafted cells or tissue. The method includes administering to a

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subject in need thereof a composition of immune cells contacted with a molecule of the invention.

[0152] 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.

[0153] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, references to "the method" includes one or more methods, and/or steps of the type described herein which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0154] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described.

[0155] As used herein "immune cells" or "immune effector cells" include T lymphocytes, B lymphocytes, natural killer (NK) cells, NKT cells, monocytes, macrophages, dendritic cells (DC), antigen presenting cells (APC).

[0156] As used herein, "neoplasm" or "tumor" including grammatical variations thereof, means new and abnormal growth of tissue, which may be benign or cancerous. In a related aspect, the neoplasm is indicative of a neoplastic disease or disorder, including but not limited, to various cancers. For example, such cancers can include prostate, pancreatic, biliary, colon, rectal, liver, kidney, lung, testicular, breast, ovarian, pancreatic, brain, and head and neck cancers, melanoma, sarcoma, multiple myeloma, leukemia, lymphoma, and the like.

[0157] A used herein, "subject," including grammatical variations thereof, means a human or vertebrate animal including a dog, cat, horse, cow, pig, sheep, goat, chicken, monkey, rat, and mouse.

[0158] As used herein, "targeting moiety" refers to a molecule that has the ability to localize and bind to a specific molecule or cellular component. The targeting moiety can be an antibody, antibody fragment, scFv, Fc-containing polypeptide, fusion antibody, polypeptide, peptide, aptamer, ligand, nucleic acid, or any combination thereof. In one embodiment, a targeting moiety can bind to a molecule present in a cell or tissue. In one aspect, the targeting moiety can bind a molecule in a diseased cell or tissue, such as a cancer cell or tumor. In, another aspect, the targeting molecule can bind a normal cell or tissue, such as an immune cell. In another aspect, the targeting moiety can bind a cellular or extracellular molecule that modulates the immune response. In another aspect, the targeting moiety binds a growth factor receptor, growth factor, cytokine receptor, cytokine, or cell surface molecule.

[0159] In another embodiment, the targeting moiety is a tumor-targeting moiety. The tumor-targeting moiety can bind a component of a tumor cell or bind in the vicinity of a tumor cell (*e.g.*, tumor vasculature or tumor microenvironment). In one embodiment, the tumor targeting moiety binds to a component of a tumor cell, tumor microenvironment, tumor vasculature, tumor-associated lymphocyte, tumor antigen, tumor-associated antigen, tumor cell surface molecule, tumor antigenic determinant, tumor antigen-containing fusion protein, tumor-associated cell, tumor-associated immune cell, or tumor vaccine.

[0160] For example, in various embodiments, a targeting moiety is specific for or binds to a molecule or component, which includes but is not limited to, epidermal growth factor receptor (EGFR, EGFR1, ErbB-1, HER1), ErbB-2 (HER2/neu), ErbB-3/HER3, ErbB-4/HER4, EGFR ligand family; insulin-like growth factor receptor (IGFR) family, IGF-binding proteins (IGFBPs), IGFR ligand family (IGF-1R); platelet derived growth factor receptor (PDGFR) family, PDGFR ligand family; fibroblast growth factor receptor (FGFR) family, FGFR ligand family, vascular endothelial growth factor receptor (VEGFR) family, VEGF family; HGF receptor family: TRK receptor family; ephrin (EPH) receptor family; AXL receptor family; leukocyte tyrosine kinase (LTK) receptor

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family; TIE receptor family, angiopoietin 1, 2; receptor tyrosine kinase-like orphan receptor (ROR) receptor family; discoidin domain receptor (DDR) family; RET receptor family; KLG receptor family; RYK receptor family; MuSK receptor family; Transforming growth factor alpha (TGF-α), TGF-α receptor; Transforming growth factor-beta (TGF-β), TGF-β receptor; Interleukin 13 receptor alpha2 chain (lL13Ralpha2), Interleukin-6 (IL-6), 1L-6 receptor, Interleukin-4, IL-4 receptor, Cytokine receptors, Class I (hematopoietin family) and Class II (interferon/lL-10 family) receptors, tumor necrosis factor (TNF) family, TNF-α, tumor necrosis factor (TNF) receptor superfamily (TNTRSF), death receptor family, TRAIL-receptor; cancer-testis (CT) antigens, lineage-specific antigens, differentiation antigens, alpha-actinin-4, ARTC1, breakpoint cluster region-Abelson (Bcr-abl) fusion products, B-RAF, caspase-5 (CASP-5), caspase-8 (CASP-8), beta-catenin (CTNNB1), cell division cycle 27 (CDC27), cyclin-dependent kinase 4 (CDK4), CDKN2A, COA-1, dek-can fusion protein, EFTUD-2, Elongation factor 2 (ELF2), Ets variant gene 6/acute myeloid leukemia 1 gene ETS (ETC6-AML1) fusion protein, fibronectin (FN), GPNMB, low density lipid receptor/GDP-L fucose: beta-Dgalactose 2-alpha-Lfucosyltraosferase (LDLR/FUT) fusion protein, HLA-A2, arginine to isoleucine exchange at residue 170 of the alpha-helix of the alpha2-domain in the HLA-A2 gene (HLA-A*201-R170I), MLA-A11, heat shock protein 70-2 mutated (HSP70-2M), KIAA0205, MART2, melanoma ubiquitous mutated 1,2, 3 (MUM-1, 2,3), prostatic acid phosphatase (PAP), neo-PAP, Myosin class 1, NFYC, OGT, OS-9, pml-RARalpha fusion protein, PRDX5, PTPRK, K-ras (KRAS2), N-ras (NRAS), HRAS, RBAF600, SIRT2, SNRPD1, SYT-SSX1 or -SSX2 fusion protein, Triosephosphate Isomerase, BAGE, BAGE-1, BAGE-2,3,4,5, GAGE-1,2,3,4,5,6,7,8, GnT-V (aberrant N-acetyl giucosaminyl transferase V, MGAT5), HERV-K-MEL, KK-LC, KM-HN-1, LAGE, LAGE-1, CTL-recognized antigen on melanoma (CAMEL), MAGE-Al (MAGE-1), MAGE-A2, MAGE-A3, MAGE-A4, MAGE-AS, MAGE-A6, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-3, MAGE-B1, MAGE-B2, MAGE-B5, MAGE-B6, MAGE-C1, MAGE-C2, mucin 1 (MUCl), MART-1/Melan-A (MLANA), gp100, gp100/Pme117 (S1LV), tyrosinase (TYR), TRP-1, HAGE, NA-88, NY-ESO-1, NY-ESO-l/LAGE-2, SAGE, Sp17, SSX-1,2,3,4, TRP2-1NT2, carcino-embryonic antigen (CEA), Kallikfein 4, mammaglobm-A, OA1, prostate specific antigen (PSA), prostate specific membrane

antigen, TRP-l/gp75, TRP-2, adipophilin, interferon inducible protein absent in nielanorna 2 (AIM-2), BING-4, CPSF, cyclin D1, epithelial cell adhesion molecule (Ep-CAM), EpbA3, fibroblast growth factor-5 (FGF-5), glycoprotein 250 (gp250intestinal carboxyl esterase (iCE), alpha-feto protein (AFP), M-CSF, mdm-2, MUCI, p53 (TP53), PBF, FRAME, PSMA, RAGE-1, RNF43, RU2AS, SOX10, STEAP1, survivin (BIRCS), human telomerase reverse transcriptase (hTERT), telomerase, Wilms' tumor gene (WT1), SYCP1, BRDT, SPANX, XAGE, ADAM2, PAGE-5, LIP1, CTAGE-1, CSAGE, MMA1, CAGE, BORIS, HOM-TES-85, AF15q14, HCA66I, LDHC, MORC, SGY-1, SPO11, TPX1, NY-SAR-35, FTHLI7, NXF2 TDRD1, TEX 15, FATE, TPTE, immunoglobulin idiotypes, Bence-Jones protein, estrogen receptors (ER), androgen receptors (AR), CD40, CD30, CD20, CD19, CD33, CD4, CD25, CD3, cancer antigen 72-4 (CA 72-4), cancer antigen 15-3 (CA 15-3), cancer antigen 27-29 (CA 27-29), cancer antigen 125 (CA 125), cancer antigen 19-9 (CA 19-9), beta-human chorionic gonadotropin, 1-2 microglobulin, squamous cell carcinoma antigen, neuron-specific enoJase, heat shock protein gp96, GM2, sargramostim, CTLA-4, 707 alanine proline (707-AP), adenocarcinoma antigen recognized by T cells 4 (ART-4), carcinoembryogenic antigen peptide-1 (CAP-1), calcium-activated chloride channel-2 (CLCA2), cyclophilin B (Cyp-B), human signet ring tumor-2 (HST-2), Human papilloma virus (HPV) proteins (HPV-E6, HPV-E7, major or minor capsid antigens, others), Epstein-Barr vims (EBV) proteins (EBV latent membrane proteins—LMP1, LMP2; others), Hepatitis B or C virus proteins, and HIV proteins. A composition of the invention can further include the foregoing as a peptide/polypeptide and/or encoding the same.

[0161] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. In one aspect, the targeting moiety specifically binds epidermal growth factor receptor (EGFR1, Erb-B1), HER2/neu (Erb-B2), CD20, Vascular endothelial growth factor (VEGF), insulin-like growth factor receptor (IGF-1R), TRAIL-receptor, epithelial cell adhesion molecule, carcino-embryonic antigen, Prostate-specific membrane antigen, Mucin-1, CD30, CD33, CD40, or a combination thereof.

[0162] Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as trastuzumab (anti-HER2/neu antibody); Pertuzumab (anti-HER2 mAb); cetuximab (chimeric monoclonal antibody to epidermal growth factor receptor EGFR); panitumumab (anti-EGFR antibody); nimotuzumab (anti-EGFR antibody); Zalutumumab (anti-EGFR mAb); Necitumumab (anti-EGFR mAb); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-447 (humanized anti-EGF receptor bispecific antibody); Rituximab (chimeric murine/human anti-CD20 mAb); Obinutuzumab (anti-CD20 mAb); Ofatumumab (anti-CD20 mAb); Tositumumab-I131 (anti-CD20 mAb); Ibritumomab tiuxetan (anti-CD20 mAb); Bevacizumab (anti-VEGF mAb); Ramucirumab (anti-VEGFR2 mAb); Ranibizumab (anti-VEGF mAb); Aflibercept (extracellular domains of VEGFR1 and VEGFR2 fused to IgG1 Fc); AMG386 (angiopoietin-1 and -2 binding peptide fused to IgG1 Fc); Dalotuzumab (anti-IGF-1R mAb); Gemtuzumab ozogamicin (anti-CD33 mAb); Alemtuzumab (anti-Campath-1/CD52 mAb); Brentuximab vedotin (anti-CD30 mAb); Catumaxomab (bispecific mAb that targets epithelial cell adhesion molecule and CD3); Naptumomab (anti-5T4 mAb); Girentuximab (anti-Carbonic anhydrase ix); or Farletuzumab (anti-folate receptor). Other examples include antibodies such as Panorex™ (17-1A) (murine monoclonal antibody); Panorex (@ (17-1A) (chimeric murine monoclonal antibody); BEC2 (ami-idiotypic mAb, mimics the GD epitope) (with BCG); Oncolym (Lym-1 monoclonal antibody); SMART M195 Ab, humanized 13' 1 LYM-1 (Oncolym), Ovarex (B43.13, anti-idiotypic mouse mAb); 3622W94 mAb that binds to EGP40 (17-1A) pancarcinoma antigen on adenocarcinomas; Zenapax (SMART Anti-Tac (IL-2 receptor); SMART M195 Ab, humanized Ab, humanized); NovoMAb-G2 (pancarcinoma specific Ab); TNT (chimeric mAb to histone antigens); TNT (chimeric mAb to histone antigens); Gliomab-H (Monoclonals—Humanized Abs); GNI-250 Mab; EMD-72000 (chimeric-EGF antagonist); LymphoCide (humanized IL.L.2 antibody); and MDX-260 bispecific, targets GD-2, ANA Ab, SMART IDIO Ab, SMART ABL 364 Ab or ImmuRAIT-CEA. Examples of antibodies include those disclosed in US5736167, US7060808, and US5821337.

[0163] In one embodiment, the targeting moiety specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: CD4; CD25 (IL-2α receptor; IL-2αR); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF-βR); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); Programmed death-1 ligand (PD-L1 or PD-L2); Receptor activator of nuclear factor-κB (RANK); Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); or Interleukin-4 receptor (IL-4R). In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

[0164] In one aspect, the targeting moiety binds a specific cytokine, cytokine receptor, co-stimulatory molecule, co-inhibitory molecule, or immunomodulatory receptor that modulates the immune system. In another aspect, the targeting moiety specifically binds one of the following molecules: tumor necrosis factor (TNF) superfamily; tumor necrosis factor-α (TNF-α); tumor necrosis factor receptor (TNFR) superfamily; Interleukin-12 (IL-12); IL-12 receptor; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNR4); OX40 ligand (OX40L; CD40; CD40 ligand (CD40L); CTLA-4; Programmed death-1 (PD-1); PD-1 ligand 1 (PD-L1; B7-H1); or PD-1 ligand 2 (PD-L2; B7-DC); B7 family; B7-1 (CD80); B7-2 (CD86); B7-H3; B7-H4; GITR/AITR; GITRL/AITRL; BTLA; CD70; CD27; LIGHT; HVEM; Toll-like receptor (TLR)(TLR 1,2,3,4,5,6,7,8,9, 10). In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

[0165] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide, or peptide that specifically binds a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a cytokine, cytokine receptor, co-stimulatory molecule,

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or co-inhibitory molecule that modulates the immune system. In another aspect, the targeting moiety specifically binds one of the following molecules: CD4; CD25 (IL-2α receptor; IL-2αR); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta receptor (TGF-βR); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); PD-1 ligand 1 (PD-L1; B7-H1); PD-1 ligand 2 (PD-L2; B7-DC); Receptor activator of nuclear factor-κB (RANK); Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); Interleukin-4 receptor (IL-4R); tumor necrosis factor (TNF) superfamily; tumor necrosis factor-α (TNF-α); tumor necrosis factor receptor (TNFR) superfamily; Interleukin-12 (IL-12); IL-12 receptor; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNR4); OX40 ligand (OX40L; CD40; CD40 ligand (CD40L); CTLA-4; B7 family; B7-1 (CD80); B7-2 (CD86); B7-H3; B7-H4; GITR/AITR; GITRL/AITRL; BTLA; CD70; CD27; LIGHT; or HVEM. In one aspect, the targeting moiety is an agonist that increases the function of the targeted molecule. In another aspect, the targeting moiety is an antagonist that inhibits the function of the targeted molecule.

[0166] Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as Zanulimumab (anti-CD4 mAb), Keliximab (anti-CD4 mAb); Ipilimumab (MDX-101; anti-CTLA-4 mAb); Tremilimumab (anti-CTLA-4 mAb); (Daclizumab (anti-CD25/IL-2R mAb); Basiliximab (anti-CD25/IL-2R mAb); MDX-1106 (anti-PD1 mAb); antibody to GITR; GC1008 (anti-TGF-β antibody); metelimumab/CAT-192 (anti- TGF-β antibody); lerdelimumab/CAT-152 (anti- TGF-β antibody); ID11 (anti- TGF-β antibody); Denosumab (anti-RANKL mAb); BMS-663513 (humanized anti-4-1BB mAb); SGN-40 (humanized anti-CD40 mAb); CP870,893 (human anti-CD40 mAb); Infliximab (chimeric anti-TNF mAb; Adalimumab (human anti-TNF mAb); Certolizumab (humanized Fab anti-TNF); Golimumab (anti-TNF); Etanercept (Extracellular domain of TNFR fused to IgG1 Fc); Belatacept (Extracellular domain of CTLA-4 fused to Fc); Abatacept (Extracellular domain of CTLA-4 fused to Fc); Belimumab (anti-B Lymphocyte stimulator); Muromonab-CD3 (anti-CD3 mAb); Otelixizumab (anti-CD3 mAb); Teplizumab (anti-CD3 mAb); Tocilizumab (anti-IL6R mAb); REGN88 (anti-IL6R mAb); Ustekinumab

(anti-IL-12/23 mAb); Briakinumab (anti-IL-12/23 mAb); Natalizumab (anti-α4 integrin); Vedolizumab (anti-α4 β7 integrin mAb); T1h (anti-CD6 mAb); Epratuzumab (anti-CD22 mAb); Efalizumab (anti-CD11a mAb); and Atacicept (extracellular domain of transmembrane activator and calcium-modulating ligand interactor fused with Fc).

[0167] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an "immunomodulatory moiety". As used herein, "immunomodulatory moiety" refers to a ligand, peptide, polypeptide, or Fc-containing polypeptide that binds a specific component of a regulatory T cell, myeloid suppressor cell, or dendritic cell and modulates the number or function of Tregs or myeloid suppressor cells. In an additional aspect, the "immunomodulatory moiety" specifically binds a cytokine, cytokine receptor, co-stimulatory molecule, or co-inhibitory molecule that modulates the immune system. In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: Transforming growth factor-beta receptor (TGF-βR); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); PD-1 ligand 1 (PD-L1; B7-H1); PD-1 ligand 2 (PD-L2; B7-DC); Receptor activator of nuclear factor-κB (RANK); or Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); or vascular endothelial growth factor (VEGF). In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR; TNFRSF18); GITRL/AITRL; 4-1BB (CD137); 4-1BB ligand (4-1BBL; CD137L); OX40 (CD134; TNR4); OX40 ligand (OX40L); B7-H3; B7-H4; BTLA; CD40; CD40 ligand (CD40L); CD70; CD27; LIGHT; or HVEM. In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: tumor necrosis factor-α (TNF-α); Interleukin-12 (IL-12); IL-12R; Interleukin-10 (IL-10); IL-10R. In another aspect, the immunoodulatory moiety comprises an extracellular domain of CTLA-4. In one aspect, the immunomodulatory moiety is an agonist that increases the function of the bound molecule. In another aspect, the immunomodulatory moiety is an antagonist that inhibits the function of the targeted molecule.

[0168] In another aspect, the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming

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

[0169] In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: Transforming growth factor-beta (TGF-β); PD-1 ligand 1 (PD-L1); PD-1 ligand 2 (PD-L2); or IL-10. In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL.

[0170] In another aspect, the immunomodulatory moiety is fused to the C-terminus of the targeting moiety. In another aspect, the immunomodulatory moiety is fused to the N-terminus of the targeting moiety. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is the targeting moiety, Fc is an immunoglobulin Fc region, and Y is the immunomodulatory moiety. In another aspect, the fusion molecule is represented by Y-Fc-X, wherein X is the targeting moiety, and Y is the immunomodulatory moiety. In one aspect, the targeting moiety may additionally be an immunomodulatory moiety.

[0171] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell, and the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF-βRII, TGF-βRIIb, or TGF-βRIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor-κB (RANK); vascular endothelial growth factor receptor (VEGFR1 or VEGFR2); or IL-10R.

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[0172] In one aspect, the targeting moiety includes an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell, and the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL.

[0173] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds a specific component of a regulatory T cell, myeloid suppressor cell, or dendritic cell, and the immunomodulatory moiety comprises an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF-βRII, TGF-βRIIb, or TGF-βRIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor-κB (RANK); or IL-10R. In another aspect, the immunomodulatory moiety comprises one or more of the following ligands or active ligand fragments: 4-1BB ligand (4-1BBL; CD137L); OX40 ligand (OX40L); IL-12; CD40L; or GITRL/AITRL. In another aspect, the specific targeted component of a regulatory T cell, myeloid suppressor cell, or dendritic cell is one of the following molecules: CD4; CD25 (IL-2α receptor; IL-2αR); cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152); Interleukin-10 (IL-10); Transforming growth factor-beta (TGF-β); Programmed Death-1 (PD-1); Programmed death-1 ligand (PD-L1 or PD-L2); Receptor activator of nuclear factor-κB (RANK) ligand (RANKL); LAG-3; glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; TNFRSF18); or Interleukin-4 receptor (IL-4R).

