



(51) International Patent Classification:

C07K 16/28 (2006.01)

(21) International Application Number:

PCT/IB2018/051067

(22) International Filing Date:

21 February 2018 (21.02.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/466,918

03 March 2017 (03.03.2017)

US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,

DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP,

KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,

OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,

TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH,

GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM),

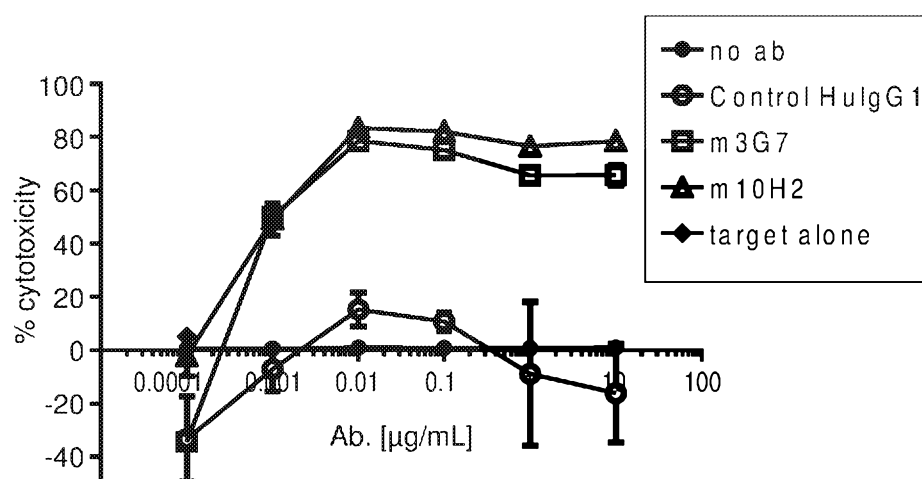
European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,

MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,

KM, ML, MR, NE, SN, TD, TG).

(54) Title: ANTI-GITR ANTIBODIES AND METHODS OF USE THEREOF

Figure 4A



(57) Abstract: The present invention provides antibodies that bind Glucocorticoid Induced Tumor necrosis factor Receptor family related protein (GITR) and methods of using same. The anti-GITR antibodies can be used therapeutically alone or in combination with other therapeutics to treat cancer and other diseases.

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description (Rules 13bis.4(d)(i) and 48.2(a)(viii))*
- *with sequence listing part of description (Rule 5.2(a))*
- *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

ANTI-GITR ANTIBODIES AND METHODS OF USE THEREOF

Field

The present invention relates to antibodies, e.g., full length antibodies that bind
5 Glucocorticoid Induced Tumor necrosis factor Receptor family related protein (GITR).
The invention further relates to compositions comprising antibodies to GITR, and
methods of using anti-GITR antibodies as a medicament. Certain embodiments relate
to methods of using anti-GITR antibodies for the treatment, prevention and/or diagnosis
of various diseases, including hyperproliferative disease, such as cancer.

Background

Glucocorticoid induced tumor necrosis factor receptor family related protein
(GITR) is expressed on the surface of NK cells and T cells, including CD8⁺, CD4⁺, or
regulatory T cells (Treg). Tregs express GITR at higher levels than other types of CD4⁺
15 T cells or CD8⁺ T cells. Expression of GITR is low and on minor populations of Tregs in
peripheral blood, whereas Treg infiltrating solid tumors have increased GITR
expression on the majority of Tregs.

Signaling via GITR increases T cell proliferation and effector functions and also
protects T cells from activation-induced cell death (AICD), which in turn increases the
20 frequency of memory T cells. See, e.g., Ronchetti et al., Euro. J. of Immunology.
34(3):613-22 (2004); and Snell et al., Immunological Reviews. 244(1):197-217 (2011).
Previous studies have shown that an agonist anti-GITR monoclonal antibody (mAb) has
anti-tumor efficacy in multiple mouse syngeneic tumor models, including Meth A
fibrosarcoma, CT26 colon carcinoma, and MB49 bladder carcinoma. See, e.g., Joshi et
25 al., Immunity, 43:1-12 (2015); and Ronchetti et al., J. of Immunology, 179:5916-5916
(2007). This efficacy has been shown to correspond with enhanced responsiveness of
effector T cells. See, e.g., Ko et al., J. Exp. Med. 202(7):885-891 (2005); Coe et al.,
Cancer Immunol. Immunother., 59(9):1367-77 (2010); and Cohen et al., Cancer Res.,
66(9):4904-12 (2006). GITR agonists have also shown synergistic anti-tumor effect
30 with an anti-CTLA-4 antibody and an anti-PD-1 antibody. See, e.g., Mitsui et al.,
Clinical Cancer Research: an Official Journal of the American Association for Cancer
research, 16(10):2781-91 (2010); and Lu et al., J. of Translational Medicine, 12:36

(2014). Accordingly, development of an antibody-based therapeutic agent that modulates GITR signaling would be of great value in treatments of cancer.

Summary

5 The invention disclosed herein is directed to antibodies that selectively bind to GITR. It is demonstrated that the anti-GITR antibodies of the present invention have the dual properties of Treg depletion and T effector cell activation, which in turn resulting in enhanced T effector to Treg ratio in tumors and tumor regression in patients.

10 In one aspect, the invention provides an isolated antibody that specifically binds to GITR and comprises: a heavy chain variable region (VH) comprising a VH complementarity determining region one (CDR1), VH CDR2, and VH CDR3 of the VH having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, or 112; and/or a light chain variable region (VL) comprising a VL CDR1, VL CDR2, and VL CDR3 of the VL having an amino acid sequence selected from the group consisting of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or 111. In some embodiments, the antibody comprises a VH region produced by the expression vector with ATCC Accession No. PTA-123632. In some embodiments, the antibody comprises a VL region produced by the expression vector with ATCC Accession No. PTA-123633.

20 In some embodiments, the antibody comprises a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 24, 25, 26, 32, 33, 34, 35, 39, 40, 41, 115, 116, 117, 63, 64, 65, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 27, 28, 44, 45, 66, or 67, and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 29, 46, or 68; and/or a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 21, 30, 36, 42, 113, or 31, a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 22, 37, 43, 114, or 61, and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 23, 38, or 62. In some embodiments, the antibody comprises a VH comprising the amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, or 112, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR. In some embodiments, the antibody comprises a VL comprising the amino acid sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or 111, or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR. In some embodiments, the

antibody comprises a VH comprising the amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, or 112, and a VL comprising the amino acid sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or 111.

In another aspect, the invention provides an isolated antibody that specifically binds to GITR and comprises: a VH comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 18, 20, 121, and 123; and/or a VL comprising a VL CDR1, VL CDR2, and VL CDR3 of the VL having an amino acid sequence selected from the group consisting of SEQ ID NO: 15, 17, 19, 120, and 122. In some embodiments, the antibody comprises a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 50, 51, 52, 56, or 57, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 53, 54, 58, or 59, and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 55, 60, or 124; and/or a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 47, a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 48, and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 49.

In another aspect, the invention provides an isolated antibody that specifically binds to GITR and comprises: a VH comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH having an amino acid sequence selected from the group consisting of SEQ ID NO: 70 and 72; and/or a VL comprising a VL CDR1, VL CDR2, and VL CDR3 of the VL having an amino acid sequence selected from the group consisting of SEQ ID NO: 69 and 71. In some embodiments, the antibody comprises a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 32, 76, 77, 84, 85, or 86, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 78 or 79, and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 80 or 87; and/or a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 73 or 81, a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 74 or 82, and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 75 or 83.

In some embodiments, the antibody can be a human antibody, a humanized antibody, or a chimeric antibody. In some embodiments, the antibody is a monoclonal antibody.

In some embodiments, the antibody comprises a constant region. In some embodiments, the antibody is of the human IgG₁, IgG₂, IgG_{2Δa}, IgG₃, IgG₄, IgG_{4Δb},

IgG_{4ΔC}, IgG₄ S228P, IgG_{4Δb} S228P, and IgG_{4ΔC} S228P subclass. In some embodiments, the antibody is of the IgG1 isotype.

In another aspect, the invention provides an isolated antibody which specifically binds to GITR and competes with and/or binds to the same or overlaps with the GITR epitope recognized by the anti-GITR antibodies as described herein.

In some embodiments, an anti-GITR antibody provided herein promotes Treg depletion and activates T effector cells (e.g., as measured in vivo using a syngeneic tumor model in CT26 cells).

In some embodiments, an anti-GITR antibody provided herein increases the release of the inflammatory cytokines IFN γ and TNF α from effector T cells (e.g., as measured in vitro in CD4⁺ and CD8⁺ T cells).

In some embodiments, an anti-GITR antibody provided herein enhances anti-tumor immune responses (e.g., inhibiting tumor growth, and enhancing the effects on non-T cells such as NK and other innate cells).

In some embodiments, an anti-GITR antibody provided herein binds human GITR.

In another aspect, the invention provides a pharmaceutical composition comprising a therapeutically effective amount of an anti-GITR antibody as described herein and a pharmaceutically acceptable carrier.

In another aspect, the invention provides an isolated polynucleotide comprising a nucleotide sequence encoding an anti-GITR antibody as described herein. In another aspect, the invention provides a vector comprising the polynucleotide.

In another aspect, the invention provides an isolated host cell that recombinantly produces an anti-GITR antibody as described herein.

In another aspect, the invention provides a method of producing an anti-GITR antibody, the method comprising: culturing a cell line that recombinantly produces the antibody as described herein under conditions wherein the antibody is produced; and recovering the antibody.

In another aspect, the invention provides a method of producing an anti-GITR antibody, the method comprising: culturing a cell line comprising nucleic acid encoding an antibody comprising a heavy chain comprising the amino acid sequence shown in SEQ ID NO: 110 and a light chain comprising the amino acid sequence shown in SEQ

ID NO: 109 under conditions wherein the antibody is produced; and recovering the antibody.

In some embodiments, the heavy and light chains are encoded on separate vectors. In other embodiments, heavy and light chains are encoded on the same vector.

5 In another aspect, the invention provides a method for treating a condition in a subject comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition as described herein. In some embodiments, the condition is a cancer. In some embodiments, the cancer is selected from the group consisting of B-cell related cancer, gastric cancer, small intestine cancer, sarcoma,
10 head and neck cancer, thymic cancer, epithelial cancer, salivary cancer, liver cancer, biliary cancer, neuroendocrine tumors, stomach cancer, thyroid cancer, lung cancer, mesothelioma, ovarian cancer, breast cancer, prostate cancer, esophageal cancer, pancreatic cancer, glioma, renal cancer, bladder cancer, cervical cancer, uterine cancer, vulvar cancer, penile cancer, testicular cancer, anal cancer, choriocarcinoma,
15 colorectal cancer, oral cancer, skin cancer, Merkel cell carcinoma, glioblastoma, brain tumor, bone cancer, eye cancer, and melanoma.

In some embodiments, the B-cell related cancer is selected from the group consisting of multiple myeloma, malignant plasma cell neoplasm, Hodgkin's lymphoma, nodular lymphocyte predominant Hodgkin's lymphoma, Kahler's disease and
20 Myelomatosis, plasma cell leukemia, plasmacytoma, B-cell prolymphocytic leukemia, hairy cell leukemia, B-cell non-Hodgkin's lymphoma (NHL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), follicular lymphoma, Burkitt's lymphoma, marginal zone lymphoma, mantle cell lymphoma, large cell lymphoma, precursor B-lymphoblastic
25 lymphoma, myeloid leukemia, Waldenstrom's macroglobulinemia, diffuse large B cell lymphoma, follicular lymphoma, marginal zone lymphoma, mucosa-associated lymphatic tissue lymphoma, small cell lymphocytic lymphoma, mantle cell lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B-cell lymphoma, lymphoplasmacytic lymphoma, Waldenström macroglobulinemia, nodal marginal zone
30 B cell lymphoma, splenic marginal zone lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, primary central nervous system lymphoma, primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell

lymphoma of the elderly, diffuse large B-cell lymphoma associated with inflammation, intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, plasmablastic lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, B-cell lymphoma unclassified with features intermediate between diffuse large
5 B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, and other B-cell related lymphoma.

In some embodiments, the cancer is relapsed or refractory.

10 In some embodiments, the cancer is locally advanced or metastatic melanoma, squamous cell head and neck cancer (SCHNC), ovarian cancer, renal cancer, gastric cancer, or lung cancer.

In another aspect, the invention provides a method of inhibiting tumor growth or progression in a subject who has a tumor, comprising administering to the subject an effective amount of the pharmaceutical composition as described herein.

15 In another aspect, the invention provides a method of inhibiting or preventing metastasis of cancer cells in a subject, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition as described herein.

In another aspect, the invention provides a method of inducing tumor regression in a subject who has a G1TR expressing tumor, comprising administering to the subject
20 an effective amount of the pharmaceutical composition as described herein.

In some embodiments, the antibody herein can be administered parenterally in a subject. In some embodiments, the subject is a human.

In some embodiments, the method can further comprise administering an effective amount of a second therapeutic agent. In some embodiments, the second
25 therapeutic agent is a biotherapeutic agent, for example, an antibody, including but not limited to, an anti-CTLA-4 antibody, an anti-4-1BB antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-TIM3 antibody, an anti-LAG3 antibody, an anti-TIGIT antibody, an anti-OX40 antibody, an IL-8 antibody, an anti-HVEM antibody, an anti-BTLA antibody, an anti-CD40 antibody, an anti-CD40L antibody, anti-CD47 antibody,
30 an anti-CSF1R antibody, an anti-CSF1 antibody, an anti-MARCO antibody, an anti-CXCR4 antibodies, an anti-VEGFR1 antibody, an anti-VEGFR2 antibody, an anti-TNFR1 antibody, an anti-TNFR2 antibody, an anti-CD3 bispecific antibody, an anti-CD19 antibody, an anti-CD20, an anti-Her2 antibody, an anti-EGFR antibody, an anti-

ICOS antibody, an anti-CD22 antibody, an anti-CD 52 antibody, an anti-CCR4 antibody, an anti-CCR8 antibody, an anti-CD200R antibody, an anti-VISG4 antibody, an anti-CCR2 antibody, an anti-LILRb2 antibody, an anti-CXCR4 antibody, an anti-CD206 antibody, an anti-CD163 antibody, an anti-KLRG1 antibody, an anti-FLT3 antibody, an anti-B7-H4 antibody, an anti-B7-H3 antibody, or a second anti-GITR antibody.

In some embodiments, the second therapeutic agent is a $\text{TNF}\alpha$, a PAP inhibitor, an oncolytic virus, a kinase inhibitor, an ALK inhibitor (e.g., sunitinib or crizotinib), a MEK inhibitor, an IDO inhibitor, a GLS1 inhibitor, a tyrosine kinase inhibitor (e.g., axitinib or palbociclib), a CAR (Chimeric Antigen Receptor)-T cell or T cell therapy, a TLR (Toll-Like Receptor) Agonist (e.g., TLR3, TLR4, TLR5, TLR7, TLR9), or a tumor vaccine.

Also provided is the use of any of the anti-GITR antibodies provided herein in the manufacture of a medicament for the treatment of cancer or for inhibiting tumor growth or progression in a subject in need thereof.

Brief Description of the Figures/Drawings

Figure 1A depicts a graph summarizing the GITR expression in human peripheral blood T cells, including CD4⁺ Foxp3⁺ T regulatory (Treg) cells, CD4⁺Foxp3⁻ T conventional (Tconv) cells, and CD8⁺ T (CD8) cells.

Figure 1B depicts a graph summarizing the density (MFI) of the anti-GITR antibody (m3G7) binding to the GITR positive cells on Treg, CD8 cells, and Tconv cells.

Figure 1C depicts a graph summarizing the frequency of Treg, Tconv and CD8 cells in human peripheral blood mononuclear cells.

Figure 2A depicts a graph summarizing the expression of GITR and OX40 by renal cell carcinoma-associated T cells, including CD4⁺ Foxp3⁺ T regulatory (Tregs) cells, CD4⁺Foxp3⁻ T conventional (Tconv) cells, and CD8⁺ T (CD8) cells.

Figure 2B depicts a graph summarizing the GITR and OX40 antibody binding density (MFI) on RCC-associated T cell subsets.

Figure 2C depicts a graph summarizing the frequency of Treg, Tconv and CD8 cells within the immune infiltrate (CD45⁺) in renal cell carcinoma tissues.

Figure 3A shows a graph summarizing anti-GITR antibodies m3G7 and m10H2 binding to activated T cells.

Figure 3B shows a graph summarizing binding of anti-GITR antibodies R5 (h3G7 R5), R9 (h3G7 N9), R13 (10H2 N13), and R14 (10H2 N14) to activated Tregs.

Figure 4A shows that GITR antibodies mediate cell cytotoxicity in the *in vitro* assay of antibody-dependent cell cytotoxicity (ADCC) mediated by un-fractionated PBMC. The hIgG1 GITR antibodies (m3G7 and m10H2) enabled macrophages to mediate a dose dependent killing of GITR+ T cells.

Figure 4B shows that GITR antibodies mediate cell cytotoxicity in the *in vitro* assay of antibody-dependent cell phagocytosis (ADCP) mediated by macrophages. The hIgG1 GITR antibodies depicted are h3G7 R5 ("R5"), h3G7 R9 ("R9"), h10H2 R13 ("R13"), and h10H2 R14 ("R14").

Figure 4C shows that GITR antibodies mediate cell cytotoxicity in the *in vitro* assay of antibody-dependent cell phagocytosis (ADCP) mediated by macrophages.

Figure 5A shows a graph summarizing that the GITR antibodies of the present invention (h3G7 R5 ("R5"), h3G7 R9 ("R9"), h10H2 R13 ("R13"), and h10H2 R14 ("R14")) caused enhanced secretion of $\text{TNF}\alpha$ from the GITR+ 3A9 cells in a dose dependent manner when plate bound.

Figure 5B also shows a graph summarizing that soluble anti-GITR antibodies R5, R9, R13, and R14 enhanced secretion of $\text{TNF}\alpha$ between in GITR+ 3A9 cells co-cultured with the B cell lymphoma line (LK35.2).

Figure 5C shows that plate-bound anti-GITR antibodies R5, R9, R13 and R14 caused dose dependent increases in secreted $\text{IFN}\gamma$, from human blood CD4+ and CD8+ T cells activated with anti-CD3 antibody.

Figure 5D shows that plate-bound anti-GITR antibodies R5, R9, R13, and R14 caused dose dependent increases in secreted $\text{TNF}\alpha$ from human blood CD4+ and CD8+ T cells activated with anti-CD3 antibody.

Figure 6A, Figure 6B, and Figure 6C show correlated density of the staining of T cell lines expressing (A) no, (B) low and (C) high human GITR with the anti-GITR antibody m10H2.

Figure 6D shows the anti-GITR m10H2 antibody flow cytometry staining of the no, low, and high GITR-expressing cell lines described in Figure 6A.

Figure 6E shows the quantitative PCR and normalization to actin B expression of T cell lines expressing no, low and high human GITR.