[0174] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds one of the following: CTLA-4; 4-1BB (CD137); OX40 (CD134; TNR4); CD40; IL-12R; or glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR; AITR; TNFRSF18); and the immunomodulatory moiety comprises a different molecule selected from the following: (i) an extracellular domain or ligand-binding sequence of one of the following receptors: Transforming growth factor-beta receptor (TGF-βRII, TGF-βRIIb, or TGF-βRIII); Programmed Death-1 (PD-1); Receptor activator of nuclear factor-κB (RANK); or IL-

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10R; or (ii) Fc-containing polypeptide or ligand that binds one of the following: CTLA-4; 4-1BB (CD137); OX40 (CD134; TNR4); CD40; IL-12R; or GITR (AITR; TNFRSF18).

[0175] In another aspect, the targeting moiety and immunomodulatory moiety are two different molecules selected from any of the following: an antibody, antibody fragment, scFv, Fc-containing polypeptide or ligand that binds TGF-β, CTLA-4, PD-1, 4-1BB (CD137), OX40 (CD134; TNR4), CD40; IL-12R, or GITR/AITR (TNFRSF18), or Toll-like receptor (TLR); an extracellular domain or ligand-binding sequence of Transforming growth factor-beta receptor (TGF-βRII, TGF-βRIIb, or TGF-βRIII), Programmed Death-1 (PD-1), Receptor activator of nuclear factor-κB (RANK), or IL-10R. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is an immunomodulatory targeting moiety and Y is a different immunomodulatory moiety.

[0176] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds one of the following molecules: CD4; CD25 (IL-2α receptor; IL-2αR); or CD20; and the immunomodulatory moiety comprises one of the following ligands or active ligand fragments: Transforming growth factor-beta (TGF-β); PD-1 ligand 1 (PD-L1); PD-1 ligand 2 (PD-L2); or IL-10.

[0177] In another aspect, the targeting moiety includes an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds tumor necrosis factor-α (TNF-α), Interleukin-12 (IL-12), IL-6R, B-lymphocyte stimulator, CD11a, CD6, or CD22; and the immunomodulatory moiety comprises one of the following: (i) ligands or active ligand fragments of Transforming growth factor-beta (TGF-β), PD-1 ligand 1 (PD-L1), or IL-10; or (ii) an extracellular domain or ligand-binding fragment of RANK, 4-1BB (CD137), OX40 (CD134; TNR4), CD40, IL-12R or GITR/AITR (TNFRSF18).

[0178] In another aspect, the targeting moiety comprises the extracellular domain of CTLA-4 fused to immunoglobulin Fc, and the immunomodulatory moiety comprises one of the following: (i) ligands or active ligand fragments of Transforming growth factor-beta (TGF-β), PD-1 ligand 1 (PD-L1), or IL-10; or (ii) ligand-binding fragment of TNFR2, RANK, 4-1BB (CD137), OX40 (CD134; TNR4), CD40, IL-12R or GITR/AITR (TNFRSF18).

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[0179] In another aspect, the targeting moiety and immunomodulatory moiety are two different molecules selected from any of the following: an antibody, antibody fragment, scFv, Fc-containing polypeptide that binds tumor necrosis factor-α (TNF-α), Interleukin-12 (IL-12), IL-6R, B-lymphocyte stimulator, CD11a, CD6, or CD22; a ligand-binding fragment of TNFR2, RANK, 4-1BB (CD137), OX40 (CD134; TNR4), CD40, IL-12R or GITR/AITR (TNFRSF18); ligands or active ligand fragments of Transforming growth factor-beta (TGF-β), PD-1 ligand 1 (PD-L1), or IL-10; or CTLA-4-Fc. In one aspect, the fusion molecule is represented by X-Fc-Y, wherein X is the immunomodulatory targeting moiety and Y is a different immunomodulatory moiety.

[0180] Antibodies: In one embodiment, the targeting moiety or fusion protein is an immunoglobulin. As used herein, the term "immunoglobulin" includes natural or artificial mono- or polyvalent antibodies including, but not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments. F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin ion can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgGl, IgG2, IgG3, IgG4, IgAl, and IgA2) or subclass of immunoglobulin molecule.

[0181] An antibody as disclosed herein includes an antibody fragment, such as, but not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdfv) and fragments including either a VL or VH domain. In one embodiment, the targeting moiety is an antibody or scFv.

[0182] An antigen-binding antibody fragment, including single-chain antibody, may include the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. An antigen-binding fragment can also include any combination of variable region(s) with a hinge region, CHI, CH2, and CH3 domains. Also includes is a Fc fragment, antigen-Fc fusion proteins, and Fc-

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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.

[0183] The antibody herein specifically include a "chimeric" antibody in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al. (1984) Proc. Natl. Acad. Sci. USA. 81:6851-6855). A chimeric antibody of interest herein includes "primatized" antibodies including variable domain antigen-binding sequences derived from a non-human primate (*e.g.*, Old World Monkey, Ape etc.) and human constant region sequences.

[0184] 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.

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[0185] In one embodiment, a hybridoma can produce a targeted fusion protein comprising a targeting moiety and an immunomodulatory moiety. In one embodiment, a targeting moiety comprising an antibody, antibody fragment, or polypeptide is linked or fused to an immunomodulatory moiety consisting of a polypeptide, with a linker or without a linker. The linker can be an amino acid linker. In one embodiment, a linker is (GGGGS)n (SEQ ID NO: 123) wherein n is 1, 2, 3, 4, 5, 6, 7, or 8. For example, GGGGSGGGGSGGGGS (SEQ ID NO: 104). In another embodiment, a linker is EPKSCDK (SEQ ID NO: 105). In another embodiment, a linker is IEGRDMD (SEQ. ID. NO: 106). In various aspects, the length of the linker may be modified to optimize binding of the target moiety or the function of the immunomodulatory moiety. In various aspects, the immunomodulatory moiety is a polypeptide that is fused to the C-terminus of the Fc region of the heavy chain of a targeting antibody or Fc-containing fusion protein. In another aspect, the immunomodulatory moiety is a polypeptide that is fused to the C-terminus of the light chain of a targeting antibody. In another aspect, the fusion protein comprises an X-Fc-Y sequence, wherein X is a targeting polypeptide and Y is an immunomodulatory polypeptide.

[0186] For example, a hybridoma can produce the polypeptides corresponding to SEQ. ID. NO: 1-69.

[0187] An antibody fragment can include a portion of an intact, antibody, *e.g.,* including the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')2, and Fv fragments; Fc fragments or Fc-fusion products; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragment(s).

[0188] An intact antibody is one which includes an antigen-binding variable region as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variant thereof tor any other modified Fc (*e.g.,* glycosylation or other engineered Fc).

[0189] The intact antibody may have one or more "effector functions" which refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region or any other modified Fc region) of an antibody. Examples of antibody effector functions include Clq binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor (BCR); and cross-presentation of antigens by antigen presenting cells or dendritic cells. In one embodiment, the targeting antibody or Fc-containing fusion protein facilitates focused or preferential delivery of a immunomodulatory moiety to a target cell. In another aspect, a targeting antibody can induce death of the targeted cell or sensitize it to immune cell-mediated cytotoxicity. In another aspect, the Fc-fusion protein or antibody can facilitate delivery of the immunomodulatory moiety or immunogenic apoptotic material from antibody-bound tumor targets, or both, to an antigen presenting cells (APC) via interactions between their Fc and Fc receptors (on APC).

[0190] Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different "classes." There arc five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" (isotypes), *e.g.*, IgG1, IgG2, IgC3, IgG4, IgA, and IgA2. The heavy-chain constant domains (hat correspond to the different classes of antibodies are called alpha (α), delta (δ), epsilon (ε), gamma (γ), and mu (μ) respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0191] Peptides: In some aspects of the invention the targeting moiety or immunomodulatory moiety is a peptide or polypeptide. A peptide includes any analog, fragment or chemical derivative of a peptide whose amino acid residue sequence is shown herein. Therefore, a present peptide can be subject to various changes, substitutions, insertions, and deletions where such changes provide for certain advantages in its use. In this regard, a peptide of this invention corresponds to, rather than is identical to, the sequence of a recited peptide where one or more changes are made and it retains the ability to function as the unmodified peptide in one or more of the assays.

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[0192] The term "analog" includes any peptide having an amino acid residue sequence substantially identical to a sequence specifically shown herein in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the activity as described herein. Examples of conservative substitutions include the substitution of one non-polar (hydrophobic) residue such as isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another.

[0193] 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.

[0194] 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.

[0195] 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.

[0196] Aptamers: In one aspect of the invention, the targeting moiety is an aptamer. In various embodiments, an aptamer is specific for a molecule on a tumor cell, tumor vasculature, and/or a tumor microenvironment. The term "aptamer" includes DNA, RNA or peptides that are selected based on specific binding properties to a particular molecule. For example, an aptamer(s) can be selected for binding a particular gene product in a tumor cell, tumor vasculature, tumor microenvironment, and/or an immune cell, as disclosed herein, where selection is made by methods known in the art and familiar to one of skill in the art. Subsequently, said aptamer(s) can be administered to a subject to modulate or regulate an immune response.

[0197] Some aptamers having affinity to a specific protein, DNA, amino acid and nucleotides have been described (*e.g.*, K. Y. Wang, et al., Biochemistry 32:1899-1904 (1993); Pitner et al., U.S. Pat. No. 5,691,145: Gold, et al., Ann. Rev. Biochem. 64:763-797 (1995); Szostak et al., U.S. Pat. No. 5,631,146). High affinity and high specificity binding aptamers have been derived from combinatorial libraries (supra, Gold, et al.). Aptamers may have high affinities, with equilibrium dissociation constants ranging from micromolar to sub-nanomolar depending on the selection used, aptamers may also exhibit high selectivity, for example, showing a thousand fold, discrimination between 7-methyl G and G (Haller and Sarnow, Proc. Natl. Acad. Sci. USA 94:8521-8526 (1997)) or between D and L-tryptophan (supra, Gold et al.). An aptamer can be selected based on the particular molecule targeted (*e.g.*, aptamer targeting EGFR or other cancer markers). Standard procedures for in vitro selection are known, such as SELEX experiments, described at Science 249 (4968) 505-510 (1990), and Nature (London), 346 (6287) 818-822 (1990) which can be followed throughout, or with modifications and improvements known in the art.

[0198] 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.

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[0199] 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.

[0200] 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.

[0201] 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.

[0202] 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.

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[0203] The following examples are provided to further illustrate the embodiments of the present invention, but are not intended to limit the scope of the invention. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

EXAMPLE 1

[0204] Counteracting Tumor Immune Tolerance via Antibody-mediated Depletion of $CD4^+$ Regulatory T Cells Facilitates the Activation of Tumor-Reactive $CD8^+$ T Cells and Enhances the *In Vivo* Antitumor Efficacy of Cytotoxic Anticancer Agents.

[0205] Immunogenic death of tumor cells by chemotherapeutic agents can induce $CD8^+$ T cell-mediated antitumor immunity. In response to specific chemotherapeutic agents, tumor cells exhibit the rapid translocation of intracellular calreticulin (CRT) to the cell surface where its aggregation provides a signal for the recognition and engulfment of dying tumor cells by antigen presenting dendritic cells (DCs). Treatment of mouse MB49 or human SW780 bladder cancer cells with doxorubicin, an anthracycline chemotherapeutic agent, induced rapid surface exposure of CRT that was detected by immunofluorescence cytometry of cells stained with Dylight 488-labeled anti-CRT antibody (FIG. 53A). To determine whether *ex vivo* treatment with doxorubicin induced an immunogenic death of tumor cells, either untreated live MB49 cells or an equivalent number of MB49 cells that were pre-treated *in vitro* with doxorubicin were injected into one flank of syngeneic immunocompetent C57BL/6 mice. Unlike mice injected with live tumor cells, mice injected with doxorubicin-treated tumor cells exhibited increased production of IFN-γ by draining lymph node (DLN) cells in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 53B). Vaccination with doxorubicin-killed MB49 cells generated a tumor-specific immune response since no corresponding increase in IFN-γ secretion by DLN cells was observed following *in vitro* exposure to an irrelevant peptide (Hemagglutinin-HA). Injection of doxorubicin-treated MB49 tumor cells protected mice against tumor growth upon challenge with untreated live MB49 tumor cells injected into the opposite flank. (FIG. 1C). The protection against tumor growth by vaccination with doxorubicin-treated tumor cells was not observed in mice that were depleted of $CD8^+$ T cells with an anti-CD8 antibody before challenge with live tumor cells (FIG. 53C). These

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observations indicate that *ex vivo* treatment with chemotherapeutic agents can induce an immunogenic death of tumor cells that generates $CD8^+$ T cell-mediated adaptive antitumor immunity.

[0206] Tumor-induced immune tolerance inhibits activation of $CD8^+$ T cells in response to chemotherapy. To examine whether *in vivo* treatment with chemotherapeutic agents can activate $CD8^+$ T cell-mediated immune responses in mice with pre-established tumors, C57BL/6 mice were injected with live syngeneic MB49 tumor cells and then administered intratumoral doxorubicin at various time points following tumor inoculation. In contrast to vaccination of naïve mice with doxorubicin-killed MB49 cells, *in vivo* treatment of mice with established MB49 tumors at d10 following tumor inoculation failed to induce a corresponding increase in IFN-γ secretion by DLN cells in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 53B). Whereas treatment with doxorubicin on d3 following tumor inoculation was able to arrest tumor growth, delayed administration of the same dose of doxorubicin on d10 failed to inhibit the progressive growth of established MB49 tumors (FIG. 53D). These results indicate that tumor-induced immune tolerance in the microenvironment of established cancers counteracts the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death.

[0207] Regulatory T cells (Treg) accumulate in the tumor microenvironment and counteract the ability of chemotherapy to activate $CD8^+$ T cell mediated antitumor immunity. To investigate whether $FoxP3^+$ Tregs are involved in enforcing immune tolerance in the tumor microenvironment, we examined the percentage of $CD4^+CD25^+FoxP3^+$ cells (Tregs) among $CD4^+$ T lymphocytes in the spleen, draining lymph nodes (DLN), and tumors of immunocompetent C57BL/6 mice at d0 and d14 after tumor inoculation. Whereas tumor-bearing mice exhibited only a minor increase in the percentage of Tregs among $CD4^+$ T cells in the spleen and DLN at d14 following tumor inoculation, a majority of tumor-infiltrating $CD4^+$ T cells at this time were $CD4^+CD25^+FoxP3^+$ cells (FIG. 53E). To investigate whether Tregs infiltrating the tumor microenvironment can suppress the activation of adaptive antitumor immunity in response to chemotherapy-induced tumor cell death, $CD4^+CD25^+$ cells isolated from tumors and DLN of tumor-bearing mice were adoptively transferred into syngeneic C57BL/6 naïve

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mice before vaccination with doxorubicin-killed MB49 cells. The adoptive transfer of tumor-infiltrating $CD4^+CD25^+$ cells into naïve mice inhibited the ability of subsequent *in vivo* vaccination with doxorubicin-treated MB49 tumor cells to increase production of IFN-γ by draining lymph node (DLN) cells in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 53F). Consistent with the ability of tumor-infiltrating $CD4^+CD25^+$ cells to suppress the tumor-specific immune response, the adoptive transfer of these cells counteracted the protection conferred by vaccination with doxorubicin-treated MB49 cells against tumor growth upon challenge with untreated live MB49 tumor cells (FIG. 53G). These results indicate that the tumor microenvironment fosters the accumulation of $FoxP3^+$ Tregs which counteract the activation of $CD8^+$ T cell mediated antitumor immunity in response to chemotherapy-induced tumor cell death.

[0208] Inhibition of TGF-β in the tumor microenvironment reduces tumor-infiltrating $FoxP3^+$ regulatory T cells and enhances the antitumor efficacy chemotherapy. TGF-β induces FoxP3 expression in naïve peripheral $CD4^+CD25^-FoxP3^-$ T cells and facilitates their conversion into 'adaptive' $FoxP3^+$ Tregs that share the immunosuppressive ability of natural $FoxP3^+$ Tregs generated in the thymus. Since human cancers frequently become refractory to the growth-inhibitory effect of TGF-β and acquire an ability to increase expression and secretion of TGF-β, we investigated whether this switch enables tumor cells to increase the number of adaptive Tregs in the tumor microenvironment. Examination of serum levels of TGF-β in mice at d0, d14, and d28 following inoculation of live MB49 tumor cells demonstrated that tumor growth resulted in a progressive increase in the level of serum TGF-β (FIG. 54A). To assess the precise source of TGF-β in tumor-bearing mice, the total amount of TGF-β in supernatants of tumor cells or draining lymph node cells isolated from tumor-bearing mice were measured following *ex vivo* culture in serum-free medium for 24h. Measurement of the level of TGF-β/10^6 cells showed that tumor cells were the dominant source of the increased level of TGF-β in tumor-bearing mice (FIG. 54B). In addition to tumor cell-autonomous expression of TGF-β, T cells from tumor-bearing mice also expressed higher levels of TGF-β compared to their counterparts from tumor-free mice (FIG. 54B). To determine whether the elevation of TGF-β is responsible for the upregulation of Tregs in the tumor microenvironment, tumor-bearing mice were treated with a soluble chimeric protein comprising the

extracellular domain of TGFβRII and the Fc portion of the murine IgG1 heavy chain (TGFβRII:Fc). This fusion protein interferes with the binding of TGF-β to endogenous TGFβRII and functions as a stable TGF-β antagonist. ELISA assays confirmed the ability of TGFβRII:Fc to sequester TGF-β in supernatants of MB49 tumor cells in a concentration-dependent manner (FIG. 54C). At 5d following inoculation of MB49 tumor cells, mice were either left untreated or treated with TGFβRII:Fc (1μg intratumoral; twice weekly) for 3 weeks followed by flow cytometric analyses of intracellular FoxP3 expression in $CD4^+CD25^+$ T cells infiltrating the tumors. *In vivo* treatment of tumors with TGFβRII:Fc resulted in a significant decline in FoxP3 expression in tumor-infiltrating $CD4^+$ T cells (FIG. 54D) and a dramatic reduction of $CD4^+CD25^+FoxP3^+$ Tregs in tumor tissue (FIG. 54E). To determine whether inhibition of TGF-β in the tumor microenvironment can improve the antitumor efficacy of chemotherapy, MB49 tumor-bearing mice were administered doxorubicin (5mg/kg i.p. weekly x 3) with or without twice weekly treatment with TGFβRII:Fc (1μg intratumoral). In contrast to treatment with either doxorubicin or TGFβRII:Fc alone, combined treatment with both agents was able to arrest the growth of MB49 tumors. These results indicate that tumor cell autonomous expression of TGF-β in the tumor microenvironment induces 'adaptive' $FoxP3^+$ Tregs and that counteracting tumor-induced TGF-β-mediated immune tolerance enhances the antitumor efficacy of chemotherapy.