Figure 6F shows the staining of FFPE (Formalin Fixed Paraffin Embedded) tissues isolated from NSCLC tumors with m10H2.

Figure 6G shows the staining of FFPE tissues isolated from NSCLC tumors with isotype control mouse antibody.

5 Figure 7A-Figure 7D show the tumor growth of individual mice treated with 5mpk (mg/kg) of mIgG2a isotype control, 0.2, 1 and 5 mpk (mg/kg) of the anti-GITR 21B6 mouse(m) Fc IgG2a, respectively.

10 Figure 7E-Figure 7H show the tumor growth of individual mice treated with 5mpk (mg/kg) of mIgG21 isotype control, 0.2, 1 and 5 mpk (mg/kg) of the anti-GITR 21B6 mouse(m) Fc IgG1, respectively.

Figure 7I shows that the IgG1 treatment did not improve survival of tumor bearing mice compared to isotype control treated mice, whereas IgG2a treatment resulted in 40%, 44.4% and 80% survival 0.2mpk, 1mpk and 5mpk, respectively.

15 Figure 7J shows that the inhibition of tumor growth mediated by 21B6 mIgG2a correlated with an increase in the ratio of CD8+ T cell : Treg ratio at 1mg/kg dose.

Figure 7K shows that the inhibition of tumor growth mediated by 21B6 mIgG2a correlated with an increase in the ratio of CD4+ Teff cell : Treg ratio at all doses.

20 Figure 7L, Figure 7M, and Figure 7N show that the 21B6 mIgG2a treated mice had a non-significant reduction in the number of tumor-infiltrating Tregs, whereas CD8+ Tcell and CD4+ Teff were unaffected.

Figure 7O shows that all doses of 21B6 mIgG2a reduced the proportion of Tregs within the CD45+ immune cells infiltrating the tumors and that the proportion of Treg in the immune infiltrate were not affected by the 21B6 mIgG1 treatment.

25 Figure 7P and Figure 7Q show that changes in the proportion of CD8+ T cells and CD4+ Teff cells in the tumor infiltrate were non-significant.

Figure 7R, Figure 7S, and Figure 7T show that the 21B6 mIgG2a treated mice had the most reduction of Tregs and CD4+ Teff proportions in the spleen when treated with the 0.2 mg/kg dose (Figure 7R and Figure 7T), and that changes at other doses were non-significant. Splenic CD8+ T cells were unaffected at any dose (figure 7S).

30

Detailed Description

Disclosed herein are antibodies that bind to GITR. Methods of making anti-GITR antibodies, compositions comprising these antibodies, and methods of using these

antibodies as a medicament are provided. Anti-GITR antibodies can be used to inhibit tumor progression, and can be used in the prevention and/or treatment of cancer and/or other diseases. It is demonstrated that the anti-GITR antibodies of the present invention have at least two mechanisms of boosting the anti-tumor immunity which in turn results in tumor regression. The first mechanism is directed to the depletion of Treg infiltrating the tumor, which leads to local immune activation, particularly in enhanced T effector cell activation. The second mechanism is direct enhancement of effector functions of the T effector cells and survival of the CD8+ cells as a GITR agonist. The combined effect of Treg depletion and T effector cell activation result in enhanced T effector to Treg ratio in cold tumors and can induce tumor regression, especially in patients that have an existing adaptive anti-tumor response.

General Techniques

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J.E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R.I. Freshney, ed., 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-1998) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold

Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J.D. Capra, eds., Harwood Academic Publishers, 1995).

Definitions

5 The following terms, unless otherwise indicated, shall be understood to have the following meanings: the term "isolated molecule" as referring to a molecule (where the molecule is, for example, a polypeptide, a polynucleotide, or an antibody) that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is substantially free of other
10 molecules from the same source, e.g., species, cell from which it is expressed, library, etc., (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a molecule that is chemically synthesized, or expressed in a cellular system different from the system from which it naturally originates, will be "isolated" from its naturally associated components. A molecule also may be rendered substantially free
15 of naturally associated components by isolation, using purification techniques well known in the art. Molecule purity or homogeneity may be assayed by a number of means well known in the art. For example, the purity of a polypeptide sample may be assayed using polyacrylamide gel electrophoresis and staining of the gel to visualize the polypeptide using techniques well known in the art. For certain purposes, higher
20 resolution may be provided by using HPLC or other means well known in the art for purification.

 An "antibody" is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin
25 molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also, unless otherwise specified, any antigen binding portion thereof that competes with the intact antibody for specific binding, fusion proteins comprising an antigen binding portion, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site. Antigen binding
30 portions include, for example, Fab, Fab', F(ab')₂, Fd, Fv, domain antibodies (dAbs, e.g., shark and camelid antibodies), fragments including complementarity determining regions (CDRs), single chain variable fragment antibodies (scFv), maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv, and

polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide. An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant
5 region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁ and IgA₂. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu,
10 respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. As known in the art, the variable regions of the heavy and light chains
15 each consist of four framework regions (FRs) connected by three complementarity determining regions (CDRs) also known as hypervariable regions, and contribute to the formation of the antigen binding site of antibodies. If variants of a subject variable region are desired, particularly with substitution in amino acid residues outside of a CDR region (i.e., in the framework region), appropriate amino acid substitution,
20 preferably, conservative amino acid substitution, can be identified by comparing the subject variable region to the variable regions of other antibodies which contain CDR1 and CDR2 sequences in the same canonical class as the subject variable region (Chothia and Lesk, J Mol Biol 196(4): 901-917, 1987).

In certain embodiments, definitive delineation of a CDR and identification of
25 residues comprising the binding site of an antibody is accomplished by solving the structure of the antibody and/or solving the structure of the antibody-ligand complex. In certain embodiments, that can be accomplished by any of a variety of techniques known to those skilled in the art, such as X-ray crystallography. In certain embodiments, various methods of analysis can be employed to identify or approximate the CDR
30 regions. In certain embodiments, various methods of analysis can be employed to identify or approximate the CDR regions. Examples of such methods include, but are not limited to, the Kabat definition, the Chothia definition, the AbM definition, the contact definition, and the conformational definition.

The Kabat definition is a standard for numbering the residues in an antibody and is typically used to identify CDR regions. See, e.g., Johnson & Wu, 2000, *Nucleic Acids Res.*, 28: 214-8. The Chothia definition is similar to the Kabat definition, but the Chothia definition takes into account positions of certain structural loop regions. See, e.g., Chothia et al., 1986, *J. Mol. Biol.*, 196: 901-17; Chothia et al., 1989, *Nature*, 342: 877-83. The AbM definition uses an integrated suite of computer programs produced by Oxford Molecular Group that model antibody structure. See, e.g., Martin et al., 1989, *Proc Natl Acad Sci (USA)*, 86:9268-9272; "AbM™, A Computer Program for Modeling Variable Regions of Antibodies," Oxford, UK; Oxford Molecular, Ltd. The AbM definition models the tertiary structure of an antibody from primary sequence using a combination of knowledge databases and ab initio methods, such as those described by Samudrala et al., 1999, "Ab Initio Protein Structure Prediction Using a Combined Hierarchical Approach," in *PROTEINS, Structure, Function and Genetics Suppl.*, 3:194-198. The contact definition is based on an analysis of the available complex crystal structures. See, e.g., MacCallum et al., 1996, *J. Mol. Biol.*, 5:732-45. In another approach, referred to herein as the "conformational definition" of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding. See, e.g., Makabe et al., 2008, *Journal of Biological Chemistry*, 283:1156-1166. Still other CDR boundary definitions may not strictly follow one of the above approaches, but will nonetheless overlap with at least a portion of the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues do not significantly impact antigen binding. As used herein, a CDR may refer to CDRs defined by any approach known in the art, including combinations of approaches. The methods used herein may utilize CDRs defined according to any of these approaches. For any given embodiment containing more than one CDR, the CDRs may be defined in accordance with any of Kabat, Chothia, extended, AbM, contact, and/or conformational definitions.

As known in the art, a "constant region" of an antibody refers to the constant region of the antibody light chain or the constant region of the antibody heavy chain, either alone or in combination.

As used herein, "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations

that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single
5 determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and
10 Milstein, 1975, Nature 256:495, or may be made by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques described in McCafferty et al., 1990, Nature 348:552-554, for example. As used herein, "humanized" antibody refers to forms of non-human (e.g. murine) antibodies that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other
15 antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. Preferably, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as
20 mouse, rat, or rabbit having the desired specificity, affinity, and capacity. . The humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance.

A "human antibody" is one which possesses an amino acid sequence which
25 corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen binding residues.

The term "chimeric antibody" is intended to refer to antibodies in which the
30 variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

The term "epitope" refers to that portion of a molecule capable of being recognized by and bound by an antibody at one or more of the antibody's antigen-binding regions. Epitopes often consist of a surface grouping of molecules such as amino acids or sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics. In some embodiments, the epitope can be a protein epitope. Protein epitopes can be linear or conformational. In a linear epitope, all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearly along the primary amino acid sequence of the protein. A "nonlinear epitope" or "conformational epitope" comprises noncontiguous polypeptides (or amino acids) within the antigenic protein to which an antibody specific to the epitope binds. The term "antigenic epitope" as used herein, is defined as a portion of an antigen to which an antibody can specifically bind as determined by any method well known in the art, for example, by conventional immunoassays. Once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope, e.g., using the techniques described in the present specification. Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes. From this information, it is then possible to competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct competition and cross-competition studies to find antibodies that compete or cross-compete with one another for binding to GITR, e.g., the antibodies compete for binding to the antigen.