[0209] Anti-CD4 antibody-mediated depletion of $CD4^+$ regulatory T cells facilitates chemotherapy-induced activation of tumor-reactive $CD8^+$ T cells and enhances the antitumor efficacy of chemotherapy. To determine whether depletion of $CD4^+$ regulatory T cells can improve the antitumor efficacy of chemotherapy by enhancing the activity of $CD8^+$ T cells in the tumor microenvironment, immunocompetent mice bearing syngeneic tumors were administered an anti-CD4 antibody (Clone GK1.5) to deplete $CD4^+$ T cells or an anti-CD8 antibody (Clone GK2.43) to deplete $CD8^+$ T cells and then treated with specific chemotherapeutic agents. Flow cytometric analyses of peripheral blood mononuclear cells from MB49 tumor-bearing mice at d7 following administration of anti-CD4 antibody or anti-CD8 antibody confirmed the target-specific depletion of either $CD4^+$ T cells or $CD8^+$ T cells, respectively (FIG. 55A). Mice treated with anti-CD4 antibody showed loss of $CD4^+CD25^+FoxP3^+$ T cells in the peripheral blood as well as among

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tumor-infiltrating cells (FIGS. 55A, 55B). To determine whether antibody-mediated depletion of $CD4^+CD25^+FoxP3^+$ cells facilitates chemotherapy-induced activation of tumor-reactive $CD8^+$ T cells in the tumor microenvironment, we evaluated the expression of IFN-γ in $CD8^+$ T cells extracted from the tumor and draining lymph node of MB49 tumor-bearing mice that were left untreated or treated with doxorubicin (with or without anti-CD4 antibody). Flow cytometric analyses showed that $CD8^+$ T cells from untreated mice did not express IFN-γ in response to *in vitro* re-challenge with MB49 cell lysates (FIG. 55C). Whereas IFN-γ^+CD8^+ T cells became evident in mice treated with doxorubicin alone, antibody-mediated depletion of $CD4^+$ T cells further enhanced the percentage of tumor-reactive $CD8^+$ T cells that expressed IFN-γ in doxorubicin-treated animals (FIG. 55C). To directly evaluate whether the activation of tumor-reactive $CD8^+$ T cells determines the *in vivo* antitumor efficacy of chemotherapy, we examined the effect of antibody-mediated depletion of $CD8^+$ T cells or $CD4^+$ T cells on the response of MB49 tumor-bearing mice to systemic treatment with doxorubicin (5mg/kg). Treatment with doxorubicin alone inhibited the growth of MB49 tumors but failed to arrest tumor progression. Whereas depletion of $CD8^+$ T cells completely impaired the *in vivo* antitumor efficacy of doxorubicin, depletion of $CD4^+$ T cells enhanced the response to doxorubicin and resulted in tumor regression (FIG. 55D).

[0210] Anti-CD4 antibody-mediated depletion of $CD4^+$ regulatory T cells augments and sustains the antitumor effect of chemotherapy by enabling activation of adaptive antitumor immunity. Whereas tumor cells treated with anthracyclins, such as doxorubicin, are particularly effective in eliciting an antitumor immune response, other chemotherapeutic agents are less effective in inducing immunogenic tumor cell death. The surface exposure of calreticulin is a key determinant of the immunogenicity of tumor cell death in response to chemotherapeutic agents. Compared to the efficient translocation of CRT to the cell surface in response to treatment with doxorubicin (FIG. 1A), treatment of MB49 tumor cells with equitoxic doses of either cisplatin or the combination of cisplatin and gemcitabine was less effective in increasing CRT exposure (FIG. 56A). Whereas tumor-reactive IFN-γ^+CD8^+ T cells were evident in tumors of MB49 tumor-bearing mice treated with doxorubicin (FIG. 55C), treatment with cisplatin was unable to induce a corresponding elevation of IFN-γ expression in $CD8^+$ T cells in response to *in*

vitro re-challenge with MB49 cell lysates (FIG. 56B). To examine whether counteraction of Treg-mediated immune tolerance enables the activation of antitumor immunity by cisplatin, immunocompetent tumor-bearing mice were treated with cisplatin following depletion of Tregs with anti-CD4 antibody. Antibody-mediated depletion of $CD4^+$ T cells enhanced the percentage of tumor-reactive IFN-γ^+CD8^+ T cells as well as $CD8^+CD62L^-$ T cells in cisplatin-treated animals (FIGS. 56B, 56C). Treatment of MB49 tumor-bearing mice with cisplatin partially inhibited tumor growth but failed to arrest tumor progression. Whereas depletion of $CD8^+$ T cells completely negated the *in vivo* antitumor effect of cisplatin, depletion of $CD4^+$ T cells enhanced the response to cisplatin and arrested tumor growth (FIG. 56D). Although treatment of tumor-bearing mice with the combination of cisplatin and gemcitabine was also able to arrest tumor growth, tumor growth rapidly resumed following termination of therapy with none of the animals (0/8) being tumor-free at d50 following tumor inoculation (FIG. 56E). In contrast, mice depleted of $CD4^+$ T cells exhibited a more sustained response to either single agent or combination chemotherapy, with 7/16 mice exhibiting complete tumor regression. The complete regression of tumors was attended with establishment of adaptive antitumor immunity since none of the cured mice (7/7) developed tumors when re-challenged with live MB49 tumor cells in the opposite flank.

[0211] Chemotherapy-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD^{4+} regulatory T cells to stimulate CD^{8+} T cell-mediated tumor regression. NKG2D (NK group 2, member D) is a lectin-like type II transmembrane stimulatory receptor used by NK cells, $\gamma\delta$-TC^{R+} T cells and $\alpha\beta$-TC^{R+} T cells for immune surveillance of tumors. Expression of mouse and human ligands for NKG2D is upregulated in transformed epithelial cell lines in response to genotoxic stress or stalled DNA replication, via activation of a DNA damage checkpoint pathway initiated by ATM (ataxia telengiectasia, mutated) or ATR (ATM- and Rad3-related) protein kinases. Treatment of CT26 mouse colon cancer cells with genotoxic chemotherapeutic agents resulted in upregulation of mouse NKG2D ligands of the retinoic acid inducible gene family (Rae1) (FIG. 57A). RT-PCR showed that Rae1 mRNA was induced in CT26 cells by 2-4h, reached a plateau after 16-24h, and began to decline after 48h of treatment with either the irinotecan or oxaliplatin (FIG. 57A). Flow cytometric analysis

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demonstrated that cell surface expression of human NKG2D ligands (MHC-I-related A and B molecules – MICA, MICB) was also upregulated on human colorectal cancer cells (HCT116) in response to treatment with irinotecan (FIG. 53B). Isogenic HCT116 cells that differ only in their p53 status demonstrated that p53 is not required for irinotecan-induced upregulation of MICA/B (FIG. 53B). To examine whether the induction of NKG2D ligands contributes to the antitumor effect of chemotherapy *in vivo*, immunocompetent Balb/C mice inoculated with syngeneic CT26 tumor cells were treated with irinotecan (50 mg/kg i.p) with or without pre-treatment with an NKG2D blocking antibody (200 μg i.p.). Whereas treatment with irinotecan alone inhibited the growth of CT26 tumors, the antitumor effect of irinotecan was negated by pre-treatment with the NKG2D blocking antibody (FIG. 1C). Since engagement of NKG2D by its ligands provides a costimulatory signal for the activation of CD^{8+} T cells, we investigated whether DNA damage-induced expression of NKG2D ligands on tumor cells cooperates with depletion of CD^{4+} regulatory T cells to stimulate CD^{8+} T cell-mediated tumor regression. Balb/C mice bearing CT26 tumors were administered an anti-CD4 antibody (Clone GK1.5) to deplete CD^{4+} T cells and/or an anti-CD8 antibody (Clone GK2.43) to deplete CD^{8+} T cells and then treated with irinotecan. Flow cytometric analyses confirmed the loss of $CD^{4+}CD2^{5+}FoxP^{3+}$ T cells in the spleen and draining lymph node of mice treated with anti-CD4 antibody (FIG. 57D). Antibody-mediated depletion of CD^{4+} T cells enhanced the percentage of tumor-reactive IFN-$\gamma^{+}CD^{8+}$ T cells in irinotecan-treated animals (FIG. 57E). Whereas treatment of CT26 tumor-bearing mice with irinotecan only slowed tumor growth, depletion of CD^{4+} T cells enhanced the response to irinotecan and arrested tumor growth (FIG. 57F). The ability of CD^{4+} T cell depletion to augment the antitumor efficacy of irinotecan was mediated by CD^{8+} T cells since antibody-mediated depletion of CD^{8+} T cells completely negated the *in vivo* antitumor effect of chemotherapy in CD^{4+} T cell-depleted mice (FIG. 57F).

[0212] These data provide the following insights: (i) activation of tumor-reactive $CD8^{+}$ T cells in response to immunogenic tumor cell death is a crucial determinant of the antitumor efficacy of chemotherapy *in vivo;* (ii) tumor-induced Tregs impair the antitumor efficacy of chemotherapy by inhibiting the activation of $CD8^{+}$ T cells in the tumor microenvironment; and (iii) Counteracting tumor-induced immune tolerance via antibody-

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

EXAMPLE 2

[0213] Exemplary Targeted Immunomodulatory Antibodies and Fusion Proteins

[0214] A targeting moiety, including an antibody, can be coupled to an immunomodulatory moiety including a polypeptide derived from the extracellular domain of TGFBR2. Crosslinkers or activating agents for such coupling or conjugation are well known in the art. Alternatively, the fusion proteins of the invention can be synthesized using recombination DNA technology well known in the art where the coding sequences of various portions of the fusion proteins can be linked together at the nucleic acid level. Subsequently the fusion proteins of the invention can be produced using a host cell well known in the art. Examples of targeted immunomodulatory antibodies and fusion proteins are shown in Figures 1-33 and briefly described below.

[0215] In one embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety specifically binds to Transforming growth factor-beta (TGF-β). SEQ ID NO: 1 provides a fusion protein including anti-HER2/neu antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 2). SEQ ID NO: 2 provides a fusion protein including anti-EGFR1 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 3). SEQ ID NO: 3 provides a fusion protein including anti-CD20 antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 4). SEQ ID NO: 4 provides a fusion protein including anti-VEGF antibody and Transforming growth factor-beta receptor II (TGFβ-RII) Extracellular domain (ECD) (FIG. 5). SEQ ID NO: 5 provides a fusion

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

[0216] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety is a molecule that specifically binds to Programmed Death-1 ligand 1 (PD-L1 or B7-H1) or Programmed Death-1 ligand 2 (PD-L2 or B7-DC). SEQ ID NO: 15 provides a fusion protein including anti-HER2/neu antibody and PD-1 Ectodomain (FIG. 13). SEQ ID NO: 16 provides a fusion protein including anti-EGFR1 antibody and PD-1 Ectodomain (FIG. 14). SEQ ID NO: 17 provides a fusion protein including anti-CD20 antibody and PD-1 Ectodomain

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(FIG. 15). SEQ ID NO: 18 provides a fusion protein including anti-VEGF antibody and PD-1 Ectodomain (FIG. 16). SEQ ID NO: 19 provides a fusion protein including anti-human CTLA-4 antibody and PD-1 Ectodomain (FIG. 17). SEQ ID NO: 20 provides a fusion protein including anti-CD25 antibody and PD-1 Ectodomain (FIG. 18A). SEQ ID NO: 21 provides a fusion protein including anti-CD25 antibody and PD-1 Ectodomain (FIG. 18B). SEQ ID NO: 22 provides a fusion protein including IL-2, Fc, and PD-1 ectodomain (IL-2 + Fc + PD-1 ectodomain; FIG. 19). SEQ ID NO: 23 provides a fusion protein including PD-1 ectodomain, Fc, and IL-2 (PD-1 ectodomain + Fc + IL-2; FIG. 19). SEQ ID NO: 24 provides a fusion protein including anti-CD4 antibody and PD-1 Ectodomain (FIG. 20). SEQ ID NO: 25 provides a fusion protein including RANK Ectodomain, Fc, and PD-1 ectodomain (RANK ectodomain + Fc + PD-1 ectodomain; FIG. 21). SEQ ID NO: 26 provides a fusion protein including PD-1 ectodomain, Fc, and RANK Ectodomain (PD-1 ectodomain + Fc + RANK ectodomain; FIG. 21).

[0217] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety is a molecule that specifically binds to Receptor activator of NF-kB ligand (RANKL). SEQ ID NO: 27 provides a fusion protein including anti-HER2/neu antibody and RANK Ectodomain (FIG. 23). SEQ ID NO: 28 provides a fusion protein including anti-EGFR1 antibody and RANK Ectodomain (FIG. 24). SEQ ID NO: 29 provides a fusion protein including anti-CD20 antibody and RANK Ectodomain (FIG. 25). SEQ ID NO: 30 provides a fusion protein including anti-VEGF antibody and RANK Ectodomain (FIG. 26). SEQ ID NO: 31 provides a fusion protein including anti-human CTLA-4 antibody and RANK Ectodomain (FIG. 27). SEQ ID NO: 32 provides a fusion protein including anti-CD25 antibody and RANK Ectodomain (FIG. 28A). SEQ ID NO: 33 provides a fusion protein including anti-CD25 antibody and RANK Ectodomain (FIG. 28B). SEQ ID NO: 34 provides a fusion protein including IL-2, Fc, and RANK ectodomain (IL-2 + Fc + RANK ectodomain; FIG. 29). SEQ ID NO: 35 provides a fusion protein including RANK ectodomain, Fc, and IL-2 (RANK ectodomain + Fc + IL-2; FIG. 29). SEQ ID NO: 36 provides a fusion protein including anti-CD4 antibody and RANK Ectodomain (FIG. 30).

[0218] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety includes a molecule that specifically binds to Programmed death-1 (PD-1). SEQ ID NO: 37 provides a fusion protein including anti-tumor necrosis factor (TNFα) antibody and PD-1 ligand 1 (FIG. 32). SEQ ID NO: 38 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and PD-1 ligand: (TNFR2 ECD + IgG Cγ1 + PD-L1; FIG. 33). SEQ ID NO: 39 provides a fusion protein including PD-1 ligand, Fc, and TNFR2 Extracellular ligand binding domain: (PD-L1 + IgG Cγ1 -TNFR2 ECD; FIG. 33). SEQ ID NO: 40 provides a fusion protein including anti-CD20 antibody and PD-1 ligand 1 (PD-L1) (FIG. 34). SEQ ID NO: 41 provides a fusion protein including anti-CD25 antibody and PD-1 ligand 1 (PD-L1) (FIG. 35A). SEQ ID NO: 42 provides a fusion protein including anti-CD25 antibody and PD-1 ligand 1 (PD-L1) (FIG. 35B). SEQ ID NO: 43 provides a fusion protein including PD-1 ligand 1 (PD-L1), Fc, and IL-2 (PD-L1 – Fc – IL2; FIG. 36). SEQ ID NO: 44 provides a fusion protein including IL-2, Fc, and PD-1 ligand 1 (PD-L1) (IL-2 – Fc – PD-L1; FIG. 36). SEQ ID NO: 45 provides a fusion protein including anti-CD4 antibody and PD-1 ligand 1 (PD-L1) (FIG. 37). SEQ ID NO: 46 provides a fusion protein including the extracellular domain of CTLA-4, Immunoglobulin Fc (IgG Cγ1), and a sequence from PD-1 ligand (PD-L1) (Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + PD-L1; FIG. 38). SEQ ID NO: 47 provides a fusion protein including the extracellular domain of PD-1 ligand (PD-L1), immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4: (PD-L1 + IgG Cγ1 + CTLA-4 ECD; FIG. 38). SEQ ID NO: 48 provides a fusion protein including Transforming growth factor-β (TGF-β), immunoglobulin Fc (IgG Cγ1), and a sequence from PD-1 ligand 1 (PD-L1) (TGFβ-1 + Fc + PD-L1; FIG. 39). SEQ ID NO: 49 provides a fusion protein including a sequence from PD-1 ligand 1 (PD-L1), immunoglobulin Fc (IgG Cγ1), and Transforming growth factor beta (TGF-□β) (PD-L1 + Fc + TGFβ-1; FIG. 39).

[0219] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety includes a

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molecule that specifically binds to Transforming growth factor-beta receptor (TGF-βR). SEQ ID NO: 50 provides a fusion protein including an antibody that binds TNF-α, and a sequence from Transforming growth factor-β (TGF-β) (FIG. 41). SEQ ID NO: 51 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and a sequence from Transforming growth factor-β (TGF-β) (TNFR2 ECD + IgG Cγ1 + TGF-β; FIG. 42). SEQ ID NO: 52 provides a fusion protein including a sequence from Transforming growth factor-β (TGF-β), Fc, and TNFR2 Extracellular ligand binding domain: (TGF-β + IgG Cγ1 + TNFR2 ECD; FIG. 42). SEQ ID NO: 53 provides a fusion protein including anti-CD20 antibody and a sequence from Transforming growth factor-β (TGF-β) (FIG. 43). SEQ ID NO: 54 provides a fusion protein including anti-CD25 antibody and a sequence from transforming growth factor-β (TGF-β) (FIG. 44A). SEQ ID NO: 55 provides a fusion protein including anti-CD25 antibody and a sequence from transforming growth factor-β (TGF-β) (FIG. 44B). SEQ ID NO: 56 provides a fusion protein including a sequence from Transforming growth factor-β (TGF-β), Fc, and IL-2 (TGF-β + Fc + IL-2; FIG. 45). SEQ ID NO: 57 provides a fusion protein including IL-2, Fc, and Transforming growth factor-β (TGF-β)(IL-2 + Fc + TGF-β; FIG. 45). SEQ ID NO: 58 provides a fusion protein including anti-CD4 antibody and a sequence from transforming growth factor-β (TGF-β) (FIG. 46). SEQ ID NO: 59 provides a fusion protein including the extracellular domain of CTLA-4, immunoglobulin Fc (IgG Cγ1), and a sequence from a sequence from transforming growth factor-β (TGF-β) (Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + TGF-β1; FIG. 47). SEQ ID NO: 60 provides a fusion protein including a sequence from Transforming growth factor-β (TGF-β), immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4: (TGF-β1 + IgG Cγ1 + CTLA-4 ECD) (FIG. 47).

[0220] In another embodiment, the present invention provides a molecule including a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety specifically binds to a target molecule, and the immunomodulatory moiety is a molecule that specifically binds to Receptor activator of NF-kB ligand (RANKL). SEQ ID NO: 61 provides a fusion protein including an antibody that binds TNF-α, and a sequence from RANK Ectodomain (FIG. 48). SEQ ID NO: 62 provides a fusion protein including TNFR2 Extracellular ligand binding domain, Fc, and a sequence from RANK Ectodomain

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(TNFR2 ECD + IgG Cγ1 + RANK Ectodomain; FIG. 49). SEQ ID NO: 63 provides a fusion protein including a sequence from RANK Ectodomain, Fc, and TNFR2 Extracellular ligand binding domain: (RANK Ectodomain + IgG Cγ1 + TNFR2 ECD; FIG. 49). SEQ ID NO: 64 provides a fusion protein including the extracellular domain of CTLA-4, immunoglobulin Fc (IgG Cγ1), and a sequence from a sequence from RANK Ectodomain (Oncostatin M signal peptide + CTLA-4 ECD + IgG Cγ1 + RANK Ectodomain; FIG. 50). SEQ ID NO: 65 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG Cγ1), and a sequence from the extracellular domain of CTLA-4: (RANK Ectodomain + IgG Cγ1 + CTLA-4 ECD) (FIG. 50). SEQ ID NO: 66 provides a fusion protein including a sequence from transforming growth factor-β (TGF-β), immunoglobulin Fc region (IgG Cγ1), and an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) (TGF-β + IgG Cγ1 + RANK Ectodomain; FIG. 51). SEQ ID NO: 67 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG Cγ1), and a sequence from transforming growth factor-β (TGF-β): (RANK Ectodomain + IgG Cγ1 + TGF-β) (FIG. 51). SEQ ID NO: 68 provides a fusion protein including a sequence from Programmed death-1 ligand 1 (PD-L1), immunoglobulin Fc region (IgG Cγ1), and an extracellular ligand-binding domain or ectodomain of Receptor activator of nuclear factor-κB (RANK) (PD-L1 + IgG Cγ1 + RANK Ectodomain; FIG. 52). SEQ ID NO: 69 provides a fusion protein including a sequence from RANK Ectodomain, immunoglobulin Fc (IgG Cγ1), and a sequence from Programmed death-1 ligand 1 (PD-L1): (RANK Ectodomain + IgG Cγ1 + PD-L1) (FIG. 52).

[0221] Although the invention has been described with reference to the above example, it will be understood that modifications and variations are encompassed within the spirit and scope of the invention. Accordingly, the invention is limited only by the following claims.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty. Docket No: JHU3260-10

In re US Application:
THE JOHNS HOPKINS UNIVERSITY
US Application No.: **18/883,719**
US Filing Date: **SEP 12, 2024**
For: **COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS**

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

STATEMENT ACCOMPANYING SEQUENCE LISTING

Dear Sir\Ma'am,

The specification further incorporates by reference the Sequence Listing submitted herewith via Patent Center. The Sequence listing .xml file, identified as **JHU3260-10,** is **174,970 bytes** in size and was created on **September 23, 2024**. The Sequence Listing, electronically filed herewith, does not extend beyond the scope of the specification, and does not contain new matter.

September 23, 2024
Date

/ankur gupta/
Ankur Gupta

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Sagacious House,
Plot No. B7/B8,
Institutional Area, Sector 32,
Gurugram, Haryana 122001
India

PATENT
Attorney Docket No.: JHU3260-10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	Not Yet Assigned
Application No.:	18/883,719	Examiner:	Not Yet Assigned
Filed:	September 12, 2024	Conf. No.	6410
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

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

STATEMENT TO SUPPORT FILING AND SUBMISSION IN ACCORDANCE WITH 37 CFR §§1.831-1.835

Sir:

Applicant hereby submits an .xml Sequence Listing in response to the Notice to Comply with Requirements for Patent Applications Containing Nucleotide and/or Amino Acid Sequence Disclosures mailed September 27, 2024.

Support for amendment of SEQ ID NO: 123 can be found in the compliant ASCII Sequence Listing incorporated by reference in parent U.S. Application Serial No. 17/694,541 filed March 14, 2022, to which the instant application claims priority to under 37 CFR §§1.78. SEQ. ID NO: 123 in the parent application is disclosed as a variant sequence that was improperly annotated with the feature key <REGION> in the original .xml document filed September 12, 2024. The .xml sequence listing submitted herewith corrects this error by identifying SEQ. ID NO: 123 with the feature key <VARIANT>. SEQ ID NO: 123 is also disclosed in parent U.S. Application Serial No. 17/694,541 filed March 14, 2022 in paragraph [0184].

In connection with the Sequence Listing submitted concurrently herewith, the undersigned hereby states that the submission, in accordance with 37 CFR §1.835(b)(5), does not include new matter.

Applicant asserts that the listing is now in compliance with 37 CFR §§1.831-1.835.

No fee is deemed necessary in connection with the filing of this paper. However, if a fee is required, the Commissioner is hereby authorized to charge any other fees associated with the filing submitted herewith, or credit any overpayments to Deposit Account No. 07-1896.

Respectfully submitted,

Date: October 1, 2024

/Pamela E. Hwang/

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PATENT
ATTORNEY DOCKET NO.: JHU3260-10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	Not Yet Assigned
Application No.:	18/883,719	Examiner:	Not Yet Assigned
Filed:	September 12, 2024	Conf. No.	6410
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

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

STATEMENT ACCOMPANYING MARKED-UP/SUBSTITUTE SPECIFICATIONS IN RESPONSE TO NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE AND/OR AMINO ACID SEQUENCE DISCLOSURES PURSUANT TO 37 C.F.R. §§1.125(b)(c)

Sir:

The marked-up and substitute specifications filed in conjunction with the Response to Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures mailed September 27, 2024 for the above-identified patent application are supported by the specification as originally filed and include no new matter. Accordingly, entry of the specifications is respectfully requested.

No fee is deemed necessary in connection with the filing of this paper. However, if a fee is required, the Commissioner is hereby authorized to charge any other fees associated with the filing submitted herewith, or credit any overpayments to Deposit Account No. 07-1896.

Respectfully submitted,

Date: October 1, 2024

/Pamela E. Hwang/
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PATENT
Attorney Docket No.: JHU3260-10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	Not Yet Assigned
Application No.:	18/883,719	Examiner:	Not Yet Assigned
Filed:	September 12, 2024	Conf. No.	6410
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

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Commissioner for Patents
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Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Sir:

In connection with the above-identified patent application in response to the Notice to Comply mailed September 27, 2024, entry of the amendments and reconsideration of the application in view of the amendments and following remarks are respectfully requested.

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

Remarks/Arguments begin on page 3 of this paper.

I. AMENDMENTS

A. In the Specification

On page 1 of the specification, please amend paragraph [0003] as follows:

[0003] The material in the accompanying sequence listing is hereby incorporated by reference into this application. The accompanying sequence listing text file, named ~~JHU3260-10_ST26.xml~~JHU3260-10.xml, was created on ~~September 11, 2024 and is 174,955 bytes in size~~September 23, 2024 and is 174,970 bytes in size.

II. REMARKS

The amendments to the specification amend the sequence listing disclosure in paragraph [0003]; therefore, no new matter is added. The sequence listing submitted herein includes no new matter since support for the sequence listing is reflected in the original application as filed. Accordingly, entry of the amendments is respectfully requested.

The Examiner is invited to contact Applicant's undersigned representative if there are any questions relating to this application. No fee is deemed necessary in connection with the filing of this paper. However, if a fee is required, the Commissioner is hereby authorized to charge any other fees associated with the filing submitted herewith, or credit any overpayments to Deposit Account No. 07-1896.

Respectfully submitted,

Date: October 1, 2024

/Pamela E. Hwang/

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Application Number: 18883719

Reviewed by: kBuLmOjFP3VzlESORWEnm76I/npXqkJwHo0JQ1qYXov9ZBL4CQRFMQ==

Note: This validation report concerns the sequence listing filed 10-01-2024 18:24:39 and indicates that NO errors were found by the USPTO during validation of the sequence listing. This validation report should not be construed as meaning that the sequence listing complies with all of the requirements of 37 CFR §§ 1.821-1.825, 37 CFR §§ 1.831-1.835 and 1.839, WIPO Standard ST.25, or WIPO Standard ST.26.

For any sequence listing related questions or concerns, the Applicant is invited to contact the USPTO Sequence Listing Help Desk at 571-272-2510 or SequenceHelpDesk@USPTO.GOV.

Timestamp: [year=2024; month=10; day=2; hr=7; min=19; sec=22; ms=883]

Validated By CRFValidator v 2.3.0

Application No: 18883719 Version No: 2.1

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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
18/883,719	09/12/2024		832	JHU3260-10	8	2

CONFIRMATION NO. 6410

UPDATED FILING RECEIPT

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

OC00000007889292I

Date Mailed: 10/04/2024

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

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Inventor(s)

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

Applicant(s)

The Johns Hopkins University, Baltimore, MD;

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

Domestic Priority data as claimed by applicant

This application is a CON of 17/694,541 03/14/2022
which is a CON of 16/601,347 10/14/2019 PAT 11,274,156
which is a CON of 15/362,632 11/28/2016 PAT 10,442,860
which is a DIV of 15/231,309 08/08/2016 PAT 9,850,306
which is a CON of 14/645,282 03/11/2015 PAT 9,441,044
which is a CON of 13/582,717 10/17/2012 PAT 8,993,524
which is a 371 of PCT/US2011/027317 03/04/2011
which claims benefit of 61/435,671 01/24/2011
and claims benefit of 61/311,255 03/05/2010

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

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

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

If Required, Foreign Filing License Granted: 09/25/2024

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 18/883,719**

Projected Publication Date: 01/09/2025

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Preliminary Class

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

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 18/883,719

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	64		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	280		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	320		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	8 minus 20 =	* 0	x =	0	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 minus 3 =	*	x =	0			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			168			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	832		TOTAL	

APPLICATION AS AMENDED - PART II

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

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

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/883,719	09/12/2024	Atul Bedi	JHU3260-10	6410

100899 7590 10/29/2024

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

EXAMINER
CENTRAL, DOCKET

ART UNIT	PAPER NUMBER
OPAP	

NOTIFICATION DATE	DELIVERY MODE
10/29/2024	ELECTRONIC

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

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

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

gtdocket@dlapiper.com

Decision Granting Request for Prioritized Examination (Track I)	**Application No.** 18/883,719	**Applicant(s)** Bedi et al.	
	Examiner ANGELA E WALKER	**Art Unit** OPET	**AIA (FITF) Status** No

1. THE REQUEST FILED 12 September 2024 IS **GRANTED** .

The above-identified application has met the requirements for prioritized examination

A. ☑ for an original nonprovisional application (Track I).
B. ☐ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination**. The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

A. filing a **petition for extension of time** to extend the time period for filing a reply;

B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims** , or a multiple dependent claim;

C. filing a **request for continued examination** ;

D. filing a notice of appeal;

E. filing a request for suspension of action;

F. mailing of a notice of allowance;

G. mailing of a final Office action;

H. completion of examination as defined in 37 CFR 41.102; or

I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to ANGELA WALKER at (571) 272-1058. In his/her absence, calls may be directed to Petition Help Desk at (571) 272-3282.

/ANGELA E WALKER/ Paralegal Specialist, OPET	

Notice of References Cited	Application/Control No. 18/883,719	Applicant(s)/Patent Under Reexamination Bedi et al.	
	Examiner BRIDGET E BUNNER	Art Unit 1647	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date YYYY-MM-DD	Name	CPC Classification	US Classification
*	A	US-8993524-B2	2015-03-31	Bedi; Atul	C07K16/2866	514/19.2
*	B	US-9441044-B2	2016-09-13	Bedi; Atul	A61K39/39558	1/1
*	C	US-9850306-B2	2017-12-26	Bedi; Atul	C07K16/2866	1/1
*	D	US-11274156-B2	2022-03-15	Bedi; Atul	A61K45/06	1/1
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date YYYY-MM-DD	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Huang et al. A Yeast Platform for the Production of Single-Chain Antibody–Green Fluorescent Protein Fusions. Appl Environmental Microbiol 72(12): 7748-7759, 2006
	V	Schmidt et al. Suppression of metastasis formation by a recombinant single chain antibody-toxin targeted to full-length and oncogenic variant EGF receptors. Oncogene 18: 1711-1721, 1999
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in YYYY-MM-DD format are publication dates. Classifications may be US or foreign.

Bicara Ex. 1002, Page 381 of 482
Bicara v. JHU - PGR2026-00025

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/883,719	Bedi et al.
	Examiner	**Art Unit**
	BRIDGET E BUNNER	1647

CPC - Searched*		
Symbol	**Date**	**Examiner**
C07K16/2863; C07K14/71; C07K19/00; C07K2319/00; C07K2319/31 ; C07K2319/32; C07K2319/33; C07K2319/35; C07K14/70596; C07K14/70578; C07K14/70575; C07K16/2866; C07K16/30; A61K39/3955; A61K2039/505; A61K38/1793; A61K38/179	11/27/2024	BEB

CPC Combination Sets - Searched*		
Symbol	**Date**	**Examiner**

US Classification - Searched*			
Class	**Subclass**	**Date**	**Examiner**

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	**Date**	**Examiner**
PE2E (JPO, EPO, USPAT, DERWENT); US-PGPUB; DAV (inventors ' names); ProQuest Dialog; see search history printout	11/27/2024	BEB
STIC search SEQ ID NO: 2, 71, 87; see search results in SCV/DAV	11/26/2024	BEB

Interference Search			
US Class/CPC Symbol	**US Subclass/CPC Group**	**Date**	**Examiner**

Bicara Ex. 1002, Page 382 of 482
Bicara v. JHU - PGR2026-00025

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CONFIRMATION NO. 6410

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
18/883,719	09/12/2024 RULE	424	1647	JHU3260-10

APPLICANTS
The Johns Hopkins University, Baltimore, MD;

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

**** CONTINUING DATA ***************************
This application is a CON of 17/694,541 03/14/2022
which is a CON of 16/601,347 10/14/2019 PAT 11274156
which is a CON of 15/362,632 11/28/2016 PAT 10442860
which is a DIV of 15/231,309 08/08/2016 PAT 9850306
which is a CON of 14/645,282 03/11/2015 PAT 9441044
which is a CON of 13/582,717 10/17/2012 PAT 8993524
which is a 371 of PCT/US2011/027317 03/04/2011
which claims benefit of 61/435,671 01/24/2011
and claims benefit of 61/311,255 03/05/2010

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED **** ** SMALL ENTITY **
09/25/2024

Foreign Priority claimed	❑ Yes ☑ No		STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
35 USC 119(a-d) conditions met	❑ Yes ☑ No	❑ Met after Allowance	MD	75	8	2
Verified and Acknowledged	/BRIDGET E BUNNER/ Examiner's Signature	BB Initials				

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

TITLE
COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

FILING FEE RECEIVED	FEES: Authority has been given in Paper	❑ All Fees
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	No._____ for following:	❑ 1.17 Fees (Processing Ext. of time)
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Bicara v. JHU - PGR2026-00025

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Set#	Searched for	Results
S1	au.exact("bedi, a" OR "bedi, a." OR "bedi, atul")	326°
S2	au.exact("ravi, r" OR "ravi, r." OR "ravi, r.; r" OR "ravi, rajani" OR "ravi, rajani k" OR "ravi, rajani k." OR "ravi, r k" OR "ravi, r. k." OR "ravi, r.k.")	7032*
S3	EGFR OR ERBB1 OR ERBB-1 OR HER1 OR (epidermal pre/2 growth pre/2 factor pre/2 receptor)	1466083*
S4	(transforming pre/2 growth pre/2 factor pre/2 beta pre/2 receptor pre/2 (2 OR II)) OR TGFbetaRII OR TGF-betaRII OR TGFR2 OR TGFR-2 OR TGF-βRII OR TGFβRII	19548*
S5	(transforming pre/2 growth pre/2 factor pre/3 receptor) OR TGFR	65387*
S6	anti-EGFR OR anti-ERBB1 OR anti-ERBB-1 OR anti-HER1	62588*
S7	(antibody OR antibodies) pre/3 (EGFR OR ERBB1 OR	21242*

Bicara Ex. 1002, Page 386 of 482
Bicara v. JHU - PGR2026-00025

	ERBB-1 OR HER1)	
S8	cetuximab	177364*
S9	(s1 or s2) and (s3 or s4 or s8)	18°
S10	((s1 or s2) and (s3 or s4 or s8)) and (pd(2003-2010))	5°
S11	(John pre/3 Hopkins pre/3 University) and (s3 or s4 or s8)	709°
S12	(John pre/3 Hopkins pre/3 University) and (s3 or s8) and (s4 or s5)	23°
S13	(s6 or s7 or s8) pre/150 (s4 or s5)	132°
S14	((s6 or s7 or s8) pre/150 (s4 or s5)) and (pd(1999-2010))	31°
S15	(s6 or s7 or s8) pre/100 (fuse or fusion or fused or joined or join or linked or conjugat*) pre/100 (s4 or s5)	12°
S16	(s6 or s7 or s8) pre/75 GGGGS	13°
S17	(s4 or s5) pre/75 GGGGS	2°

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(Use as many sheets as necessary)

Sheet 1 of 5

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	September 12, 2024
First Named Inventor	Atul Bedi
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	JHU3260-10

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1	7,317,091	01-08-2008	Lazar et al.	
	2	7,527,791	05-05-2009	Adams et al.	
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Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] "Number[4] "Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
	22	WO 2009/062399 A1	05-22-2009	Shanghai Jiaotong University		
	23	WO 2009/152610 A1	12-23-2009	The Royal Institution For The Advancement Of Learning/Mcgill University		
	24	WO 2010/003118 A1	01-07-2010	Trubion Pharmaceuticals, Inc.		

Examiner Signature		Date Considered	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 2 of 5

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	September 12, 2024
First Named Inventor	Atul Bedi
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	JHU3260-10

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	25	Alignment of SEQ ID No. 10 of D3 with SEQ ID No. 87 of B1 no date provided	
	26	Alignment of SEQ ID No. 744 of D2 with SEQ ID No. 87 of B1 no date provided	
	27	Canadian Office Action dated Mar. 28, 2018, regarding CA 2,791,383	
	28	Carter et al., "Identification and Validation of Cell Surface Antigens for Antibody Targeting in Oncology", Endocr. Relat. Cancer, December 2004, 11(4):659-687.	
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	32	EP Third Party Observation in European Application No. EP20200205164 dated April 5, 2022, 10 pages.	
	33	European Search Report and Search Opinion Received for EP Application No. 17168491.3, mailed on November 24, 2017, 5 pages	
	34	Fernandez-Botran et al., "Soluble cytokine receptors in biological therapy"; Expert Opinion on Biological Therapy, 2002, vol. 2, Issue 6, p. 585-605	

Examiner Signature		Date Considered	

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Sheet 3 of 5

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	September 12, 2024
First Named Inventor	Atul Bedi
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	JHU3260-10

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	35	Fonseca et al., "Capitalizing on the immunogenicity of Dying Tumor Cells"; Clinical Cancer Research 2008, 14 (6), p. 1603-1608	
	36	Francisco et al, "PD-L1 Regulates the Development, Maintenance, and Function of Induced Regulatory T Cells", J. Exp. Med., December 2009, 206(13):3015-3029	
	37	Haier et al., "Cell Surface Molecules and Their Prognostic Values in Assessing Colorectal Carcinomas", Annal Surgery, January 2000, 231(1):11-24	
	38	IN Examination Report in Indian Application No. 201948021756 dated January 24, 2022, 6 pages	
	39	International Preliminary Report on Patentability received for PCT Patent Application No. PCT/US2011/027317, mailed on September 20, 2012, 9 pages	
	40	International Search Report and Written Opinion received for PCT Patent Application No. PCT/US2011/027317, mailed on February 08. 2012, 11 pages	
	41	Japanese Office Action issued on May 27, 2020, regarding JP 2018-140050	
	42	JP Office Action in Japanese Application No. 2021-094042 dated August 1, 2022, 6 pages (with English translation)	
	43	Jones et al., "Degree of CD25 expression in T-Cell lymphoma is dependent on tissue site: implications for targeted therapy", Clinical Cancer Research, 2004, 10:5587-5594	

Examiner Signature		Date Considered	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 4 of 5

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	September 12, 2024
First Named Inventor	Atul Bedi
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	JHU3260-10

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	44	Jung et al.: "Double anti-angiogenic and anti-inflammatory protein valpha targeting VEGF-A and TNF-.alpha. in retinopathy and psoriasis"; JBC, vol. 286, No. 16, Feb. 23, 2011, pp. 14410-14418	
	45	Koh et al.: "Double anti-angiogenic protein, DAAP, targeting VEGF-A and angiopoietins in tumor angiogenesis, metastasis, and vascular leakage"; Cancer Cell, vol. 18, Issue 2, Aug. 16, 2010, p. 171-184. Supplementary data, 28 pages	
	46	Kwiatkowska et al.: "Expression of soluble recombinant TGF-.beta. type II receptor fused with the Fc portion of human IgG1 (sT.beta.11-Fc) in NSO cells"; Acta Biochimica Polonica, 2006, 53(2), pp. 361-369	
	47	Letourneau et al,: "IL-2- and CD25-dependent immunoregulatory mechanisms in the homeostasis of T-cell subsets"; J Allergy Clin Immunol. 2009, 123(4), pp. 758-762	
	48	Malek et al.: "Interleukin-2 receptor signaling: at the interface between tolerance and immunity"; Immunity 2010, vol. 33, Issue 2, p. 153-165	
	49	Office Action received for European Application No. 11751481.0, mailed on January 22, 2016, 3 pages	
	50	Podar et al.: "Inhibition of the TGF-.beta. signaling pathway in tumor cells"; Recent Results in Cancer Research, 2007, vol. 172, p. 77-97	
	51	Regnault et al.: "Fc.gamma. Receptor-mediated Induction of Dendritic Cell Maturation and Major Histocompatibility Complex Class I-restricted Antigen Presentation after Immune Complex Internalization"; J. Exp. Med., 1999, vol. 189, No. 2, p. 371-380	
	52	Rose-John et al. 2007. Expert Opinion in Therapeutic Targets. 11:613-624	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.
[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.
This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /BEB/

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 5 of 5

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	September 12, 2024
First Named Inventor	Atul Bedi
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	JHU3260-10