As used herein, the term "GITR" refers to any form of GITR and variants thereof that retain at least part of the activity of GITR. Unless indicated differently, such as by specific reference to human GITR, GITR includes all mammalian species of native sequence GITR, e.g., human, canine, feline, equine, and bovine. One exemplary human GITR is found as Uniprot Accession Number Q9Y5U5 (SEQ ID NO: 108).

The term "agonist" refers to a substance which promotes (i.e., induces, causes, enhances, or increases) the biological activity or effect of another molecule. The term agonist encompasses substances which bind receptor, such as an antibody, and substances which promote receptor function without binding thereto (e.g., by activating an associated protein).

The term "antagonist antibody" refers to an antibody that binds to a target and prevents or reduces the biological effect of that target. In some embodiments, the term

can denote an antibody that prevents the target to which it is bound from performing a biological function.

The terms "polypeptide", "oligopeptide", "peptide" and "protein" are used interchangeably herein to refer to chains of amino acids of any length. The chain may be linear or branched, it may comprise modified amino acids, and/or may be interrupted by non-amino acids. The terms also encompass an amino acid chain that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. It is understood that the polypeptides can occur as single chains or associated chains.

As known in the art, "polynucleotide," or "nucleic acid," as used interchangeably herein, refer to chains of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a chain by DNA or RNA polymerase. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the chain. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of modifications include, for example, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by

phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, alpha- or beta-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S("thioate"), P(S)S ("dithioate"), (O)NR₂ ("amidate"), P(O)R, P(O)OR', CO or CH₂ ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

An antibody that "preferentially binds" or "specifically binds" (used interchangeably herein) to an epitope is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule is said to exhibit "specific binding" or "preferential binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody "specifically binds" or "preferentially binds" to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to a GPCR epitope is an antibody that binds this epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other GPCR epitopes or non-GPCR epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" or

"preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

As used herein, "substantially pure" refers to material which is at least 50% pure (i.e., free from contaminants), more preferably, at least 90% pure, more preferably, at least 95% pure, yet more preferably, at least 98% pure, and most preferably, at least 99% pure.

A "host cell" includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

As known in the art, the term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain. The "Fc region" may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991. The Fc region of an immunoglobulin generally comprises two constant domains, CH2 and CH3. As is known in the art, an Fc region can be present in dimer or monomeric form.

As used in the art, "Fc receptor" and "FcR" describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. FcRs are reviewed in Ravetch and Kinet, 1991, Ann. Rev. Immunol., 9:457-92; Capel et al., 1994, Immunomethods, 4:25-34; and de Haas et al., 1995, J. Lab. Clin. Med., 126:330-41. "FcR" also includes the neonatal receptor, FcRn, which is responsible for

the transfer of maternal IgGs to the fetus (Guyer et al., 1976, J. Immunol., 117:587; and Kim et al., 1994, J. Immunol., 24:249).

The term “compete”, as used herein with regard to an antibody, means that a first antibody, or an antigen-binding portion thereof, binds to an epitope in a manner sufficiently similar to the binding of a second antibody, or an antigen-binding portion thereof, such that the result of binding of the first antibody with its cognate epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the binding of the second antibody to its epitope is also detectably decreased in the presence of the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody with its cognate epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to “cross-compete” with each other for binding of their respective epitope(s). Both competing and cross-competing antibodies are encompassed by the present invention. Regardless of the mechanism by which such competition or cross-competition occurs (e.g., steric hindrance, conformational change, or binding to a common epitope, or portion thereof), the skilled artisan would appreciate, based upon the teachings provided herein, that such competing and/or cross-competing antibodies are encompassed and can be useful for the methods disclosed herein.

A “functional Fc region” possesses at least one effector function of a native sequence Fc region. Exemplary “effector functions” include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity; phagocytosis; down-regulation of cell surface receptors (e.g. B cell receptor), etc. Such effector functions generally require the Fc region to be combined with a binding domain (e.g. an antibody variable domain) and can be assessed using various assays known in the art for evaluating such antibody effector functions.

A “native sequence Fc region” comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. A “variant Fc region” comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, yet retains at least one effector function of the native sequence Fc region. Preferably, the variant Fc region has at least

one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g. from about one to about ten amino acid substitutions, and preferably, from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably, at least about 90% sequence identity therewith, more preferably, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity therewith.

As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: reducing the proliferation of (or destroying) neoplastic or cancerous cells, inhibiting metastasis of neoplastic cells, shrinking or decreasing the size of a tumor, remission of cancer, decreasing symptoms resulting from cancer, increasing the quality of life of those suffering from cancer, decreasing the dose of other medications required to treat cancer, delaying the progression of cancer, curing a cancer, and/or prolong survival of patients having cancer.

"Ameliorating" means a lessening or improvement of one or more symptoms as compared to not administering an anti-GITR antibody. "Ameliorating" also includes shortening or reduction in duration of a symptom.

As used herein, an "effective dosage" or "effective amount" of drug, compound, or pharmaceutical composition is an amount sufficient to effect any one or more beneficial or desired results. In more specific aspects, an effective amount prevents, alleviates or ameliorates symptoms of disease, and/or prolongs the survival of the subject being treated. For prophylactic use, beneficial or desired results include eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as reducing one or more symptoms of a disease such as, for example, cancer including, for example without limitation, B-cell related cancer, gastric cancer, small intestine cancer, sarcoma, head and neck cancer, thymic cancer,

epithelial cancer, salivary cancer, liver cancer, biliary cancer, neuroendocrine tumors, stomach cancer, thyroid cancer, lung cancer, mesothelioma, ovarian cancer, breast cancer, prostate cancer, esophageal cancer, pancreatic cancer, glioma, renal cancer (e.g., renal cell carcinoma), bladder cancer, cervical cancer, uterine cancer, vulvar
5 cancer, penile cancer, testicular cancer, anal cancer, choriocarcinoma, colorectal cancer, oral cancer, skin cancer, Merkel cell carcinoma, glioblastoma, brain tumor, bone cancer, eye cancer, and melanoma, decreasing the dose of other medications required to treat the disease, enhancing the effect of another medication, and/or delaying the progression of the cancer in patients. An effective dosage can be
10 administered in one or more administrations. For purposes of this invention, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another
15 drug, compound, or pharmaceutical composition. Thus, an "effective dosage" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

An "individual" or a "subject" is a mammal, more preferably, a human. Mammals
20 also include, but are not limited to, farm animals (e.g., cows, pigs, horses, chickens, etc.), sport animals, pets, primates, horses, dogs, cats, mice and rats.

As used herein, "vector" means a construct, which is capable of delivering, and, preferably, expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA
25 expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

As used herein, "expression control sequence" means a nucleic acid sequence that directs transcription of a nucleic acid. An expression control sequence can be a
30 promoter, such as a constitutive or an inducible promoter, or an enhancer. The expression control sequence is operably linked to the nucleic acid sequence to be transcribed.

As used herein, "pharmaceutically acceptable carrier" or "pharmaceutical acceptable excipient" includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity and is non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Preferred diluents for aerosol or parenteral administration are phosphate buffered saline (PBS) or normal (0.9%) saline. Compositions comprising such carriers are formulated by well-known conventional methods (see, for example, Remington's Pharmaceutical Sciences, 18th edition, A. Gennaro, ed., Mack Publishing Co., Easton, PA, 1990; and Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing, 2000).

The term "effector function" refers to the biological activities attributable to the Fc region of an antibody. Examples of antibody effector functions include, but are not limited to, antibody-dependent cell-mediated cytotoxicity (ADCC), Fc receptor binding, complement dependent cytotoxicity (CDC), phagocytosis, C1q binding, and down regulation of cell surface receptors (e.g., B cell receptor; BCR). See, e.g., U.S. Pat No. 6,737,056. Such effector functions generally require the Fc region to be combined with a binding domain (e.g., an antibody variable domain) and can be assessed using various assays known in the art for evaluating such antibody effector functions. An exemplary measurement of effector function is through Fcγ3 and/or C1q binding.

As used herein "antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (e.g. natural killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. ADCC activity of a molecule of interest can be assessed using an *in vitro* ADCC assay, such as that described in U.S. Patent No. 5,500,362 or 5,821,337. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and NK cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in an animal model such as that disclosed in Clynes et al., 1998, *PNAS (USA)*, 95:652-656.

"Complement dependent cytotoxicity" or "CDC" refers to the lysing of a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g. an

antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., *J. Immunol. Methods*, 202: 163 (1996), may be performed.

5 The term " k_{on} " or " k_a ", as used herein, refers to the rate constant for association of an antibody to an antigen. Specifically, the rate constants (k_{on} or k_a and k_{off} or k_d) and equilibrium dissociation constants are measured using whole antibody (i.e. bivalent) and monomeric GITR proteins.

The term " k_{off} " or " k_d ", as used herein, refers to the rate constant for dissociation of an antibody from the antibody/antigen complex.

10 The term " K_D ", as used herein, refers to the equilibrium dissociation constant of an antibody-antigen interaction.

Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X." Numeric ranges are inclusive of the numbers defining the range. Generally speaking, the term "about" refers to the indicated value of the variable and to all values of the variable that are within the experimental error of the indicated value (e.g. within the 95% confidence interval for the mean) or within 10 percent of the indicated value, whichever is greater. Where the term "about" is used within the context of a time period (years, months, weeks, days etc.), the term "about" means that period of time plus or minus one amount of the next subordinate time period (e.g. about 1 year means 11-13 months; about 6 months means 6 months plus or minus 1 week; about 1 week means 6-8 days; etc.), or within 10 per cent of the indicated value, whichever is greater.