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	53	Schrama et al.: "Antibody targeted drugs as cancer therapeutics"; Nature Reviews Drug Discovery, Jan. 20, 2006, vol. 5, p. 147-159	
	54	Supplementary European Search Report received for EP Patent Application No. 11751481.0, mailed on June 27, 2013, 16 pages	
	55	Trail et al.: "Monoclonal antibody drug conjugates in the treatment of cancer"; Current Opinion in Immunology, 1999, vol. 11, Issue 5, p. 584-588	
	56	U.S. Appl. No. 61/311;255, Mar. 5, 2010, Atul Bedi	
	57	U.S. Appl. No. 61/435,671, Jan. 24, 2011, Atul Bedi	
	58	Wang et al.: "Tumor necrosis factor and cancer, buddies or foes?"; Acta Pharmacologica Sinica, 2008, 29 (11), p. 1275-1288	
	59	Yang et al.: "The role of T.sub.reg in the cancer immunological response"; American Journal of Immunology, 2009, 5 (1), p. 17-28	
	60	Yao and Chen: "Contribution of B7-H1/PD-1 Co-inhibitory Pathway to T-Ce11 Dysfunction in Cancer"; D.I. Gabrilovich, A.A. Hurwitz (eds.), Tumor-Induced Immune Suppression. pp. 29-40 .COPYRGT. Springer 2008	
	61	Zhang et al., "Design and Optimization of a Linker for Fusion Protein Construction," Prog. Natural Sci. (2009), 19:1197-1200, Elsevier	
	62	Zhang et al: "Expression of a Soluble TGF-.beta. Receptor by Tumor Cells Enhances Dendritic Cell/Tumor FusionVaccine Efficacy.sup.1"; J. Immunology, 2008, 181, pp. 3690-3697	

Examiner Signature	/BRIDGET E BUNNER/	Date Considered	11/27/2024

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.
[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.
This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /BEB/

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L1	21	((("BEDI") near3 ("Atul")) OR (("RAVI") near3 ("Rajani"))).INV.	(US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:14 PM
L2	6786	((("The") near3 ("Johns") near3 ("Hopkins") near3 ("University"))).AS,AANM.	(US-PGPUB; USPAT)	OR	ON	ON	2024/11/27 01:14 PM
L3	6786	((("The") near3 ("Johns") near3 ("Hopkins") near3 ("University"))).AS,AANM.	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:14 PM
L4	124911	EGFR OR ERBB1 OR ERBB-1 OR HER1 OR (epidermal adj2 growth adj2 factor adj2 receptor)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:27 PM
L5	1928	(transforming adj2 growth adj2 factor adj2 beta adj2 receptor adj2 (2 OR II)) OR TGFbetaRII OR TGF-betaRII OR TGFR2 OR TGFR-2 OR TGF-βRII OR TGFβRII	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:29 PM
L6	7481	(transforming adj2 growth adj2 factor adj3 receptor) OR TGFR	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:30 PM
L7	16	L1 AND L4	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:31 PM
L8	499	L3 AND L4	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:31 PM
L9	5	L3 AND L4 AND L5	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:31 PM
L10	41	L3 AND L4 AND L6	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:31 PM
L11	0	L4 SAME L5 SAME (fusion OR fused OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:34 PM
L12	0	L4 same2 L5 same2 (fusion OR fused OR join OR joined OR link	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:34 PM

		OR linked OR conjugat$)					
L13	0	L4 same2 L5	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:34 PM
L14	0	L4 same6 L5	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:34 PM
L15	2167	L4 same6 L6	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:35 PM
L16	1122	L4 same2 L6 same2 (fusion OR fused OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:35 PM
L17	663	L4 SAME L6 SAME (fusion OR fused OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:36 PM
L18	167	L4 WITH L6 WITH (fusion OR fused OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:37 PM
L19	34484	L4 SAME (fusion OR fused OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:38 PM
L20	0	L19 SAME L5	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:38 PM
L21	28101	L19 SAME EGFR	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:38 PM
L22	1928	TGFbetaRII OR TGF-betaRII OR TGFR2 OR TGFR-2 OR TGF-βRII OR TGFβRII	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:39 PM
L23	149	L19 SAME L22	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:39 PM
L25	294	L4 same2 L22 same2 (fusion OR fused OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:39 PM
L26	149	L4 SAME L22 SAME (fusion OR fused OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:40 PM

L29	173894	(C07K16/2863 OR C07K14/71 OR C07K19/00 OR C07K2319/00 OR C07K2319/31 OR C07K2319/32 OR C07K2319/33 OR C07K2319/35 OR C07K14/70596 OR C07K14/70578 OR C07K14/70575 OR C07K16/2866 OR C07K16/30 OR A61K39/3955 OR A61K2039/505 OR A61K38/1793 OR A61K38/179).cpc.	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:48 PM
L30	20522	anti-EGFR OR anti-ERBB1 OR anti-ERBB-1 OR anti-HER1	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:54 PM
L31	23085	(antibody OR antibodies) near3 (EGFR OR ERBB1 OR ERBB-1 OR HER1)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:55 PM
L32	15177	(L30 OR L31) same3 (fuse OR fusion OR fused OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:56 PM
L33	48	(L30 OR L31) same3 (fuse OR fusion OR fused OR join OR joined OR link OR linked OR conjugat$) same3 L22	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:56 PM
L34	15177	(L30 OR L31) same3 (fuse OR fusion OR fused OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:57 PM
L35	65	cetuximab same3 (fuse OR fusion OR fused OR join OR joined OR link OR linked OR conjugat$) same3 L22	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 01:57 PM
L36	35	cetuximab SAME (fuse OR fusion OR fused OR join OR joined OR link OR linked OR conjugat$) same3 L22	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 02:17 PM
L37	180	L17 AND L29	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 02:25 PM
L38	43	L18 AND L29	(US-PGPUB; USPAT; EPO; JPO;	OR	ON	ON	2024/11/27 02:25 PM

			DERWENT)				
L39	79	L23 AND L29	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 02:26 PM
L40	159	L25 AND L29	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 02:26 PM
L41	79	L26 AND L29	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 02:26 PM
L42	46	L33 AND L29	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 02:26 PM
L43	43	L35 AND L29	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 02:26 PM
L44	70732	GGGGS	(US-PGPUB; USPAT; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 02:36 PM
L46	0	L44 SAME (L5 OR L6)	(US-PGPUB; USPAT; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 02:36 PM
L47	0	L44 same3 (L5 OR L6)	(US-PGPUB; USPAT; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO,	OR	ON	ON	2024/11/27 02:36 PM

			MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); EPO; JPO; DERWENT)				
L48	986	L44 same3 (L30 OR L31)	(US-PGPUB; USPAT; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 02:37 PM
L49	439	L44 SAME (L30 OR L31)	(US-PGPUB; USPAT; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 02:37 PM
L50	116	L44 SAME (L30 OR L31)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2024/11/27 02:37 PM

PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/883,719	09/12/2024	Atul Bedi	JHU3260-10	6410

100899 7590 12/03/2024

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

EXAMINER	
BUNNER, BRIDGET E	

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
12/03/2024	ELECTRONIC

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

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

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

gtdocket@dlapiper.com

Office Action Summary	Application No. 18/883,719	Applicant(s) Bedi et al.	
	Examiner BRIDGET E BUNNER	Art Unit 1647	AIA (FITF) Status No

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

Period for Reply

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

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

Status

1)☑ Responsive to communication(s) filed on 01 October 2024.

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

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

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

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

Disposition of Claims*

5) ☑ Claim(s) 1-8 is/are pending in the application.

5a) Of the above claim(s) 4-8 is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 1-3 is/are rejected.

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

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

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

Application Papers

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

11)☑ The drawing(s) filed on 12 September 2024 is/are: a)☑ accepted or b)☐ objected to by the Examiner.

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

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

Priority under 35 U.S.C. § 119

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

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

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

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

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

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

Attachment(s)

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

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date 09/12/24.

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

4) ☐ Other: _____.

Bicara Ex. 1002, Page 399 of 482
Bicara v. JHU - PGR2026-00025

DETAILED ACTION

Notice of Pre-AIA or AIA Status

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

Status of Application, Amendments and/or Claims

Claims 1-8 are pending.

Election/Restriction

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

I. Claims 1-3, drawn to an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety comprises an antibody, scFv, or Fc-containing polypeptide that targets EGFR; and the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of TGF-βRII, classified in C07K 16/2863, for example.

II. Claims 4-8, drawn to a method of treating cancer in a subject in need thereof comprising administering a therapeutically effective amount of a molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety comprises an antibody, scFv, or Fc-containing polypeptide that targets EGFR; and the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of TGF-βRII, classified in A61K 39/39558.

2. The inventions are independent or distinct, each from the other because:

a. Inventions I and II are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially

different process of using that product. See MPEP § 806.05(h). In the instant case, the isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety claimed in Group I can be used in materially different methods, such as in protein purification methods or cell culture assays.

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

• the inventions have acquired a separate status in the art in view of their different classification

• the inventions require a different field of search (e.g., searching different classes /subclasses or electronic resources, or employing different search strategies or search queries)

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

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

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to

petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

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

4. During a telephone conversation with Pamela Hwang on 22 November 2024, a provisional election was made without traverse to prosecute the invention of Group I, claims 1-3. Affirmation of this election must be made by applicant in replying to this Office action. Claims 4-8 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

Applicant is reminded that upon the cancelation of claims to a non-elected invention, the inventorship must be corrected in compliance with 37 CFR 1.48(a) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. A request to correct inventorship under 37 CFR 1.48(a) must be accompanied by an application data sheet in accordance with 37 CFR 1.76 that identifies each inventor by his or her legal name and by the processing fee required under 37 CFR 1.17(i).

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

In the event of rejoinder, the requirement for restriction between the product/apparatus claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product/apparatus are found allowable, an otherwise proper restriction requirement between product/apparatus claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product/apparatus claim will not be rejoined. See MPEP § 821.04. Additionally, in order for rejoinder to occur, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product/apparatus claims. **Failure to do so may result in no rejoinder.** Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on 12 September 2024 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Claim Objections

5. Claims 1 and 2 are objected to because of the following informalities:

5a. Claim 1 recites the acronyms "EGFR" without first defining what it represents. While the claims can reference acronyms, the material presented by the acronym must be clearly set forth at the first use of the acronym and/or in each independent claim.

5b. In claim 2, the linker is an amino acid sequence, not a sequence identifier (SEQ ID NO:). Thus, for clarity, the claim should be amended to recite, for example, "...a linker comprising the amino acid sequence of SEQ ID NO: 104".

Appropriate correction is required.

Claim Rejections - 35 USC § 112(b)

The following is a quotation of 35 U.S.C. 112(b):
(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

6. Claims 2 and 3 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject

matter which the inventor or a joint inventor (or for applications subject to pre-AIA 35 U.S.C. 112, the applicant), regards as the invention.

6a. Claims 2 and 3 are rejected as being indefinite because the elements comprising the isolated molecule are unclear, as well as the structure of the molecule. Claim 2 recites the molecule of claim 1, wherein the molecule comprises a light chain, from N-terminus to C-terminus, an amino acid sequence comprising SEQ ID NO: 71, a linker comprising SEQ ID NO: 104, and an amino acid sequence comprising SEQ ID NO: 87.

For reference, claim 1 recites a targeting moiety fused with an immunomodulatory moiety, wherein (a) the targeting moiety comprises an antibody, antibody fragment, scFv, or Fc-containing polypeptide comprising amino acids 1-119 of SEQ ID NO: 2 and amino acids 1-107 of SEQ ID NO: 71, and that specifically binds EGFR; and (b) the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of TGF-βRII comprising SEQ ID NO: 87.

Therefore, regarding claim 2, it is not clear how a light chain comprises an amino acid sequence of SEQ ID NO: 87. The amino acid sequence of SEQ ID NO: 87 is an immunomodulatory moiety, specifically, the extracellular domain of TGF-βRII, and not a light chain.

It is also not clear how amino acids 1-119 of SEQ ID NO: 2 recited in claim 1 are structured/associated with the light chain of claim 2. Or, is the light chain recited in claim 2 an additional component of the isolated molecule of claim 1? Likewise, regarding claim 3, is the heavy chain an additional component of the isolated molecule of claim 1? Or, is Applicant attempting to limit the structure of the targeting moiety? One of ordinary skill in the art would not be apprised of the scope of the invention.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP § 2146 *et seq.* for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The filing of a terminal disclaimer by itself is not a complete reply to a nonstatutory double patenting (NSDP) rejection. A complete reply requires that the terminal disclaimer be accompanied by a reply requesting reconsideration of the prior Office action. Even where the NSDP rejection is provisional the reply must be complete. See MPEP § 804, subsection I.B.1. For a reply to a non-final Office action, see 37 CFR 1.111(a). For a reply to final Office action, see 37 CFR 1.113(c). A request for reconsideration while not provided for in 37 CFR 1.113(c) may be filed after final for consideration. See MPEP §§ 706.07(e) and 714.13.

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The actual filing date of the application in

which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/apply/applying-online/eterminal-disclaimer.

7. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-9 of **U.S. Patent No. 9,441,044**. Although the claims at issue are not identical, they are not patentably distinct from each other because both sets of claims are directed to an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety.

Claim 1 of the instant application is directed to an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein: (a) the targeting moiety comprises an antibody, antibody fragment, scFv, or Fc-containing polypeptide comprising amino acids 1-119 of SEQ ID NO: 2 and amino acids 1-107 of SEQ ID NO: 71, and that specifically binds EGFR; and (b) the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of TGF-βRII comprising SEQ ID NO: 87.

Meanwhile, claim 1 of the ‘044 patent recites an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein: (a) the targeting moiety specifically binds EGFR; and (b) the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of TGF-βRII. Claim 2 of the ‘044 patent recites that the targeting moiety comprises an antibody, antibody fragment, scFv, or Fc-containing polypeptide that specifically binds EGFR or a fragment thereof. Claim 3 of the ‘044 patent recites that the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of TGF-βRII selected from the group consisting of SEQ ID NO: 79-91. Claim 41 of the ‘044 patent recites that the immunomodulatory moiety comprises the amino acid sequence corresponding to SEQ ID NO: 87

or a binding fragment thereof. It is noted that the amino acid sequence of SEQ ID NO: 87 of the '044 patent is 100% identical the amino acid sequence of SEQ ID NO: 87 of the instant claims.

Furthermore, claim 5 of the '044 patent recites that the molecule comprises the amino acid sequence of SEQ ID NO: 2 or a binding fragment thereof. It is noted that the amino acid sequence of SEQ ID NO: 2 of the '044 patent is 100% identical to the amino acid sequence of SEQ ID NO: 2 of the instant application.

Lastly, claims 7-9 of the '044 patent recite that the isolated molecule comprises a linker at the CH3 region of the Fc, wherein the linker comprises the amino acid sequence of SEQ ID NO: 104 (GGGGSGGGGSGGGGS). It is noted that the amino acid sequence of SEQ ID NO: 104 of the '044 patent is 100% identical to the amino acid sequence of SEQ ID NO: 104 as recited in instant 2.

8. Claim 1-3 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-12 of copending **Application No. 18/811,627** in view of **Huang et al.** (App Environ Microbiol 72(12): 7748-7759, 2006).

Instant claim 1 is directed to an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein: (a) the targeting moiety comprises an antibody, antibody fragment, scFv, or Fc-containing polypeptide comprising amino acids 1-119 of SEQ ID NO: 2 and amino acids 1-107 of SEQ ID NO: 71, and that specifically binds EGFR; and (b) the immunomodulatory moiety comprises an amino acid sequence of the extracellular domain of TGF-βRII comprising SEQ ID NO: 87.

Meanwhile, claim 1 of the ‘627 application recites a method of treating a subject having cancer, comprising: administering to the subject a therapeutically effective amount of (a) a fusion protein comprising a targeting moiety fused to an immunomodulatory moiety, wherein:

(i) the targeting moiety comprises amino acids 1-119 of SEQ ID NO: 225 and amino acids 1-107 of SEQ ID NO: 226, and

(ii) the immunomodulatory moiety comprises a ligand binding fragment of the extracellular domain (ecd) of transforming growth factor β receptor II (TGFβRIIecd) having the amino acid sequence of SEQ ID NO: 6,

and (b) pembolizumab.

It is noted that amino acids 1-119 of SEQ ID NO: 225 of the ‘627 application are 100% identical to amino acids 1-119 of SQ ID NO: 2 of the instant application (see sequence alignment, below).

Qy=instant SEQ ID NO: 2
Db= SEQ ID NO: 225 of 18/811,627

```
RESULT 1
US-18-811-627-225

  Query Match             100.0%;  Score 3253;  DB 1;  Length 601;
  Best Local Similarity   100.0%;
  Matches  601;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYN 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYN 60

Qy         61 TPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVSAA 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 TPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVSAA 120

Qy        121 STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG 180

Qy        181 LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGP 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Db        181 LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGP 240

Qy        241 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS 300

Qy        301 TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        301 TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL 360

Qy        361 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        361 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ 420

Qy        421 QGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSTIPPHVQKSVNNDMIV 480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        421 QGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSTIPPHVQKSVNNDMIV 480

Qy        481 TDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLE 540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        481 TDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLE 540

Qy        541 TVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNP 600
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        541 TVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNP 600

Qy        601 D 601
              |
Db        601 D 601
```

Additionally, amino acids 1-107 of SEQ ID NO: 226 of the ‘627 application are 100% identical to amino acids 1-107 of SQ ID NO: 71 of the instant application (see sequence alignment, below).

Qy=instant SEQ ID NO: 71
Db= SEQ ID NO: 226 of 18/811,627

```
RESULT 1
US-18-811-627-226

  Query Match             100.0%;  Score 1109;  DB 1;  Length 379;
  Best Local Similarity   100.0%;
  Matches  214;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPS 60

Qy         61 RFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPP 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPP 120
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Qy        121 SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT 180

Qy        181 LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 214
              |||||||||||||||||||||||||||||||||
Db        181 LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 214
```

Lastly, the amino acid sequence of SEQ ID NO: 6 of the ‘627 application is 100% identical to the amino acid sequence of SEQ ID NO: 87 of the instant application (see sequence alignment, below).

Qy=instant SEQ ID NO: 87
Db= SEQ ID NO: 6 of 18/811,627

```
RESULT 1
US-18-811-627-6

  Query Match             100.0%;  Score 764;  DB 1;  Length 137;
  Best Local Similarity   100.0%;
  Matches  137;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE 60

Qy         61 VCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 VCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE 120

Qy        121 CNDNIIFSEEYNTSNPD 137
              |||||||||||||||||
Db        121 CNDNIIFSEEYNTSNPD 137
```

Claims 5 and 6 of the ‘627 application recite that the fusion protein comprises a linker fused between the targeting moiety and the immunomodulatory moiety; and that the linker comprises the amino acid sequence of SEQ ID NO: 4.

The only difference between the fusion protein of the ‘627 claims and the fusion protein of the instant application is that the claims of the ‘627 application do not recite that the linker

comprises the amino acid sequence of SEQ ID NO: 104 $(GGGGS)_3$, as required by instant claim 2.

Huang et al. teach the generation of scFv and single-chain TCR fused to GFP via a linker, $(GGGGS)_3$ (abstract; page 7749, column 1, 1st full paragraph; page 7751, column 1; Figure 1 (Type II, III); Table 1). Huang et al. disclose that secretion yields were not substantially different, but fusion proteins with the extended linker of $(GGGGS)_3$ demonstrated higher fluorescence per molecule, indicating less interference between the folded protein domains (page 7757, top of column 2).