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The term "immune-effector-cell enhancer" or "IEC enhancer" refers to a substance capable of increasing or enhancing the number, quality, or function of one or more types of immune effector cells of a mammal. Examples of immune effector cells include cytolytic CD8 T cells, CD4 T cells, NK cells, and B cells.

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The term "immune modulator" refers to a substance capable of altering (e.g., inhibiting, decreasing, increasing, enhancing, or stimulating) the immune response (as defined herein) or the working of any component of the innate, humoral or cellular immune system of a host mammal. Thus, the term "immune modulator" encompasses the "immune-effector-cell enhancer" as defined herein and the "immune-suppressive-

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cell inhibitor" as defined herein, as well as substance that affects other components of the immune system of a mammal.

The term "immune response" refers to any detectable response to a particular substance (such as an antigen or immunogen) by the immune system of a host mammal, such as innate immune responses (e.g., activation of Toll receptor signaling cascade), cell-mediated immune responses (e.g., responses mediated by T cells, such as antigen-specific T cells, and non-specific cells of the immune system), and humoral immune responses (e.g., responses mediated by B cells, such as generation and secretion of antibodies into the plasma, lymph, and/or tissue fluids).

The term "immunogenic" refers to the ability of a substance to cause, elicit, stimulate, or induce an immune response, or to improve, enhance, increase or prolong a pre-existing immune response, against a particular antigen, whether alone or when linked to a carrier, in the presence or absence of an adjuvant.

The term "immune-suppressive-cell inhibitor" or "ISC inhibitor" refers to a substance capable of reducing or suppressing the number or function of immune suppressive cells of a mammal. Examples of immune suppressive cells include regulatory T cells ("Treg"), myeloid-derived suppressor cells, and tumor-associated macrophages.

The term "intradermal administration," or "administered intradermally," in the context of administering a substance to a mammal including a human, refers to the delivery of the substance into the dermis layer of the skin of the mammal. The skin of a mammal is composed of an epidermis layer, a dermis layer, and a subcutaneous layer. The epidermis is the outer layer of the skin. The dermis, which is the middle layer of the skin, contains nerve endings, sweat glands and oil (sebaceous) glands, hair follicles, and blood vessels. The subcutaneous layer is made up of fat and connective tissue that houses larger blood vessels and nerves. In contrast in intradermal administration, "subcutaneous administration" refers to the administration of a substance into the subcutaneous layer and "topical administration" refers to the administration of a substance onto the surface of the skin.

The term "neoplastic disorder" refers to a condition in which cells proliferate at an abnormally high and uncontrolled rate, the rate exceeding and uncoordinated with that of the surrounding normal tissues. It usually results in a solid lesion or lump known as "tumor." This term encompasses benign and malignant neoplastic disorders. The term

“malignant neoplastic disorder”, which is used interchangeably with the term “cancer” in the present disclosure, refers to a neoplastic disorder characterized by the ability of the tumor cells to spread to other locations in the body (known as “metastasis”). The term “benign neoplastic disorder” refers to a neoplastic disorder in which the tumor cells lack the ability to metastasize.

The term “preventing” or “prevent” refers to (a) keeping a disorder from occurring or (b) delaying the onset of a disorder or onset of symptoms of a disorder.

The term “tumor-associated antigen” or “TAA” refers to an antigen which is specifically expressed by tumor cells or expressed at a higher frequency or density by tumor cells than by non-tumor cells of the same tissue type. Tumor-associated antigens may be antigens not normally expressed by the host; they may be mutated, truncated, misfolded, or otherwise abnormal manifestations of molecules normally expressed by the host; they may be identical to molecules normally expressed but expressed at abnormally high levels; or they may be expressed in a context or milieu that is abnormal. Tumor-associated antigens may be, for example, proteins or protein fragments, complex carbohydrates, gangliosides, haptens, nucleic acids, or any combination of these or other biological molecules.

The term “vaccine” refers to an immunogenic composition for administration to a mammal for eliciting an immune response against a particular antigen in the mammal. A vaccine typically contains an agent (known as “antigen” or “immunogen”) that resembles, or is derived from, the target of the immune response, such as a disease-causing micro-organism or tumor cells. A vaccine intended for the treatment of a tumor, such as a cancer, typically contains an antigen that is derived from a TAA found on the target tumor and is able to elicit immunogenicity against the TAA on the target tumor.

It is understood that wherever embodiments are described herein with the language “comprising,” otherwise analogous embodiments described in terms of “consisting of” and/or “consisting essentially of” are also provided.

Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, the present invention encompasses not only the entire group listed as a whole, but each member of the group individually and all possible subgroups of the main group, but also the main group absent one or more of the group members. The present invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

Unless otherwise defined, 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. In case of conflict, the present specification, including definitions, will control. Throughout this specification and claims, the word "comprise," or variations
5 such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Any example(s) following the term "e.g." or "for example" is not meant to be exhaustive or limiting.

10 Exemplary methods and materials are described herein, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention. The materials, methods, and examples are illustrative only and not intended to be limiting.

15 Anti-GITR antibodies

Provided herein are anti-GITR antibodies that have the properties of boosting the anti-tumor immunity which results in tumor regression. An anti-GITR antibody of the present invention exhibits the following characteristics: (a) deplete tumor infiltrating Tregs through the function of antibody dependent cytotoxicity (ADCC) and/or antibody
20 dependent phagocytosis (ADCP); (b) bind to GITR on the T effector cells and directly enhance the T effector cell activation; and/or (c) enhance effector functions of the T effector cells and the survival of CD8+ T cells.

The antibodies useful in the present invention can encompass monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, Fab', F(ab')₂, Fv, Fc, etc.), chimeric antibodies, bispecific antibodies, heteroconjugate antibodies, single
25 chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion (e.g., a domain antibody), humanized antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants
30 of antibodies, and covalently modified antibodies. The antibodies may be murine, rat, human, or any other origin (including chimeric or humanized antibodies). In some embodiments, the anti-GITR antibody is a monoclonal antibody. In some embodiments, the antibody is a human or humanized antibody.

The anti-GITR antibodies may be made by any method known in the art. General techniques for production of human and mouse antibodies are known in the art and/or are described herein.

Anti-GITR antibodies can be identified or characterized using methods known in the art, whereby GITR binding activity is detected. In some embodiments, an anti-GITR antibody is identified by incubating a candidate agent with GITR and monitoring binding. The binding assay may be performed with, e.g., purified GITR polypeptide(s), or with cells naturally expressing (e.g., various strains), or transfected to express, GITR polypeptide(s). In one embodiment, the binding assay is a competitive binding assay, where the ability of a candidate antibody to compete with a known anti-GITR antibody for GITR binding is evaluated. The assay may be performed in various formats, including the ELISA format. In some embodiments, an anti-GITR antibody is identified by incubating a candidate antibody with GITR and monitoring binding.

Following initial identification, the activity of a candidate anti-GITR antibody can be further confirmed and refined by bioassays, known to test the targeted biological activities. In some embodiments, an in vitro cell assay is used to further characterize a candidate anti-GITR antibody.

The anti-GITR antibodies may be characterized using methods well known in the art. For example, one method is to identify the epitope to which it binds, or "epitope mapping." There are many methods known in the art for mapping and characterizing the location of epitopes on proteins, including solving the crystal structure of an antibody-antigen complex, competition assays, gene fragment expression assays, and synthetic peptide-based assays, as described, for example, in Chapter 11 of Harlow and Lane, Using Antibodies, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999. In an additional example, epitope mapping can be used to determine the sequence to which an anti-GITR antibody binds. Epitope mapping is commercially available from various sources, for example, Pepscan Systems (Edelhertweg 15, 8219 PH Lelystad, The Netherlands). The epitope can be a linear epitope, i.e., contained in a single stretch of amino acids, or a conformational epitope formed by a three-dimensional interaction of amino acids that may not necessarily be contained in a single stretch. Peptides of varying lengths (e.g., at least 4-6 amino acids long) can be isolated or synthesized (e.g., recombinantly) and used for binding assays with an anti-GITR antibody. In another example, the epitope to which

the anti-GITR antibody binds can be determined in a systematic screening by using overlapping peptides derived from the GITR sequence and determining binding by the anti-GITR antibody. According to the gene fragment expression assays, the open reading frame encoding GITR is fragmented either randomly or by specific genetic constructions and the reactivity of the expressed fragments of GITR with the antibody to be tested is determined. The gene fragments may, for example, be produced by PCR and then transcribed and translated into protein in vitro, in the presence of radioactive amino acids. The binding of the antibody to the radioactively labeled GITR fragments is then determined by immunoprecipitation and gel electrophoresis. Certain epitopes can also be identified by using large libraries of random peptide sequences displayed on the surface of phage particles (phage libraries) or yeast (yeast display). Alternatively, a defined library of overlapping peptide fragments can be tested for binding to the test antibody in simple binding assays. In an additional example, mutagenesis of an antigen, domain swapping experiments and alanine scanning mutagenesis can be performed to identify residues required, sufficient, and/or necessary for epitope binding. For example, alanine scanning mutagenesis experiments can be performed using a mutant GITR in which various residues of the GITR polypeptide have been replaced with alanine. By assessing binding of the antibody to the mutant GITR, the importance of the particular GITR residues to antibody binding can be assessed.

Yet another method which can be used to characterize an anti-GITR antibody is to use competition assays with other antibodies known to bind to the same antigen, i.e., various fragments of GITR, to determine if the anti-GITR antibody binds to the same epitope as other antibodies. Competition assays are well known to those of skill in the art, including in an ELISA format.

The binding affinity (K_D) of an anti-GITR antibody to GITR can be about 0.001 to about 200 nM. In some embodiments, the binding affinity is any of about 200 nM, about 150 nM, about 100 nM, about 50 nM, about 20 nM, about 19 nM, about 18 nM, about 17 nM, about 16 nM, about 15 nM, about 14 nM, about 13 nM, about 12 nM, about 11 nM, about 10 nM, about 9 nM, about 8 nM, about 7 nM, about 6 nM, about 5 nM, about 4 nM, about 3 nM, about 2 nM, about 1 nM, about 500 pM, about 100 pM, about 60 pM, about 50 pM, about 20 pM, about 15 pM, about 10 pM, about 5 pM, about 2 pM, or about 1 pM. In some embodiments, the binding affinity is less than any of about 250 nM, about 200 nM, about 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500

pM, about 100 pM, about 50 pM, about 20 pM, about 10 pM, about 5 pM, or about 2 pM.

Accordingly, the invention provides any of the following, or compositions (including pharmaceutical compositions) comprising an antibody having a partial light chain sequence and a partial heavy chain sequence as found in Table 1, or variants thereof. In Table 1, the underlined sequences are CDR sequences according to Kabat and in bold according to Chothia.

Table 1: Variable Regions Sequences of Anti-GITR Antibodies

mAb	Light Chain	Heavy Chain
m3G7	DIVLTQSPASLAVSLGQRATISCR <u>ASESVDNFGINFMN</u> WFQQKSGQ PPKLLIY <u>AASNQGS</u> GVPARFSGS GSGTDFSLDIHPMEEDDTAMYFC <u>QQSKELPWT</u> FGGGTKLEIK (SEQ ID NO: 1)	EVQLQQSGPERVKPGASMKISCK <u>VSGYSFTDYTMN</u> WVKQSHGKNL EWIGL <u>INPYNGG</u> IRYNQKFKGKAS LTVDKSSNTAYMELLSTSEDSAV YYCAR <u>IGGYYDSMDY</u> WGQGTSTV TVSS (SEQ ID NO: 2)
h3G7 VL1.1	DIQMTQSPSSLSASVGDRTITC <u>RASESVDNFGINFLN</u> WFQQKPG KAPKLLIY <u>AASNQGS</u> GVPSPRFSG GSGTDFTLTISSLQPEDFATYYC <u>QQSKELPWT</u> FGGGTKVEIK (SEQ ID NO: 3)	EVQLVESGGGLVQPGGSLRLSCA <u>VSGYSFTDYTMN</u> WVRQAPGKGL EWVAL <u>INPYNGG</u> IRYNQKFKGRF TISVDNAKNSLYLQMNSLRAEDTA VYYCAR <u>IGGYYDSMDY</u> WGQGTL VTVSS (SEQ ID NO: 4)
h3G7 H1	DIQMTQSPSSLSASVGDRTITC <u>RASESVDNFGINFLN</u> WYQQKPG KAPKLLIY <u>AASNQGS</u> GVPSPRFSG GSGTDFTLTISSLQPEDFATYYC <u>QQSKELPWT</u> FGGGTKVEIK (SEQ ID NO: 5)	EVQLVESGGGLVQPGGSLRLSCA <u>ASGFTFSDYTMN</u> WVRQAPGKGL EWVAL <u>INPYNGG</u> IRYNQKFKGRF TISRDNKNSLYLQMNSLRAEDT AVYYCAR <u>IGGYYDSMDY</u> WGQGT LTVSS (SEQ ID NO: 6)
h3G7 H2	EIVLTQSPGTLSPGERATLSCR <u>ASESVDNFGINFMN</u> WYQQKPGQ APRLLIY <u>AASNQGS</u> GIPDRFSGS GSGTDFTLTISRLEPEDFAVYYC <u>QQSKELPWT</u> FGQGTKVEIK (SEQ ID NO: 7)	QVQLVQSGAEVKKPGASVKVSC KASGYTFT <u>DYTMN</u> WVRQAPGQG LEWMGL <u>INPYNGG</u> IRYNQKFKGR VTMTRDTSTSTVYMELSSLRSED TAVYYCAR <u>IGGYYDSMDY</u> WGQG TLTVSS (SEQ ID NO: 8)
h3G7 AM	DIQMTQSPSSLSASVGDRTITC <u>RASESVDPRGINFLN</u> WYQQKPG KAPKLLIY <u>AASNQAK</u> GVPSPRFSG GSGTDFTLTISSLQPEDFATYYC <u>QQAELPWT</u> FGGGTKVEIK (SEQ ID NO: 9)	QVQLVQSGAEVKKPGASVKVSC KASGYTFT <u>GATMN</u> WVRQAPGQG LEWMGL <u>INPYNGG</u> IRYNQKFKGR VTMTRDTSTSTVYMELSSLRSED TAVYYCAR <u>IGGYYDSMDY</u> WGQG TLTVSS (SEQ ID NO: 10)

mAb	Light Chain	Heavy Chain
h3G7 R5	DIQMTQSPSSLSASVGDRVTITC <u>RASESVEPRGINFL</u> NWYQQKPG KAPKLLIY <u>AASQASK</u> GVPSRFSG SGSGTDFTLTISSLQPEDFATYYC <u>QQAELPWT</u> FGGGTKVEIK (SEQ ID NO: 11)	QVQLVQSGAEVKKPGASVKVSC <u>KASGYTFTGATMN</u> WVRQAPGQG LEWMGL <u>INPYTGG</u> IRYNQKFKGR VTMTRDTSTSTVYMELSSLRSED TAVYYCAR <u>IGGYYDSMDY</u> WGQG TLVTVSS (SEQ ID NO: 12)
h3G7 LF	DIQMTQSPSSLSASVGDRVTITC <u>RASESVEPRGINFL</u> NWYQQKPG KAPKLLIY <u>AASQASK</u> GVPSRFSG SGSGTDFTLTISSLQPEDFATYYC <u>QQAELPWT</u> FGGGTKVEIK (SEQ ID NO: 13)	QVQLVQSGAEVKKPGASVKVSC <u>KASGYTFTGATMN</u> WVRQAPGQG LEWMGL <u>INPYTGG</u> IRYNQKFKGR VTMTRDTSTSTVYMELSSLRSED TAVYYCAR <u>IGGYYDTMDY</u> WGQG TLVTVSS (SEQ ID NO: 14)
h3G7 N9	DIQMTQSPSSLSASVGDRVTITC <u>RASESVQPRGINFL</u> NWYQQKPG KAPKLLIY <u>AASNPSK</u> GVPSRFSG SGSGTDFTLTISSLQPEDFATYYC <u>QQAELPWT</u> FGGGTKVEIK (SEQ ID NO: 111)	QVQLVQSGAEVKKPGASVKVSC <u>KASGYTFTGYTVS</u> WVRQAPGQG LEWMGL <u>INPYTGG</u> IRYNQKFKGR VTMTRDTSTSTVYMELSSLRSED TAVYYCAR <u>IGGYYDSMDY</u> WGQG TLVTVSS (SEQ ID NO: 112)
m10H2	DILLTQSPAILSVPGERVSFSCR <u>ASQSIGTSIH</u> WYQQRTNGSPRLL IKY <u>ASESVS</u> GIPSRFSGSGSGTD FTLFINSVESEDIADYYC <u>QQTYS</u> <u>WPAT</u> FGAGTKLELK (SEQ ID NO: 15)	QVQLQQSGAELMKPGASVKISCT AT <u>GYTISRYW</u> IEWWKQRPGHGLE WIGEIL <u>PGSGVT</u> NYNEKFKGKATF TADTSSNTAYMQLSSLTSEDSAV YSCARK <u>KGTYYAMDY</u> WGQGTSVT VSS (SEQ ID NO: 16)
h10H2 HU	EIVLTQSPGTLSSLSPGERATLSCR <u>ASQSIGTSIH</u> WYQQKPGQAPRLL IYY <u>ASESVS</u> GIPDRFSGSGSGTD FTLTISRLEPEDFAVYYC <u>QQTYS</u> <u>WPAT</u> FGQGTKVEIK (SEQ ID NO: 17)	QVQLVQSGAEVKKPGSSVKVSC <u>KASGGTFSRYW</u> IEWWRQAPGQG LEWMGEIL <u>PGSGVT</u> NYNEKFKGR VTITADESTSTAYMELSSLRSED AVYYCARK <u>KGTYYAMDY</u> WGQGTS VTVSS (SEQ ID NO: 18)
h10H2 AM	EIVLTQSPGTLSSLSPGERATLSCR <u>ASQSIGTSIH</u> WYQQKPGQAPRLL IYY <u>ASESVS</u> GIPDRFSGSGSGTD FTLTISRLEPEDFAVYYC <u>QQTYS</u> <u>WPAT</u> FGQGTKVEIK (SEQ ID NO: 19)	QVQLVQSGAEVKKPGSSVKVSC <u>KASGGTFSRYW</u> IEWWRQAPGQG LEWMGEIL <u>PGSGVT</u> FENEKFKGR VTITADESTSTAYMELSSLRSED AVYYCARK <u>KGRYYAMDY</u> WGQGT LTVSS (SEQ ID NO: 20)