It would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the administered fusion protein comprising a targeting moiety fused to an immunomodulatory moiety, wherein the fusion protein comprises a linker fused between the two moieties as required by the claims of the '627 application by fusing the moieties with a $(GGGGS)_3$ (instant SEQ ID NO: 104) linker as taught by Huang et al. The person of ordinary skill in the art would have been motivated to make that modification because the $(GGGGS)_3$ linker is flexible, increasing spatial separation between the domains and minimizing potential folding interference (see Huang et al., page 7751, column 1, 1st full paragraph). The person of ordinary skill in the art reasonably would have expected success because the $(GGGGS)_3$ linker was a common flexible linker for fusion proteins at the time the invention of the '627 claims was made.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Conclusion

No claims are allowable.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure:

Schmidt et al. Oncogene 18: 1711-1721, 1999 (teach a fusion protein comprising the variable domains of anti-EGFR antibody, 14E1, fused to a truncated form of *Pseudomonas* exotoxin A via a $(GGGGS)_3$ linker (abstract; page 1712, column 1, 1st paragraph under Results)

Any inquiry concerning this communication or earlier communications from the examiner should be directed to BRIDGET E BUNNER whose telephone number is (571)272-0881. The examiner can normally be reached Monday-Friday 9:00 am-6:00 pm.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

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

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BEB
Art Unit 1647
26 November 2024

/BRIDGET E BUNNER/
Primary Examiner, Art Unit 1647

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/883,719	**12/10/2024 07:27:53 PM Z ET**	**JHU3260-10**

Title of Invention

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	6410	FILED BY	Karen LePari
PATENT CENTER #	68315729	FILING DATE	09/12/2024
CUSTOMER #	100899	FIRST NAMED INVENTOR	Atul Bedi
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Lisa Haile

Documents

TOTAL DOCUMENTS: 1

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
JHU3260-10 AUTHORIZATION FOR INTERNET COMMUNICATIONS 10DEC2024pdf.pdf	2	Internet Communications Authorization	205 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
JHU3260-10 AUTHORIZATION FOR INTERNET COMMUNICATIONS 10DEC2024pdf.pdf	85D66ED66A2E8F2314EDAB389E10B453CBB18F84D41888A2532080D082FD3B93392E9D1EA7C9761CB71A17A4CCC5093996B7E3BA670CDD15AD10E861CA3C3A24

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized

by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

Doc Code: ECOMM.AUTH/ECOMM.WTDW
Doc Description: Internet Communications Authorization/Internet Communications Authorization Withdrawal

PTO/SB/439 (11-15)

AUTHORIZATION FOR INTERNET COMMUNICATIONS IN A PATENT APPLICATION OR REQUEST TO WITHDRAW AUTHORIZATION FOR INTERNET COMMUNICATIONS		
	Application No.	18/883,719
	Filing Date	September 12, 2024
	First Named Inventor	Atul Bedi
	Art Unit	1647
	Examiner Name	Bridget E. Bunner
	Practitioner Docket No.	JHU3260-10

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

I. To authorize permission for Internet Communications.

☒ Recognizing that Internet communications are not secure, I hereby authorize the USPTO to communicate with the undersigned and practitioners in accordance with 37 CFR 1.33 and 37 CFR 1.34 concerning any subject matter of this application via video conferencing, instant messaging, or electronic mail. I understand that a copy of these communications will be made of record in the application file. (MPEP 502.03)

II. To withdraw authorization for Internet Communications.

☐ The authorization given on _____, to the USPTO to communicate with the undersigned and any practitioner in accordance with 37 CFR 1.33 and 37 CFR 1.34 concerning any subject matter of this application via Internet communications such as video conferencing, instant messaging, or electronic mail is hereby withdrawn. I understand that the withdrawal is effective when approved rather than when received.

I am the

☐ applicant.

☒ attorney or agent of record. Registration number 38347.

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.

/Lisa A. Haile /
Signature

December 10, 2024
Date

Lisa A. Haile
Typed or printed name

+1 858.677.1456
Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Juristic entities must be represented by a patent practitioner (see 37 CFR 1.31, which is applicable to any paper filed on or after September 16, 2012, that is presented on behalf of a juristic entity, regardless of application filing date). Submit multiple forms if more than one signature is required, see below*.

☒ * Total of one forms are submitted.

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

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

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
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5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/883,719	09/12/2024	Atul Bedi	JHU3260-10	6410

100899 7590 12/23/2024

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

EXAMINER	
BUNNER, BRIDGET E	

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
12/23/2024	ELECTRONIC

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

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

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

gtdocket@dlapiper.com

Applicant-Initiated Interview Summary	**Application No.** 18/883,719	**Applicant(s)** Bedi et al.		
	Examiner BRIDGET E BUNNER	**Art Unit** 1647	**AIA (First Inventor to File) Status** No	**Page** 1 of 1

All Participants (applicant, applicants representative, PTO personnel)	**Title**	**Type**
BRIDGET E BUNNER	Primary Examiner	Video Conference
PAMELA HWANG	Attorney of Record	

Date of Interview: 17 December 2024

Issues Discussed:

Proposed Amendment(s)

Ms. Hwang discussed proposed amendments to claims 1 and 2 (as well as the addition of new dependent and independent claims) to address the claim objections and rejections set forth in the previous Office Action of 03 December 2024. Specifically, the proposed amendments would clarify the elements that comprise the claimed isolated molecule. Ms. Hwang also indicated the submission of a terminal disclaimer to address the nonstatutory double patenting rejections. The Examiner stated that such amendments (and terminal disclaimer submission) sounded reasonable to overcome the objections /rejections of record, but that such would be considered once officially made of record.

	/BRIDGET E BUNNER/ Primary Examiner, Art Unit 1647

Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04
Please further see:
MPEP 713.04
Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b)
37 CFR § 1.2 Business to be transacted in writing

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

REMARKS

Prior to this Communication, claims 1-9 were pending. With the present communication, claims 1 and 2 have been amended, claims 3-8 have been canceled without prejudice or disclaimer and new claims 9-15 have been added. Support for the claim amendments and new claims may be found generally throughout the application and claims as originally filed. No new matter is added. After entry of the amendments, claims 1-2 and 9-15 will be pending and under consideration.

Examiner's Interview

Applicant thanks Examiner Bunner for the telephone interview with Applicant's representatives on December 17, 2024. During the interview, proposed claim amendments were discussed to address clarity and indefiniteness rejections and the submission of a terminal disclaimer to address the obviousness type double patenting rejection were discussed. The Examiner stated that the proposed amendments and filing of a terminal disclaimer were reasonable to overcome the objections/rejections, but that such would be considered once the response is made of record.

Election/Restriction

The Examiner contacted Applicant's representative regarding a telephonic restriction requirement. The Examiner restricted the claims into two groups:

I. Claims 1-3 drawn to an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety comprises an antibody, scFv, or Fc-containing polypeptide that targets EGFR; and the immunomodulatory moiety comprises an amino acid sequence of extracellular domain of TGF-BRII; and

II. Claims 4-8 drawn to a method of treating cancer in a subject in need thereof comprising administering a therapeutically effective amount of a molecule comprising a targeting moiety fused with an immunomodulatory moiety,

wherein the targeting moiety comprises an antibody, scFv, or Fc-containing polypeptide that targets EGFR; and the immunomodulatory moiety comprises an amino acid sequence of extracellular domain of TGF-BRII.

During a telephone conversion with Applicant's representative on November 22, 2024, a provisional election to pursue the invention of Group I, claims 1-3, without traverse was made. Applicant affirms the election of Group I, claims 1-3 without traverse.

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

Claim Objections

The Examiner objected to claim 1 stating that the acronym "EGFR" is recited without first defining what it represents.

Applicant amended claim 1 to define "EGFR" as Epidermal Growth Factor Receptor". submits that this amendment renders the objection moot and respectfully requests withdrawal of the objection.

The Examiner objected to claim 2 not reciting a sequence identifier for the amino acid sequence of the linker and suggested that the claim be amended to recite "… linker comprising the amino acid sequence of SEQ ID NO: 104."

Applicant amended claim 2 to recite "wherein the targeting moiety comprises an antibody fragment selected from a Fab or scFv." Applicant submits that these amendments render the objection moot and respectfully request withdrawal of the objection.

Rejections under 35 USC § 112, second paragraph

Claims 2 and 3 stand rejected under 35 USC § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. Applicant respectfully traverses this rejection.

The Examiner states that claims 2 and 3 are indefinite because the elements comprising the isolated molecule are unclear, as well as the structure of the molecule.

As an initial matter, claim is canceled. Applicant amended claim 2 to recite "wherein the targeting moiety comprises an antibody fragment selected from a Fab or scFv." Applicant submits that these amendments and cancelations render the rejection moot and respectfully requests reconsideration and withdraw of the rejection.

Double Patenting Rejection

Claims 1-3 are provisionally rejected on the ground of non-statutory obviousness-type double patenting over claims 1-9 of U.S. Patent No. 9,441,044. Applicants respectfully traverse this rejection.

Without acquiescing to the Examiner's assertion and to expedite prosecution of the claimed invention, a terminal disclaimer is hereby submitted together with this paper to overcome the non-statutory obviousness-type double patenting rejections over U.S. Patent No. 9,441,044. Accordingly, withdrawal of the double patenting rejection is respectfully requested.

Claims 1-3 are provisionally rejected on the ground of non-statutory obviousness-type double patenting over claims 1-12 of U.S. Patent Appl. Serial No. 18/811,627 in view of Huang et al. (App Environ Microbiol 72(12):7748-7759, 2006). Applicants respectfully traverse this rejection.

Without acquiescing to the Examiner's assertion and to expedite prosecution of the claimed invention, a terminal disclaimer is hereby submitted together with this paper to overcome the non-statutory obviousness-type double patenting rejections over U.S. Patent Appl. Serial No. 18/811,627. Accordingly, withdrawal of the double patenting rejection is respectfully requested.

CONCLUSION

In view of the amendments and above remarks, it is submitted that the claims are in condition for allowance, and a notice to that effect is respectfully requested. The Examiner is invited to contact Applicant's undersigned representative if there are any questions relating to this application.

No fee is believed due in connection with this submission. The Commissioner is hereby authorized to charge any additional fees required by this submission, or make any credits or overpayments, to Deposit Account No. 07-1896 referencing the above-identified attorney docket number.

Respectfully submitted,

Date: December 23, 2024

/Pamela E. Hwang/
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PTO/SB/06 (09-11)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 18/883,719	Filing Date 09/12/2024	☐ To be Mailed

ENTITY: ☐ LARGE ☑ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $40 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $192 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	12/23/2024	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 9	Minus	** 20	= 0		x $40 =	0
	Independent (37 CFR 1.16(h))	* 2	Minus	*** 3	= 0		x $192 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

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

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TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A PRIOR PATENT

APPLICATION #	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET #
18883719	**09/12/2024**	**Atul Bedi**	**JHU3260-10**

Title of Invention

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

✓	Filing of terminal disclaimer does not obviate requirement for response under 37 CFR 1.111 to outstanding Office Action
✓	This electronic Terminal Disclaimer is not being used for a Joint Research Agreement.

Owner	Percent interest
THE JOHNS HOPKINS UNIVERSITY	100%
Total	**100%**

The owner(s) of percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending reference Application Number(s)

Application #	Filing Date

as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the reference application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.
In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said reference application, "as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application," in the event that any such patent granted on the pending reference application: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

The owner(s) of percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of prior patent number(s)

Patent #
9441044

as the term of said prior patent is presently shortened by any terminal disclaimer. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the prior patent are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.
In making the above disclaimer, the owner does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the prior patent, "as the term of said prior patent is presently shortened by any terminal disclaimer," in the event that said prior patent later:
- expires for failure to pay a maintenance fee;
- is held unenforceable;
- is found invalid by a court of competent jurisdiction;
- is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
- has all claims canceled by a reexamination certificate;
- is reissued; or
- is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

Terminal disclaimer fee under 37 CFR 1.20(d) included with Electronic Terminal Disclaimer request.

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

I certify, in accordance with 37 CFR 1.4(d)(4) that I am: An attorney or agent registered to practice before the Patent and Trademark Office who is of record in this application

Signature	Name	Registration #
/Pamela E. Hwang/	Pamela Hwang	54984

* Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner). Form PTO/SB/96 may be used for making this certification. See MPEP 324.

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ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/883,719	**12/23/2024 06:54:09 PM Z ET**	**JHU3260-10**

Title of Invention

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	6410	FILED BY	Karen LePari
PATENT CENTER #	68501247	AUTHORIZED BY	Pamela Hwang
CUSTOMER #	100899	FILING DATE	09/12/2024
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Atul Bedi

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
DA / 071896	**E2024BMI55036861**	**Pamela Hwang**

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
2814	STATUTORY DISCLAIMER, INCLUDING TERMINAL DISCLAIMER	170.00	1	170.00
			TOTAL AMOUNT:	**$170.00**

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

New Applications Under 35 U.S.C. 111

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

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

New International Application Filed with the USPTO as a Receiving Office

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

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APPROVAL LETTER

APPLICATION #	FILING DATE	APPLICANT/PATENT UNDER REEXAMINATION
18/883,719	09/12/2024	Atul Bedi

Title of Invention

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Electronic terminal disclaimer filed on 12/23/2024

☑ Approved

This patent is subject to a Terminal Disclaimer

Approved / Disapproved by: Electronic Terminal Disclaimer automatically approved

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/883,719	**12/23/2024 06:54:09 PM Z ET**	**JHU3260-10**

Title of Invention

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

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CONFIRMATION #	6410	FILED BY	Karen LePari
PATENT CENTER #	68501247	FILING DATE	09/12/2024
CUSTOMER #	100899	FIRST NAMED INVENTOR	Atul Bedi
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Pamela Hwang

Documents

TOTAL DOCUMENTS: 2

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
petition-request.pdf	3	Terminal Disclaimer-Filed (Electronic)	44 KB
grantLetter.pdf	1	Terminal Disclaimer-Electronic-Approved	18 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
petition-request.pdf	AD2EB9D219559B26F7A263E6318842F1802E1A9165AAA02F5 E1322D51C3AB32C8A3462AC0E5D74FEA51AAABB6D2358D0E 66F0F1B876CB5D5E1BF92D5B35975E4

grantLetter.pdf

5BF39B46E2CCBEBC02F82225C92A2DA67149FE2820D6F3827
23895D66DE516255BF70DB3729436CC7EF83FEC57CC2A814
CAB6A0A6279E77C2AABE084A6A76342

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New Applications Under 35 U.S.C. 111

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

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

New International Application Filed with the USPTO as a Receiving Office

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

PATENT
Attorney Docket No. JHU3260-10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	1644
Application No.:	18/883,719	Examiner:	Bridget E. Bunner
Filed:	September 12, 2024	Conf. No.:	6410
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

Mail Stop AMENDMENT
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RESPONSE TO NON-FINAL OFFICE ACTION

Sir:

In response to the Non-Final Office Action mailed December 3, 2024, please enter the following amendment and remarks in the above-identified application.

Amendments to the Claims are reflected in the listing of claims which begins on page **2** of this paper.

Remarks/Arguments begin on page **5** of this paper.

Amendments to the Claims

Please amend claims 1 and 2 as shown in the listing of claims.

Please cancel claims 3-8.

Please add new claims 9-15.

This listing of claims will replace all prior versions, and listings of claims in the application.

Listing of Claims:

1. (Currently Amended) An isolated molecule comprising ~~a targeting moiety fused with an immunomodulatory moiety, wherein~~:

(a) ~~the~~ a targeting moiety comprising [[es]] an antibody[[,]] or antibody fragment thereof, ~~scFv, or Fc-containing~~ comprising amino acids 1-119 of SEQ ID NO: 2 and the amino acid[[s]] sequence ~~1-107~~ of SEQ ID NO: 71, ~~and that~~ which specifically binds Epidermal Growth Factor Receptor (EGFR); and

(b) ~~the~~ an immunomodulatory moiety comprising [[es]] an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor II (TGF-βRII) comprising the amino acid sequence of SEQ ID NO: 87.

2. (Currently Amended) The molecule of claim 1, wherein the ~~molecule~~ targeting moiety comprises an antibody fragment selected from a Fab[[,]] or scFv ~~or Fc fragment a light chain, wherein the light chain comprises, from N-terminus to C-terminus, an amino acid sequence comprising SEQ ID NO: 71, a linker comprising SEQ ID NO: 104, and an amino acid sequence comprising SEQ ID NO: 87~~.

3-8. (Canceled)

9. (New) The molecule of claim 2, wherein the antibody fragment comprises a light chain domain or a variable heavy chain domain.

10. (New) The molecule of claim 9, wherein the antibody fragment comprises a light chain domain comprising the amino acid sequence of SEQ ID NO: 71.

11. (New) The molecule of claim 10, further comprising a polypeptide linker comprising the amino acid sequence of SEQ ID NO: 104.

12. (New) The molecule of claim 11, wherein the light chain domain comprising the amino acid sequence of SEQ ID NO: 71 is fused to an N-terminus of the polypeptide linker comprising the amino acid sequence of SEQ ID NO: 104.

13. (New) The molecule of claim 12, wherein the immunomodulatory moiety comprising the amino acid sequence of SEQ ID NO: 87 is fused to a C-terminus of the polypeptide linker comprising the amino acid sequence of SEQ ID NO: 104.

14. (New) The molecule of claim 9, wherein the variable heavy chain domain comprises amino acids 1-119 of SEQ ID NO: 2.

15. (New) An isolated molecule comprising:

(a) a targeting moiety comprising an antibody fragment which specifically binds Epidermal Growth Factor Receptor (EGFR) comprising a light chain domain comprising the amino acid sequence of SEQ ID NO: 71 and a variable heavy chain domain comprising amino acids 1-119 of SEQ ID NO: 2;

(b) an immunomodulatory moiety comprising an amino acid sequence of the extracellular domain of Transforming growth factor-beta receptor II (TGF-βRII) comprising the amino acid sequence of SEQ ID NO: 87; and

(c) a polypeptide linker comprising the amino acid sequence of SEQ ID NO: 104;

wherein the light chain domain comprising the amino acid sequence of SEQ ID NO: 71 is fused to an N-terminus of the polypeptide linker comprising the amino acid of SEQ ID NO: 104, and

wherein the immunomodulatory moiety comprising the amino acid sequence of SEQ ID NO: 87 is fused to a C-terminus of the polypeptide linker comprising the amino acid sequence of SEQ ID NO: 104.

REMARKS

Prior to this Communication, claims 1-9 were pending. With the present communication, claims 1 and 2 have been amended, claims 3-8 have been canceled without prejudice or disclaimer and new claims 9-15 have been added. Support for the claim amendments and new claims may be found generally throughout the application and claims as originally filed. No new matter is added. After entry of the amendments, claims 1-2 and 9-15 will be pending and under consideration.

Examiner's Interview

Applicant thanks Examiner Bunner for the telephone interview with Applicant's representatives on December 17, 2024. During the interview, proposed claim amendments were discussed to address clarity and indefiniteness rejections and the submission of a terminal disclaimer to address the obviousness type double patenting rejection were discussed. The Examiner stated that the proposed amendments and filing of a terminal disclaimer were reasonable to overcome the objections/rejections, but that such would be considered once the response is made of record.

Election/Restriction

The Examiner contacted Applicant's representative regarding a telephonic restriction requirement. The Examiner restricted the claims into two groups:

I. Claims 1-3 drawn to an isolated molecule comprising a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety comprises an antibody, scFv, or Fc-containing polypeptide that targets EGFR; and the immunomodulatory moiety comprises an amino acid sequence of extracellular domain of TGF-BRII; and

II. Claims 4-8 drawn to a method of treating cancer in a subject in need thereof comprising administering a therapeutically effective amount of a molecule comprising a targeting moiety fused with an immunomodulatory moiety,

wherein the targeting moiety comprises an antibody, scFv, or Fc-containing polypeptide that targets EGFR; and the immunomodulatory moiety comprises an amino acid sequence of extracellular domain of TGF-BRII.

During a telephone conversion with Applicant's representative on November 22, 2024, a provisional election to pursue the invention of Group I, claims 1-3, without traverse was made. Applicant affirms the election of Group I, claims 1-3 without traverse.

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

Claim Objections

The Examiner objected to claim 1 stating that the acronym "EGFR" is recited without first defining what it represents.

Applicant amended claim 1 to define "EGFR" as Epidermal Growth Factor Receptor". submits that this amendment renders the objection moot and respectfully requests withdrawal of the objection.

The Examiner objected to claim 2 not reciting a sequence identifier for the amino acid sequence of the linker and suggested that the claim be amended to recite "… linker comprising the amino acid sequence of SEQ ID NO: 104."

Applicant amended claim 2 to recite "wherein the targeting moiety comprises an antibody fragment selected from a Fab or scFv." Applicant submits that these amendments render the objection moot and respectfully request withdrawal of the objection.

Rejections under 35 USC § 112, second paragraph

Claims 2 and 3 stand rejected under 35 USC § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. Applicant respectfully traverses this rejection.

The Examiner states that claims 2 and 3 are indefinite because the elements comprising the isolated molecule are unclear, as well as the structure of the molecule.

As an initial matter, claim is canceled. Applicant amended claim 2 to recite "wherein the targeting moiety comprises an antibody fragment selected from a Fab or scFv." Applicant submits that these amendments and cancelations render the rejection moot and respectfully requests reconsideration and withdraw of the rejection.

Double Patenting Rejection

Claims 1-3 are provisionally rejected on the ground of non-statutory obviousness-type double patenting over claims 1-9 of U.S. Patent No. 9,441,044. Applicants respectfully traverse this rejection.

Without acquiescing to the Examiner's assertion and to expedite prosecution of the claimed invention, a terminal disclaimer is hereby submitted together with this paper to overcome the non-statutory obviousness-type double patenting rejections over U.S. Patent No. 9,441,044. Accordingly, withdrawal of the double patenting rejection is respectfully requested.

Claims 1-3 are provisionally rejected on the ground of non-statutory obviousness-type double patenting over claims 1-12 of U.S. Patent Appl. Serial No. 18/811,627 in view of Huang et al. (App Environ Microbiol 72(12):7748-7759, 2006). Applicants respectfully traverse this rejection.

Without acquiescing to the Examiner's assertion and to expedite prosecution of the claimed invention, a terminal disclaimer is hereby submitted together with this paper to overcome the non-statutory obviousness-type double patenting rejections over U.S. Patent Appl. Serial No. 18/811,627. Accordingly, withdrawal of the double patenting rejection is respectfully requested.

CONCLUSION

In view of the amendments and above remarks, it is submitted that the claims are in condition for allowance, and a notice to that effect is respectfully requested. The Examiner is invited to contact Applicant's undersigned representative if there are any questions relating to this application.

No fee is believed due in connection with this submission. The Commissioner is hereby authorized to charge any additional fees required by this submission, or make any credits or overpayments, to Deposit Account No. 07-1896 referencing the above-identified attorney docket number.

Respectfully submitted,

Date: December 23, 2024

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/883,719	**12/23/2024 07:18:32 PM Z ET**	**JHU3260-10**

Title of Invention

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	6410	FILED BY	Karen LePari
PATENT CENTER #	68501957	FILING DATE	09/12/2024
CUSTOMER #	100899	FIRST NAMED INVENTOR	Atul Bedi
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Pamela Hwang

Documents

TOTAL DOCUMENTS: 3

DOCUMENT		PAGES	DESCRIPTION	SIZE (KB)
JHU3260-1 RspToNFOA 23DEC2024.pdf		8	-	169 KB
JHU3260-1 RspToNFOA 23DEC2024-A....pdf	(1-1)	1	Amendment/Request for Reconsideration-After Non-Final Rejection	108 KB
JHU3260-1 RspToNFOA 23DEC2024-CLM.pdf	(2-4)	3	Claims	154 KB
JHU3260-1 RspToNFOA 23DEC2024-REM.pdf	(5-8)	4	Applicant Arguments/Remarks Made in an Amendment	118 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
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JHU3260-1 RspToNFOA 23DEC2024-A....pdf	69585FD50527E595C3ED7DB41F820DD8F7EC5023B0A3B93F4 D661C53D6D78186CAE554CA78DDF79F06ECB644BB57D8D4 E1437D2C5F9007D806052074D59133BA
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JHU3260-1 RspToNFOA 23DEC2024-REM.pdf	DDAB5F168E01EADF06672C72F95E03736258BB033CEB7BAD 363EB1D4AA8AF6E7C855A4C1E1F99BF0079BD986250AA9CA C6B5A5EF6BE51672A63943746F88A7AB

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
18/883,719	09/12/2024	Atul Bedi	JHU3260-10

CONFIRMATION NO. 6410

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

PUBLICATION NOTICE

OC00000000828515 29

Date Mailed: 01/10/2025

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

Publication No.US-2025-0009772-A1
Publication Date:01/09/2025

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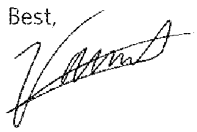

Vaishali Udupa
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Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/883,719	Bedi et al.
	Examiner	**Art Unit**
	BRIDGET E BUNNER	1647

CPC						
Symbol					**Type**	**Version**
A61K	/	31	/	704	F	2013-01-01
C07K	/	14	/	70532	I	2013-01-01
C07K	/	14	/	70575	I	2013-01-01
C07K	/	14	/	70578	I	2013-01-01
C07K	/	14	/	70596	I	2013-01-01
C07K	/	14	/	71	I	2013-01-01
C07K	/	16	/	28	I	2013-01-01
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C07K	/	2317	/	526	A	2013-01-01
C07K	/	2319	/	70	A	2013-01-01
A61K	/	2039	/	572	A	2013-01-01

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	9	
/BRIDGET E BUNNER/ Primary Examiner, Art Unit 1647	10 January 2025	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

U.S. Patent and Trademark Office — Part of Paper No.: 20250110

Bicara Ex. 1002, Page 445 of 482
Bicara v. JHU - PGR2026-00025

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/883,719	Bedi et al.
	Examiner	**Art Unit**
	BRIDGET E BUNNER	1647

CPC Combination Sets					
Symbol		**Type**	**Set**	**Ranking**	**Version**
	/ /				

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	9	
/BRIDGET E BUNNER/ Primary Examiner, Art Unit 1647	10 January 2025	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

U.S. Patent and Trademark Office — Part of Paper No.: 20250110

Bicara Ex. 1002, Page 446 of 482
Bicara v. JHU - PGR2026-00025

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/883,719	Bedi et al.
	Examiner	**Art Unit**
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INTERNATIONAL CLASSIFICATION				
CLAIMED				
C07K	/	14	/	71
C07K	/	16	/	28
C07K	/	16	/	30
C07K	/	19	/	00

NON-CLAIMED				
A61K	/	39	/	395
A61K	/	38	/	18

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	9	
/BRIDGET E BUNNER/ Primary Examiner, Art Unit 1647	10 January 2025	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

U.S. Patent and Trademark Office — Part of Paper No.: 20250110

Bicara Ex. 1002, Page 447 of 482
Bicara v. JHU - PGR2026-00025

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/883,719	Bedi et al.
	Examiner	**Art Unit**
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☑ Claims renumbered in the same order as presented by applicant ☐ CPA ☑ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	9	
/BRIDGET E BUNNER/ Primary Examiner, Art Unit 1647	10 January 2025	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

U.S. Patent and Trademark Office Part of Paper No.: 20250110

Bicara Ex. 1002, Page 448 of 482
Bicara v. JHU - PGR2026-00025

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/883,719	Bedi et al.
	Examiner	Art Unit
	BRIDGET E BUNNER	1647

CPC - Searched*		
Symbol	**Date**	**Examiner**
C07K16/2863; C07K14/71; C07K19/00; C07K2319/00; C07K2319/31 ; C07K2319/32; C07K2319/33; C07K2319/35; C07K14/70596; C07K14/70578; C07K14/70575; C07K16/2866; C07K16/30; A61K39/3955; A61K2039/505; A61K38/1793; A61K38/179	11/27/2024; updated 01/11/2025	BEB

CPC Combination Sets - Searched*		
Symbol	**Date**	**Examiner**

US Classification - Searched*			
Class	**Subclass**	**Date**	**Examiner**

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	**Date**	**Examiner**
PE2E (JPO, EPO, USPAT, DERWENT); US-PGPUB; DAV (inventors ' names); ProQuest Dialog; see search history printout	11/27/2024	BEB
STIC search SEQ ID NO: 2, 71, 87; see search results in SCV/DAV	11/26/2024	BEB
ProQuest Dialog; see search history printout	01/11/2025	BEB

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Bicara v. JHU - PGR2026-00025

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/883,719	Bedi et al.
	Examiner	Art Unit
	BRIDGET E BUNNER	1647

Interference Search			
US Class/CPC Symbol	**US Subclass/CPC Group**	**Date**	**Examiner**
	PE2E (USPAT, US-PGPUV); DAV (inventors' names); see search history printout	01/11/2025	BEB
	STIC search SEQ ID NOs: 2, 71, 87, 104; see search results in SCV/DAV	01/10/2025	BEB

Bicara Ex. 1002, Page 450 of 482
Bicara v. JHU - PGR2026-00025

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L51	23	((("BEDI") near3 ("Atul")) OR (("RAVI") near3 ("Rajani"))).INV.	(US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 11:40 AM
L52	6846	((("The") near3 ("Johns") near3 ("Hopkins") near3 ("University"))).AS,AANM.	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 11:40 AM
L53	6846	((("The") near3 ("Johns") near3 ("Hopkins") near3 ("University"))).AS,AANM.	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 11:40 AM
L54	126275	EGFR OR ERBB1 OR ERBB-1 OR HER1 OR (epidermal adj2 growth adj2 factor adj2 receptor)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 11:40 AM
L55	2291	(transforming adj2 growth adj2 factor adj2 beta adj2 receptor adj2 (2 OR II)) OR TGFbetaRII OR TGF-betaRII OR TGFR2 OR TGFR-2 OR TGF-βRII OR TGFβRII	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 11:40 AM
L56	7304	(transforming adj2 growth adj2 factor adj3 receptor) OR TGFR	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 11:40 AM
L57	46	L51 AND L54	(US-PGPUB; USPAT; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:06 PM
L58	18	L51 AND L54	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:06 PM
L59	508	L53 AND L54	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:07 PM

L60	21	L53 AND L54 AND L55	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:09 PM
L61	44	L53 AND L54 AND L56	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:10 PM
L63	0	L54 SAME (L55 OR L56) SAME (fusion OR fused OR fuse OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:13 PM
L64	0	L54 SAME2 (L55 OR L56) SAME2 (fusion OR fused OR fuse OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:14 PM
L65	2291	TGFbetaRII OR TGF-betaRII OR TGFR2 OR TGFR-2 OR TGF-βRII OR TGFβRII	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:15 PM
L66	199	L54 SAME L65 SAME (fusion OR fused OR fuse OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:15 PM
L67	657	L54 SAME L56 SAME (fusion OR fused OR fuse OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:15 PM
L68	20775	anti-EGFR OR anti-ERBB1 OR anti-ERBB-1 OR anti-HER1	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:22 PM
L69	23336	(antibody OR antibodies) near3 (EGFR OR ERBB1 OR ERBB-1 OR HER1)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:22 PM
L70	13	(L68 OR L69) SAME L65 SAME (fusion OR fused OR fuse OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:23 PM
L71	81	(L68 OR L69) SAME2 L65 SAME2 (fusion OR fused OR fuse OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:32 PM
L72	94	(L68 OR L69) SAME3 L65 SAME (fusion OR fused OR fuse OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:32 PM
L73	175352	(C07K16/2863 OR C07K14/71 OR	(US-PGPUB; USPAT; EPO; JPO;	OR	ON	ON	2025/01/11 12:32 PM

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Bicara v. JHU - PGR2026-00025

		C07K19/00 OR C07K2319/00 OR C07K2319/31 OR C07K2319/32 OR C07K2319/33 OR C07K2319/35 OR C07K14/70596 OR C07K14/70578 OR C07K14/70575 OR C07K16/2866 OR C07K16/30 OR A61K39/3955 OR A61K2039/505 OR A61K38/1793 OR A61K38/179).cpc.	DERWENT)				
L74	78	L72 AND L73	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:33 PM
L75	130	cetuximab same3 (fuse OR fusion OR fused OR join OR joined OR link OR linked OR conjugat$) same3 L65	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:35 PM
L76	25	cetuximab SAME (fuse OR fusion OR fused OR join OR joined OR link OR linked OR conjugat$) SAME L65	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:35 PM
L77	94	L75 AND L73	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:35 PM
L78	18	(L68 OR L69) SAME L56 SAME (fusion OR fused OR fuse OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:36 PM
L79	144	(L68 OR L69) SAME3 L56 SAME3 (fusion OR fused OR fuse OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:36 PM
L80	64	L79 AND L73	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:36 PM
L81	162	(L68 OR L69) SAME5 L56 SAME5 (fusion OR fused OR fuse OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:37 PM
L82	70	L81 AND L73	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:37 PM
L83	16	L51 AND (L55 OR L56)	(US-PGPUB; USPAT; EPO; JPO;	OR	ON	ON	2025/01/11 12:42 PM

			DERWENT)				
L84	16	L51 AND (L55 OR L56) AND L54	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:42 PM
L85	47	L53 AND (L55 OR L56) AND L54	(US-PGPUB; USPAT; EPO; JPO; DERWENT)	OR	ON	ON	2025/01/11 12:42 PM

PE2E SEARCH - Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
N1	18	((("BEDI") near3 ("Atul")) OR (("RAVI") near3 ("Rajani"))).INV.	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:13 PM
N2	6846	((("The") near3 ("Johns") near3 ("Hopkins") near3 ("University"))).AS,AANM.	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:13 PM
N3	106787	EGFR OR ERBB1 OR ERBB-1 OR HER1 OR (epidermal adj2 growth adj2 factor adj2 receptor)	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:14 PM
N4	2223	TGFbetaRII OR TGF-betaRII OR TGFR2 OR TGFR-2 OR TGF-βRII OR TGFβRII	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:14 PM
N5	6199	(transforming adj2 growth adj2 factor adj3 receptor) OR TGFR	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:14 PM
N6	18	N1 AND N3	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:23 PM
N7	16	N1 AND (N4 OR N5)	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:23 PM
N8	16	N1 AND (N4 OR N5) AND N3	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:23 PM
N9	508	N2 AND N3	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:23 PM
N10	73	N2 AND (N4 OR N5)	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:23 PM
N11	47	N2 AND (N4 OR N5) AND N3	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:23 PM
N12	686	N3 SAME (N4 OR N5) SAME (fusion OR fused OR fuse OR join OR joined OR link OR linked OR conjugat$)	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:24 PM
N13	19750	anti-EGFR OR anti-ERBB1 OR anti-ERBB-1 OR anti-HER1	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:25 PM

N14	21728	(antibody OR antibodies) near3 (EGFR OR ERBB1 OR ERBB-1 OR HER1)	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:25 PM
N15	13	(N13 OR N14) SAME N4 SAME (fusion OR fused OR fuse OR join OR joined OR link OR linked OR linker OR conjugat$)	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:26 PM
N16	81	(N13 OR N14) SAME2 N4 SAME2 (fusion OR fused OR fuse OR join OR joined OR link OR linked OR linker OR conjugat$)	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:27 PM
N17	104	(N13 OR N14) SAME3 N4 SAME3 (fusion OR fused OR fuse OR join OR joined OR link OR linked OR linker OR conjugat$)	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:27 PM
N18	104466	(C07K16/2863 OR C07K14/71 OR C07K19/00 OR C07K2319/00 OR C07K2319/31 OR C07K2319/32 OR C07K2319/33 OR C07K2319/35 OR C07K14/70596 OR C07K14/70578 OR C07K14/70575 OR C07K16/2866 OR C07K16/30 OR A61K39/3955 OR A61K2039/505 OR A61K38/1793 OR A61K38/179).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:27 PM
N19	88	N17 AND N18	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:28 PM
N20	69	N16 AND N18	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:28 PM
N21	130	cetuximab same3 (fuse OR fusion OR fused OR join OR joined OR link OR linked OR conjugat$) same3 N4	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:29 PM
N22	94	N21 AND N18	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:29 PM
N23	25	cetuximab SAME (fuse OR fusion OR fused OR join OR joined OR link OR linked OR conjugat$) SAME N4	(US-PGPUB; USPAT)	OR	ON	ON	2025/01/11 01:29 PM

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Search Strategy from Dialog

January 11 2025 17:34

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Search Strategy

Databases: ABI/INFORM® Professional Advanced, Abstracts in New Technology & Engineering, AdisInsight: Drugs, AdisInsight: Trials, Adis Pharmacoeconomics & Outcomes News, AGRICOLA, AGRIS, Allied & Complementary Medicine™, Analytical Abstracts, APA PsycInfo®, Aqualine, Aquatic Science & Fisheries Abstracts (ASFA), Australian Education Index, BIOSIS® Toxicology, BIOSIS Previews®, British Library Inside Conferences, British Nursing Index, Business & Industry, CAB ABSTRACTS, Chemical Business Newsbase, Chemical Engineering & Biotechnology Abstracts, Chemical Safety Newsbase, Civil Engineering Abstracts, ClinicalTrials.gov, Current Contents® Search, Derwent Drug File, Derwent Drug Registry, DH-DATA: Health Administration, Medical Toxicology & Environmental Health, DIOGENES® FDA Regulatory Updates, Drug Information Fulltext, Earthquake Engineering Abstracts, EconLit, Ei Compendex®, Embase®, Embase® French Local Literature, Embase Preprints, EMCare®, ERIC, ESPICOM Pharmaceutical & Medical Device News, FDAnews, FLUIDEX (Fluid Engineering Abstracts), Foodline®: MARKET, Foodline®: PRODUCT, Foodline®: SCIENCE, FSTA®, Gale Group Computer Database™, Gale Group Health Periodicals Database, Gale Group New Product Announcements / Plus®, Gale Group Newsletter Database™, Gale Group PharmaBiomed Business Journals, Gale Group PROMT®, Gale Group Trade & Industry Database™, GEOBASE□, GeoRef, Global Health, HSELINE: Health and Safety, ICONDA - International Construction Database, IMS Company Profiles, IMS New Product Focus, IMS Pharma Trademarks, IMS R&D Focus, IMS R&D Focus Drug News, Inspec®, International Pharmaceutical Abstracts, Jane's Defense & Aerospace News, King's Fund, KOSMET: Cosmetic Science, Lancet Titles, Mechanical & Transportation Engineering Abstracts, MEDLINE®, Meteorological & Geoastrophysical Abstracts, Morressier Life Science Conference Abstracts and Posters, New England Journal of Medicine, Northern Light Life Sciences Conference Abstracts, NTIS: National Technical Information Service, Oceanic Abstracts, PAIS International, Paperbase, PAPERCHEM, ProQuest Advanced Tech & Aerospace Professional, ProQuest Biological & Health Science Professional, ProQuest Dissertations and Theses Professional, ProQuest Environmental Science Professional, ProQuest Materials Research Professional, ProQuest Newsstand Professional, ProQuest Technology Research Professional, Prous Science Daily Essentials, Prous Science Drug Data Report, Prous Science Drugs Of The Future™, Publicly Available Content, Registry of Toxic Effects of Chemical Substances (RTECS®), SciSearch®: a Cited Reference Science Database, Social SciSearch®, ToxFile®, Transport Research International Documentation, TULSA™ (Petroleum Abstracts), UBM Computer Full Text, Weldasearch®, Zoological Record

Set#	Searched for	Results
S1	au.exact("bedi, atul" OR "bedi, a" OR "bedi, a.")	326°
S2	au.exact("ravi, r." OR "ravi, r. k." OR "ravi, r.; r" OR "ravi, r.k." OR "ravi, rajani" OR "ravi, rajani k" OR "ravi, rajani k.")	7054*
S3	EGFR or ERBB1 or ERBB-1 or HER1 or (epidermal pre/2 growth pre/2 factor pre/2 receptor)	1476821*
S4	(transforming pre/2 growth pre/2 factor pre/2 beta pre/2 receptor pre/2 (2 or II)) or TGFbetaRII or TGF-betaRII or TGFR2 or TGFR-2 or TGF-βRII or TGFβRII	19335*
S5	(transforming pre/2 growth pre/2 factor pre/3 receptor) or TGFR	60230*
S6	anti-EGFR or anti-ERBB1 or anti-ERBB-1 or anti-HER1	62979*
S7	(antibody or antibodies) pre/3 (EGFR or ERBB1 or ERBB-1 or HER1)	19675*

S8	(s1 or s2) and s3	16°
S9	(s1 or s2) and (s4 or s5)	1°
S10	(s1 or s2) and (s6 or s7)	3°
S11	(Johns pre/3 Hopkins) and s3	27482*
S12	(Johns pre/3 Hopkins) and (s6 or s7)	932°
S13	(Johns pre/3 Hopkins) and (s6 or s7) and (s4 or s5)	57°
S14	((Johns pre/3 Hopkins) and (s6 or s7) and (s4 or s5)) and (pd(19910101-20110301))	22°
S15	(s6 or s7) pre/50 (s4 or s5)	15°
S16	((s6 or s7) pre/50 (s4 or s5)) and (pd(2001-2002))	3°
S17	(s6 or s7) pre/100 (s4 or s5) pre/100 (fuse or fused or fusion or joined or join or linked or conjugat*)	3°
S18	cetuximab pre/100 (s4 or s5) pre/100 (fuse or fused or fusion or joined or join or linked or conjugat*)	1°
S19	cetuximab pre/100 (s4 or s5)	34°
S20	(cetuximab pre/100 (s4 or s5)) and (pd(2001-2011))	7°

* Duplicates are removed from the search, but included in the result count.
° Duplicates are removed from the search and from the result count.