mAb	Light Chain	Heavy Chain
h10H2 N13	EIVLTQSPGTL ~~SL~~ SPGERATL ~~SCR~~ <u>ASQSIGTSIH</u> WYQQKPGQAPRL IYYASESVSGIPDRFSGSGSGTD FTLTISRLEPEDFAVYYC <u>QQTYS</u> <u>WPAT</u> FGQGTKVEIK (SEQ ID NO: 120)	QVQLVQSGAEVKKPGSSSVKVSC KASGGTFSRYWIEWRQAPGQG LEWMGEIL <u>PGSGV</u> EYNEKFKG RVTITADESTSTAYMELSSLRSED TAVYYCARK <u>KGRGYAMDY</u> WGQG TSVTVSS (SEQ ID NO: 121)
h10H2 N14	EIVLTQSPGTL ~~SL~~ SPGERATL ~~SCR~~ <u>ASQSIGTSIH</u> WYQQKPGQAPRL IYYASESVSGIPDRFSGSGSGTD FTLTISRLEPEDFAVYYC <u>QQTYS</u> <u>WPAT</u> FGQGTKVEIK (SEQ ID NO: 122)	QVQLVQSGAEVKKPGSSSVKVSC KASGGTFSRYWIEWRQAPGQG LEWMGEIL <u>PGSGV</u> TFENEKFKGR VTITADESTSTAYMELSSLRSED AVYYCARK <u>KGRYAMDY</u> WGQGT SVTVSS (SEQ ID NO: 123)
r18H12	DIVMTQSPSSLA ~~VS~~ AGETVTINCK <u>SSQSLLYSGNQKSYLA</u> WYQQKP GQSPKLLIY <u>WASPRQS</u> GVPDRFI GSGSGTDFTLTIS ~~SV~~ QAEDMATY YC <u>QQYYDAPPT</u> FGGGTKLEIK (SEQ ID NO: 69)	EVQLVESDGLVQPGKSLTLSCA ASGFTFSDYYMAWVRQAPTGL EWWATISYDDITTFYRDSVKGRFT ISRDTAKSTLYLQMDSLRSEDAT YYCTR <u>GLQWPYVMDA</u> WGQGal VTVSS (SEQ ID NO: 70)
r21B6	EIVLTQSPTTMAASPGEKVTITCR <u>ASSSLNYMH</u> WFQQKSGTSPKP WIYDTSELASGVPDRFSGSGSG TSYSLTISSMEADAATYYC <u>LQK</u> <u>NGYPLT</u> FGSGTKLEIK (SEQ ID NO: 71)	EAQLVESGGGLVQPGRSLKLSCE ASGFTFTKYGMAWVRQAPTGL EWWASIINSGGNTYYRDSVKGRF TISRDNASTLYLQMDSLRSEDAT TYYCAT <u>DYYDGSYHSDVVD</u> AWG QGVLT ~~TVSS~~ (SEQ ID NO: 72)

The invention also provides CDR portions of antibodies to G1TR. Determination of CDR regions is well within the skill of the art. It is understood that in some embodiments, CDRs can be a combination of the Kabat and Chothia CDR (also termed "combined CDRs" or "extended CDRs"). In another approach, referred to herein as the "conformational definition" of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding. See, e.g., Makabe et al., 2008, Journal of Biological Chemistry, 283:1156-1166. In general, "conformational CDRs" include the residue positions in the Kabat CDRs and Vernier zones which are constrained in order to maintain proper loop structure for the antibody to bind a specific

antigen. Determination of conformational CDRs is well within the skill of the art. In some embodiments, the CDRs are the Kabat CDRs. In other embodiments, the CDRs are the Chothia CDRs. In other embodiments, the CDRs are the extended, AbM, conformational, or contact CDRs. In other words, in embodiments with more than one
5 CDR, the CDRs may be any of Kabat, Chothia, extended, AbM, conformational, contact CDRs or combinations thereof.

In some embodiments, the antibody comprises three CDRs of any one of the heavy chain variable regions shown in Table 1. In some embodiments, the antibody comprises three CDRs of any one of the light chain variable regions shown in Table 1.

10 In some embodiments, the antibody comprises three CDRs of any one of the heavy chain variable regions shown in Table 1, and three CDRs of any one of the light chain variable regions shown in Table 1.

In some embodiments, provided is an isolated antibody that specifically binds to GITR and comprises: a heavy chain variable region (VH) comprising a VH CDR1, VH
15 CDR2, and VH CDR3 of the VH having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, or 112; and/or a light chain variable region (VL) comprising a VL CDR1, VL CDR2, and VL CDR3 of the VL having an amino acid sequence selected from the group consisting of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or 111.

20 In some embodiments, the antibody comprises a VH comprising the amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, or 112, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR. In some embodiments, the antibody comprises a VL comprising the amino acid sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or 111, or a variant thereof with
25 one or several amino acid substitutions in amino acids that are not within a CDR.

In some embodiments, provided is an isolated antibody that specifically binds to GITR and comprises: a VH comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH having an amino acid sequence selected from the group consisting of SEQ ID NO: 16,
18, 20, 121, and 123; and/or a VL comprising a VL CDR1, VL CDR2, and VL CDR3 of
30 the VL having an amino acid sequence selected from the group consisting of SEQ ID NO: 15, 17, 19, 120, and 122.

In some embodiments, provided is an isolated antibody that specifically binds to GITR and comprises: a VH comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH

having an amino acid sequence selected from the group consisting of SEQ ID NO: 70 and 72; and/or a VL comprising a VL CDR1, VL CDR2, and VL CDR3 of the VL having an amino acid sequence selected from the group consisting of SEQ ID NO: 69 and 71.

In some embodiments, the antibody comprises the full-length heavy chain, with or without the C-terminal lysine, and/or the full-length light chain of anti-GITR antibody. For example, the anti-GITR antibody comprises a heavy chain comprising the amino acid sequence shown in SEQ ID NO: 110, and a light chain comprising the amino acid sequence shown in SEQ ID NO: 109.

Table 2 provides examples of CDR sequences of anti-GITR antibodies provided herein.

Table 2. Anti-GITR antibodies (mAbs) and their antigen-binding CDR sequences according to Kabat and Chothia

mAb		CDR1	CDR2	CDR3
m3G7	L	RASESVDNFGINFMN (SEQ ID NO: 21)	AASNQGS (SEQ ID NO: 22)	QQSKELPWT (SEQ ID NO: 23)
	H	GYSFTDY (SEQ ID NO: 24) (Chothia) DYTMN (SEQ ID NO: 25) (Kabat) GYSFTDYTMN (SEQ ID NO: 26) (Extended)	NPYNGG (SEQ ID NO: 27) (Chothia) LINPYNGGIRYNQKFKG (SEQ ID NO: 28) (Kabat)	IGGYYDSMDY (SEQ ID NO: 29)
h3G7V L1.1	L	RASESVDNFGINFLN (SEQ ID NO: 30)	AASNQGS (SEQ ID NO: 22)	QQSKELPWT (SEQ ID NO: 23)
	H	GYSFTDY (SEQ ID NO: 24) (Chothia) DYTMN (SEQ ID NO: 25) (Kabat) GYSFTDYTMN (SEQ ID NO: 26) (Extended)	NPYNGG (SEQ ID NO: 27) (Chothia) LINPYNGGIRYNQKFKG (SEQ ID NO: 28) (Kabat)	IGGYYDSMDY (SEQ ID NO: 29)
h3G7 H1	L	RASESVDNFGINFLN (SEQ ID NO: 30)	AASNQGS (SEQ ID NO: 22)	QQSKELPWT (SEQ ID NO: 23)
	H	GFTFSDY (SEQ ID NO: 32) (Chothia) DYTMN (SEQ ID NO: 25) (Kabat) GFTFSDYTMN (SEQ ID NO: 33) (Extended)	NPYNGG (SEQ ID NO: 27) (Chothia) LINPYNGGIRYNQKFKG (SEQ ID NO: 28) (Kabat)	IGGYYDSMDY (SEQ ID NO: 29)

mAb		CDR1	CDR2	CDR3
h3G7 H2	L	RASESVDNFGINFMN (SEQ ID NO: 21)	AASNQGS (SEQ ID NO: 22)	QQSKELPWT (SEQ ID NO: 23)
	H	GYTFTDY (SEQ ID NO: 34) (Chothia) DYTMN (SEQ ID NO: 25) (Kabat) GYTFTDYTMN (SEQ ID NO: 35) (Extended)	NPYNGG (SEQ ID NO: 27) (Chothia) LINPYNGGIRYNQKFKG (SEQ ID NO: 28) (Kabat)	IGGYYDSMDY (SEQ ID NO: 29)
h3G7 AM	L	RASESVDPGRGINFLN (SEQ ID NO: 36)	AASNQAK (SEQ ID NO: 37)	QQALELPWT (SEQ ID NO: 38)
	H	GYTFTGA (SEQ ID NO: 39) (Chothia) GATMN (SEQ ID NO: 40) (Kabat) GYTFTGATMN (SEQ ID NO: 41) (Extended)	NPYNGG (SEQ ID NO: 27) (Chothia) LINPYNGGIRYNQKFKG (SEQ ID NO: 28) (Kabat)	IGGYYDSMDY (SEQ ID NO: 29)
h3G7 R5	L	RASESVEPRGINFLN (SEQ ID NO: 42)	AASQASK (SEQ ID NO: 43)	QQALELPWT (SEQ ID NO: 38)
	H	GYTFTGA (SEQ ID NO: 39) (Chothia) GATMN (SEQ ID NO: 40) (Kabat) GYTFTGATMN (SEQ ID NO: 41) (Extended)	NPYTGG (SEQ ID NO: 44) (Chothia) LINPYTGGIRYNQKFKG (SEQ ID NO: 45) (Kabat)	IGGYYDSMDY (SEQ ID NO: 29)
h3G7 LF	L	RASESVEPRGINFLN (SEQ ID NO: 42)	AASQASK (SEQ ID NO: 43)	QQALELPWT (SEQ ID NO: 38)
	H	GYTFTGA (SEQ ID NO: 39) (Chothia) GATMN (SEQ ID NO: 40) (Kabat) GYTFTGATMN (SEQ ID NO: 41)	NPYTGG (SEQ ID NO: 44) (Chothia) LINPYTGGIRYNQKFKG (SEQ ID NO: 45) (Kabat)	IGGYYDTMDY (SEQ ID NO: 46)
h3G7 N9	L	RASESVQPRGINFLN (SEQ ID NO: 113)	AASNPSK (SEQ ID NO: 114)	QQALELPWT (SEQ ID NO: 38)
	H	GYTFTGY (SEQ ID NO: 116) (Chothia) GYTVS (SEQ ID NO: 117) GYTFTGYTVS (SEQ ID NO: 115)	NPYTGG (SEQ ID NO: 44) (Chothia) LINPYTGGIRYNQKFKG (SEQ ID NO: 45) (Kabat)	IGGYYDSMDY (SEQ ID NO: 29)
m10H2	L	RASQSIGTSIH (SEQ ID NO: 47)	YASESVS (SEQ ID NO: 48)	QQTYSWPAT (SEQ ID NO: 49)