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NOTICE OF ALLOWANCE AND FEE(S) DUE

100899 7590 01/29/2025

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

EXAMINER	
BUNNER, BRIDGET E	
ART UNIT	PAPER NUMBER
1647	

DATE MAILED: 01/29/2025

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/883,719	09/12/2024	Atul Bedi	JHU3260-10	6410

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

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$516	$0.00	$0.00	$516	04/29/2025

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 40% the amount of undiscounted fees, and micro entity fees are 20% the amount of undiscounted fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PTOL-85 (Rev. 11/23)

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via the USPTO patent electronic filing system.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

100899 7590 01/29/2025
The John Hopkins University
c/o DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via the USPTO patent electronic filing system or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/883,719	09/12/2024	Atul Bedi	JHU3260-10	6410

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

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$516	$0.00	$0.00	$516	04/29/2025

EXAMINER	ART UNIT	CLASS-SUBCLASS
BUNNER, BRIDGET E	1647	424-134100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1_____
2_____
3_____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. Fees submitted: ❑Issue Fee ❑Publication Fee (if required)

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via the USPTO patent electronic filing system ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

❑ Applicant asserting small entity status. See 37 CFR 1.27

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

❑ Applicant changing to regular undiscounted fee status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

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Bicara v. JHU - PGR2026-00025

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/883,719	09/12/2024	Atul Bedi	JHU3260-10	6410

100899 7590 01/29/2025

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

EXAMINER	
BUNNER, BRIDGET E	
ART UNIT	**PAPER NUMBER**
1647	

DATE MAILED: 01/29/2025

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

PTOL-85 (Rev. 11/23)

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. The United States Patent and Trademark Office (USPTO) collects the information in this record under authority of 35 U.S.C. 2. The USPTO's system of records is used to manage all applicant and owner information including name, citizenship, residence, post office address, and other information with respect to inventors and their legal representatives pertaining to the applicant's/ owner's activities in connection with the invention for which a patent is sought or has been granted. The applicable Privacy Act System of Records Notice for the information collected in this form is COMMERCE/PAT-TM-7 Patent Application Files, available in the Federal Register at 78 FR 19243 (March 29, 2013).
https://www.govinfo.gov/content/pkg/FR-2013-03-29/pdf/2013-07341.pdf

Routine uses of the information in this record may include disclosure to:

1) law enforcement, in the event that the system of records indicates a violation or potential violation of law;
2) a federal, state, local, or international agency, in response to its request;
3) a contractor of the USPTO having need for the information in order to perform a contract;
4) the Department of Justice for determination of whether the Freedom of Information Act (FOIA) requires disclosure of the record;
5) a Member of Congress submitting a request involving an individual to whom the record pertains, when the individual has requested the Member's assistance with respect to the subject matter of the record;
6) a court, magistrate, or administrative tribunal, in the course of presenting evidence, including disclosures to opposing counsel in the course of settlement negotiations;
7) the Administrator, General Services Administration (GSA), or their designee, during an inspection of records conducted by GSA under authority of 44 U.S.C. 2904 and 2906, in accordance with the GSA regulations and any other relevant (i.e., GSA or Commerce) directive, where such disclosure shall not be used to make determinations about individuals;
8) another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c));
9) the Office of Personnel Management (OPM) for personnel research purposes; and
10) the Office of Management and Budget (OMB) for legislative coordination and clearance.

If you do not furnish the information requested on this form, the USPTO may not be able to process and/or examine your submission, which may result in termination of proceedings, abandonment of the application, and/or expiration of the patent.

Notice of Allowability	Application No. 18/883,719	Applicant(s) Bedi et al.	
	Examiner BRIDGET E BUNNER	Art Unit 1647	AIA (FITF) Status No

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

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to the response of 23 December 2024.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

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

3. ☑ The allowed claim(s) is/are 1-2 and 9-15 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information , please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

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

   Certified copies:

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☑ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/BRIDGET E BUNNER/
Primary Examiner, Art Unit 1647

Bicara Ex. 1002, Page 463 of 482
Bicara v. JHU - PGR2026-00025

DETAILED ACTION

Notice of Pre-AIA or AIA Status

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

Status of Application, Amendments and/or Claims

The amendment of 23 December 2024 has been entered in full. Claims 1 and 2 are amended. Claims 3-8 are cancelled. Claims 9-15 are added.

Claims 1, 2, and 9-15 are under consideration in the instant application.

Withdrawn Objections and/or Rejections

1. The objections to claims 1 and 2 as set forth at page 6 of the previous Office Action of 03 December 2024 are *withdrawn* in view of the amended claims (23 December 2024).

2. The rejection of claims 2 and 3 under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as set forth at pages 6-7 of the previous Office Action of 03 December 2024 is *withdrawn* in view of amended claim 2 and cancelled claim 3 (23 December 2024).

3. The rejection of claims 1-3 on the ground of nonstatutory double patenting as being unpatentable over claims 1-9 of U.S. Patent No. 9,441,044 as set forth at pages 9-10 of the previous Office Action of 03 December 2024 is *withdrawn* in view of the submission of a terminal disclaimer (23 December 2024).

4. The provisional rejection of claims 1-3 on the ground of nonstatutory double patenting as being unpatentable over claims 1-12 of copending Application No. 18/811,627 in view of Huang et al. (App Environ Microbiol 72(12): 7748-7759, 2006) as set forth at pages 10-14 of the previous Office Action of 03 December 2024 is *withdrawn* in view of the submission of a terminal disclaimer (23 December 2024).

Conclusion

Claims 1, 2, and 9-15 are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to BRIDGET E BUNNER whose telephone number is (571)272-0881. The examiner can normally be reached Monday-Friday 9:00 am-6:00 pm.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

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

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: https://patentcenter.uspto.gov. Visit https://www.uspto.gov/patents/apply/patent-center for more information about Patent Center and https://www.uspto.gov/patents/docx for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

BEB
Art Unit 1647
10 January 2025

/BRIDGET E BUNNER/
Primary Examiner, Art Unit 1647

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/883,719	09/12/2024	Atul Bedi	JHU3260-10	6410

7590 02/10/2025

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

EXAMINER	
BUNNER, BRIDGET E	
ART UNIT	PAPER NUMBER
1647	
NOTIFICATION DATE	DELIVERY MODE
02/10/2025	ELECTRONIC

NOTICE REQUIRING INVENTOR'S OATH OR DECLARATION

❑ An inventor's oath or declaration in compliance with 37 CFR 1.63 or substitute statement in compliance with 37 CFR 1.64 executed by or with respect to each inventor has not yet been submitted.

The inventor's oath or declaration or substitute statement dated 09/12/2024 is defective. The inventor's oath or declaration does not comply with 37 CFR 1.63 or the substitute statement does not comply with 37 CFR 1.64 because it:

❑ does not include a statement that the person executing the oath or declaration or substitute statement believes the named inventor or joint inventor to be the original inventor or an original joint inventor of a claimed invention in the application for which the oath or declaration is being submitted.

❑ does not state that the application was made or was authorized to be made by the person executing the oath or declaration or substitute statement.

❑ does not acknowledge that any willful false statement made in such oath or declaration or substitute statement is punishable under section 1001 of title 18 by fine or imprisonment of not more than 5 years, or both.

☒ does not identify the person executing the oath or declaration or substitute statement by his or her legal name.

❑ does not identify the application to which the oath or declaration or substitute statement is directed.

☒ Other: Pleasen verify the inventors legal name the Oath list Rajani Ravil and the ADS list Rajani Ravi

The substitute statement dated _____ does not comply with 37 CFR 1.64 because it:

❑ does not identify the inventor with respect to whom the statement applies.

❑ does not identify the relationship to the non-signing inventor.

❑ does not identify the circumstances permitting execution of the statement.

❑ was signed by someone other than the applicant.

❑ Other: _____

An oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each inventor MUST be filed **no later than the date on which the issue fee is paid.** See 35 U.S.C. 115(f). Failure to timely comply will result in ABANDONMENT of this application. This period for reply is not extendable under 37 CFR 1.136(a).

Questions relating to this Notice should be directed to the Application Assistance Unit at 571-272-4200.

(571)-272-4200 or 1(888)-786-0101
Patent Publication Branch
Office of Data Management

PTO/AIA/01 (06-12)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

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

As the below named inventor, I hereby declare that:

This declaration is directed to:

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

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

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

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

WARNING:

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

LEGAL NAME OF INVENTOR

Inventor: Rajani RAVI Date (Optional): _____

Signature: Rajani Ravi

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

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/883,719	09/12/2024	Atul Bedi	JHU3260-10	6410

7590 02/10/2025

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

EXAMINER	
BUNNER, BRIDGET E	
ART UNIT	PAPER NUMBER
1647	
NOTIFICATION DATE	DELIVERY MODE
02/10/2025	ELECTRONIC

NOTICE REQUIRING INVENTOR'S OATH OR DECLARATION

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❑ does not include a statement that the person executing the oath or declaration or substitute statement believes the named inventor or joint inventor to be the original inventor or an original joint inventor of a claimed invention in the application for which the oath or declaration is being submitted.

❑ does not state that the application was made or was authorized to be made by the person executing the oath or declaration or substitute statement.

❑ does not acknowledge that any willful false statement made in such oath or declaration or substitute statement is punishable under section 1001 of title 18 by fine or imprisonment of not more than 5 years, or both.

☒ does not identify the person executing the oath or declaration or substitute statement by his or her legal name.

❑ does not identify the application to which the oath or declaration or substitute statement is directed.

☒ Other: Pleasen verify the inventors legal name the Oath list Rajani Ravil and the ADS list Rajani Ravi

The substitute statement dated _____ does not comply with 37 CFR 1.64 because it:

❑ does not identify the inventor with respect to whom the statement applies.

❑ does not identify the relationship to the non-signing inventor.

❑ does not identify the circumstances permitting execution of the statement.

❑ was signed by someone other than the applicant.

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/883,719	**02/11/2025 08:19:45 PM Z ET**	**JHU3260-10**

Title of Invention

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	6410	FILED BY	Karen LePari
PATENT CENTER #	69145270	FILING DATE	09/12/2024
CUSTOMER #	100899	FIRST NAMED INVENTOR	Atul Bedi
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Pamela Hwang

Documents

TOTAL DOCUMENTS: 3

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
JHU3260-10 TRANSMITTAL 11FEB2025.pdf	1	Transmittal Letter	110 KB
JHU3260-10 NTC REQUIRING INVENTOR OATH OR DECLARATION.pdf	1	Miscellaneous Incoming Letter	58 KB
JHU3260-10 INVENTOR RAVI DECLARATION FROM PARENT.pdf	1	Oath or Declaration filed	59 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
JHU3260-10 TRANSMITTAL 11FEB2025.pdf	BA03C1AF43BF90BC94AC03124CC7FF3128781D2F59FE9F8F6346DF70CA5DAB69B1490E4D3171D09BD8881B9FA0360008289CF3B34BE237ABF0478EAFD27213A0
JHU3260-10 NTC REQUIRING INVENTOR OATH OR DECLARATION.pdf	79F12423664DA071398034E907144FF4D1D6F2FFBB1CD4FC9623195E44CC01D31A714A0B2FE81227FEDF0B792AADCC0AA6B0D5EC27BE997D7D13AFD8E4321A65
JHU3260-10 INVENTOR RAVI DECLARATION FROM PARENT.pdf	D06669088FCC2D1F99D5411CDF02694649C2EC266D3DF06649401DBE8A564FB1769C4A27ABB41B91511B58EF13CAE99A081B16DE6C1570BC522E157B7D8DEF7C

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

PATENT
Attorney Docket No. JHU3260-10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Bedi and Ravi	Art Unit:	1647
Application No.:	18/883,719	Examiner:	Bridget E. Bunner
Filing Date:	September 12, 2024	Conf. No.	6410
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

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

TRANSMITTAL LETTER

Dear Sir:

Transmitted herewith in Response to the Notice Requiring Inventor's Oath or Declaration for the above-identified application:

- Notice Requiring Inventor's Oath or Declaration (1 page) and
- Executed Declaration for inventor Rajani Ravi (1 page).

No fee is deemed necessary however, the Commissioner is hereby authorized to charge any other fees associated with the filing submitted herewith or credit any overpayments to Deposit Account No. 07-1896 referencing the above-identified attorney docket number.

Respectfully submitted,

Date: February 11, 2025

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

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

1617134098.1
331323.001436

UNITED STATES
PATENT AND TRADEMARK OFFICE

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/883,719	**04/11/2025 07:28:26 PM Z ET**	**JHU3260-10**

Title of Invention

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	6410	FILED BY	Aldon Griffis
PATENT CENTER #	69976255	FILING DATE	09/12/2024
CUSTOMER #	100899	FIRST NAMED INVENTOR	Atul Bedi
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	-

Documents

TOTAL DOCUMENTS: 3

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
JHU3260-10 Issue Fee Transmittal EFS.pdf	1	Transmittal Letter	113 KB
JHU3260-10 Issue Fee Response EFS.pdf	1	Transmittal Letter	112 KB
JHU3260-10 Issue Fee Form PTOL-85b.pdf	1	Issue Fee Payment (PTO-85B)	118 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
JHU3260-10 Issue Fee	33983180DB12FE4DEC20C1BD44C5081178BD49E6EADCBCF

Transmittal EFS.pdf	E524746E129DCDD846A2FF8E4D923FB5A98ABE7B53ECCAC33E3AF796FF36E41BBE519E06D16220B7A
JHU3260-10 Issue Fee Response EFS.pdf	1E02555FB0D7BB450EED6BA12F6CE186B9917D4264087A899DBB50A2F835852581A51F8D289855E76B4416ADD5E5C9BC076929F22CF80485D44CF7A0AD360D8D
JHU3260-10 Issue Fee Form PTOL-85b.pdf	8F22C36F4C55C4E5C7EAE029170A660BED8B174D2033D486A82F52D23F2300CDE29A3B3DC291ACD3F962A822164D5F7533C6BB7E17158470E1852CFD011D6FF8

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

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

New International Application Filed with the USPTO as a Receiving Office

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

PATENT
Attorney Docket No.: JHU3260-10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	1644
Application No.:	18/883,719	Examiner:	B.E. Bunner
Filed:	September 12, 2024	Conf. No.:	6410
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

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

RESPONSE TO NOTICE OF ALLOWANCE

Sir:

In response to the Notice of Allowance mailed January 29, 2025, attached is the completed Issue Fee Transmittal Form PTOL-85B.

The Commissioner is hereby authorized to charge $516.00 to Deposit Account No. 07-1896 consisting of $516.00 for the required Issue Fee payment. The Commissioner is also authorized to charge any other fees associated with the filing submitted herewith or credit any overpayment to the aforementioned Deposit Account.

Respectfully submitted,

Date: April 11, 2025

/Pamela E. Hwang/
Pamela E. Hwang, J.D., Ph.D.
Registration No. 54,984

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

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

1616850814.2
331323.001436

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via the USPTO patent electronic filing system.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

100899 7590 01/29/2025
The John Hopkins University
c/o DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via the USPTO patent electronic filing system or by facsimile to (571) 273-2885, on the date below.

Aldon Griffis	(Typed or printed name)
/Aldon Griffis/	(Signature)
April 11, 2025	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/883,719	09/12/2024	Atul Bedi	JHU3260-10	6410

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

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$516	$0.00	$0.00	$516	04/29/2025

EXAMINER	ART UNIT	CLASS-SUBCLASS
BUNNER, BRIDGET E	1647	424-134100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 DLA Piper LLP (US)
2
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: The Johns Hopkins University

(B) RESIDENCE: (CITY and STATE OR COUNTRY): Baltimore, Maryland

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. Fees submitted: ☒ Issue Fee ☐ Publication Fee (if required)

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

☒ Electronic Payment via the USPTO patent electronic filing system ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)

☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. 07-1896

5. **Change in Entity Status** (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

☐ Applicant asserting small entity status. See 37 CFR 1.27

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

☐ Applicant changing to regular undiscounted fee status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Pamela E. Hwang/ Date April 11, 2025

Typed or printed name Pamela E. Hwang, J.D., Ph.D. Registration No. 54,984

PTOL-85 Part B (11/23) Approved for use through 03/31/2026 OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
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ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/883,719	**04/11/2025 07:28:26 PM Z ET**	**JHU3260-10**

Title of Invention

COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	6410	FILED BY	Aldon Griffis
PATENT CENTER #	69976255	AUTHORIZED BY	-
CUSTOMER #	100899	FILING DATE	09/12/2024
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Atul Bedi

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
DA / 071896	**E20254AJ31019281**	**Aldon Griffis**

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
2501	UTILITY ISSUE FEE	516.00	1	516.00
			TOTAL AMOUNT:	**$516.00**

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

New Applications Under 35 U.S.C. 111

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

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

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage

submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

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

PATENT
Attorney Docket No.: JHU3260-10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Bedi and Ravi	Art Unit:	1644
Application No.:	18/883,719	Examiner:	B.E. Bunner
Filed:	September 12, 2024	Conf. No.:	6410
Title:	COMPOSITIONS AND METHODS FOR TARGETED IMMUNOMODULATORY ANTIBODIES AND FUSION PROTEINS		

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

TRANSMITTAL SHEET

Sir:

Transmitted herewith for the above-identified application, please find:

1. Response to Notice of Allowance mailed January 29, 2025 (1 page); and
2. Form PTOL-85B Issue Fee Transmittal (1 page).

The fees for this filing total $516.00, which consist of $516.00 for the Issue Fee payment pursuant to 37 CFR §1.18(a). The Commissioner is hereby authorized to charge this fee and any other fees associated with the filing submitted herewith or credit any overpayment to Deposit Account No. 07-1896.

Respectfully submitted,

Date: April 11, 2025

/Pamela E. Hwang/
Pamela E. Hwang, J.D., Ph.D.
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Lisa A. Haile, J.D., Ph.D.
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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/883,719	05/13/2025	12295968	JHU3260-10	6410

100899 7590 04/30/2025

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

ISSUE NOTIFICATION

The projected patent number and issue date are specified above. The patent will issue electronically. The electronically issued patent is the official patent grant pursuant to 35 U.S.C. § 153. The patent may be accessed on or after the issue date through Patent Center at https://patentcenter.uspto.gov/. The patent will be available in both the public and the private sides of Patent Center. Further assistance in electronically accessing the patent, or about Patent Center, is available by calling the Patent Electronic Business Center at 1-888-217-9197.

The USPTO is implementing electronic patent issuance with a transition period, during which period the USPTO will mail a ceremonial paper copy of the electronic patent grant to the correspondence address of record. Additional copies of the patent (i.e., certified and presentation copies) may be ordered for a fee from the USPTO's Certified Copy Center at https://certifiedcopycenter.uspto.gov/index.html. The Certified Copy Center may be reached at (800)972-6382.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Center (https://patentcenter.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Patents Stakeholder Experience (OPSE), Stakeholder Support Division (SSD) at (571)-272-4200.

INVENTOR(s) (Please see PATENT CENTER site https://patentcenter.uspto.gov for additional inventors):

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

APPLICANT(s) (Please see PATENT CENTER site https://patentcenter.uspto.gov for additional applicants):

The Johns Hopkins University, Baltimore, MD;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/883,719	09/12/2024	Atul Bedi	JHU3260-10	6410

100899 7590 05/13/2025

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San Diego, CA 92121-2133

EXAMINER	
BUNNER, BRIDGET E	

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
05/13/2025	ELECTRONIC

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

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

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

gtdocket@dlapiper.com

APPLICATION NO.	ISSUE DATE	PATENT NO.
18/883,719	13-May-2025	12295968

The John Hopkins University
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San Diego, CA 92121-2133

EGRANT NOTIFICATION

Your electronic patent grant (eGrant) is now available, which can be accessed via Patent Center at https://patentcenter.uspto.gov

The electronic patent grant is the official patent grant under 35 U.S.C. 153. For more information, please visit https://www.uspto.gov/electronicgrants