mAb		CDR1	CDR2	CDR3
	H	GYTISRY (SEQ ID NO: 51) (Chothia) RYWIE (SEQ ID NO: 52) (Kabat) GYTISRYWIE (SEQ ID NO: 50) (Extended)	LPGSGV (SEQ ID NO: 54) (Chothia) EILPGSGVTNYNEKFKG (SEQ ID NO: 53) (Kabat)	KGTYYYAMDY (SEQ ID NO: 55)
h10H2 HU	L	RASQSIGTSIH (SEQ ID NO: 47)	YASESVS (SEQ ID NO: 48)	QQTYSWPAT (SEQ ID NO: 49)
	H	GGTFSRY (SEQ ID NO: 57) (Chothia) RYWIE (SEQ ID NO: 52) (Kabat) GGTFSRYWIE (SEQ ID NO: 56) (Extended)	LPGSGV (SEQ ID NO: 54) (Chothia) EILPGSGVTNYNEKFKG (SEQ ID NO: 53) (Kabat)	KGTYYYAMDY (SEQ ID NO: 55)
h10H2 AM	L	RASQSIGTSIH (SEQ ID NO: 47)	YASESVS (SEQ ID NO: 48)	QQTYSWPAT (SEQ ID NO: 49)
	H	GGTFSRY (SEQ ID NO: 57) (Chothia) RYWIE (SEQ ID NO: 52) (Kabat) GGTFSRYWIE (SEQ ID NO: 56) (Extended)	LPGSGV (SEQ ID NO: 59) (Chothia) EILPGSGVTFENEKFKG (SEQ ID NO: 58) (Kabat)	KGRYYAMDY (SEQ ID NO: 60)
h10H2 N13	L	RASQSIGTSIH (SEQ ID NO: 47)	YASESVS (SEQ ID NO: 48)	QQTYSWPAT (SEQ ID NO: 49)
	H	GGTFSRY (SEQ ID NO: 57) (Chothia) RYWIE (SEQ ID NO: 52) (Kabat) GGTFSRYWIE (SEQ ID NO: 56) (Extended)	LPGSGV (SEQ ID NO: 54) (Chothia) EILPGSGVTNYNEKFKG (SEQ ID NO: 53) (Kabat)	KGRGYAMDY (SEQ ID NO: 124)
h10H2 N14	L	RASQSIGTSIH (SEQ ID NO: 47)	YASESVS (SEQ ID NO: 48)	QQTYSWPAT (SEQ ID NO: 49)
	H	GGTFSRY (SEQ ID NO: 57) (Chothia) RYWIE (SEQ ID NO: 52) (Kabat) GGTFSRYWIE (SEQ ID NO: 56) (Extended)	LPGSGV (SEQ ID NO: 59) (Chothia) EILPGSGVTFENEKFKG (SEQ ID NO: 58) (Kabat)	KGRYYAMDY (SEQ ID NO: 60)
r18H1 2	L	KSSQSLLYSGNQKSY LA (SEQ ID NO: 73)	WASPRQS (SEQ ID NO: 74)	QQYYDAPPT (SEQ ID NO: 75)

mAb		CDR1	CDR2	CDR3
	H	GFTFSDY (SEQ ID NO: 32) (Chothia) DYDMA (SEQ ID NO: 76) (Kabat) GFTFSDYDMA (SEQ ID NO: 77) (Extended)	SYDDIT (SEQ ID NO: 78) (Chothia) TISYDDITTFYRDSVKG (SEQ ID NO: 79) (Kabat)	GLQWPYVMDA (SEQ ID NO: 80)
r21B6	L	RASSSLNYMH (SEQ ID NO: 81)	DTSELAS (SEQ ID NO: 82)	LQKNGYPLT (SEQ ID NO: 83)
	H	GFTFTKY (SEQ ID NO: 86) (Chothia) KYDMA (SEQ ID NO: 85) (Kabat) GFTFTKYDMA (SEQ ID NO: 84) (Extended)	SYDDIT (SEQ ID NO: 78) (Chothia) TISYDDITTFYRDSVKG (SEQ ID NO: 79) (Kabat)	DYYDGSYHSDV VDA (SEQ ID NO: 87)

In some embodiments, the antibody comprises three light chain CDRs and three heavy chain CDRs from Table 2.

An alignment of light chain CDRs from a selective number of anti-GITR antibodies is provided in Table 3. Variable residues are shown in bold. Consensus light chain CDR sequences are provided in the last row of Table 3.

Table 3. Alignment of anti-GITR light chain CDRs

mAb	VL CDR1	SEQ ID NO:	VL CDR2	SEQ ID NO:	VL CDR3	SEQ ID NO:
m3G7	RASESV DN FGINFMN	21	AASN QGS	22	QQ SK ELPWT	23
h3G7 V L1.1; h3G7 H1	RASESV DN FGINFLN	30	AASN QGS	22	QQ SK ELPWT	23
h3G7 H2	RASESV DN FGINFMN	21	AASN QGS	22	QQ SK ELPWT	23
h3G7 AM	RASESV DPR GINFLN	36	AASN QAK	37	QQ AL ELPWT	38

mAb	VL CDR1	SEQ ID NO:	VL CDR2	SEQ ID NO:	VL CDR3	SEQ ID NO:
h3G7 R5; h3G7 LF	RASES VE PRGINFLN	42	AAS QASK	43	QQ A LELPWT	38
	RASESV X₁X₂X₃ GINF X₄ N , wherein X₁ is D, Q, or E, X₂ is N or P, X₃ is F or R, X₄ is M or L	31	AAS X₁X₂X₃X₄ , wherein X₁ is N or Q, X₂ is Q, P, or A, X₃ is G, A, or S, X₄ is S or K	61	QQ X₁X₂ ELPWT, wherein X₁ is S or A, and X₂ is K or L	62

An alignment of heavy chain CDRs from a selective number of anti-GITR antibodies is provided in Table 4. Variable residues are shown in bold. Consensus heavy chain CDR sequences are provided in the last row of Table 4.

5

Table 4. Alignment of anti-GITR heavy chain CDRs

mAb	VH CDR1	SEQ ID NO:	VH CDR2	SEQ ID NO:	VH CDR3	SEQ ID NO:
m3G 7; h3G7 V L1.1;	GYSFTDY (Chothia)	24	NPYNGG (Chothia)	27	IGGYYD SMDY	29
	DYTMN (Kabat)	25	LINPYNGGIRYNQKFKG (Kabat)	28		
	GYSFTDYTMN (Extended)	26				

mAb	VH CDR1	SEQ ID NO:	VH CDR2	SEQ ID NO:	VH CDR3	SEQ ID NO:
h3G7 H1	GFTFSDY (Chothia) DYTMN (Kabat) GFTFSDYTMN (Extended)	32 25 33	NPYNGG (Chothia) LINPYNGGIRYNQKFKG (Kabat)	27 28	IGGYYDSMDY	29
h3G7 H2	GYTFTDY (Chothia) DYTMN (Kabat) GYTFTDYTMN (Extended)	34 25 35	NPYNGG (Chothia) LINPYNGGIRYNQKFKG (Kabat)	27 28	IGGYYDSMDY	29
h3G7 AM	GYTFTGA (Chothia) GATMN (Kabat) GYTFTGATMN (Extended)	39 40 41	NPYNGG (Chothia) LINPYNGGIRYNQKFKG (Kabat)	27 28	IGGYYDSMDY	29
H3G7 R5	GYTFTGA (Chothia) GATMN (Kabat) GYTFTGATMN (Extended)	39 40 41	NPYTGG (Chothia) LINPYTGGIRYNQKFKG (Kabat)	44 45	IGGYYDSMDY	29

mAb	VH CDR1	SEQ ID NO:	VH CDR2	SEQ ID NO:	VH CDR3	SEQ ID NO:
h3G7 LF	GYTFTGA (Chothia) GATMN (Kabat) GYTFTGATMN (Extended)	39 40 41	NPYTGG (Chothia) LINPYTGGIRYNQKFKG (Kabat)	44 45	IGGYYDTMDY	46
h3G7 N9	GYTFTGY (Chothia) GATVS (Kabat) GYTFTGATMN (Extended)	39 40 41	NPYTGG (Chothia) LINPYTGGIRYNQKFKG (Kabat)	44 45	IGGYYDTMDY	46

mAb	VH CDR1	SEQ ID NO:	VH CDR2	SEQ ID NO:	VH CDR3	SEQ ID NO:
	GX₁X₂FX₃X₄X₅, wherein X₁ is Y or F, X₂ is S or T, X₃ is T or S, X₄ is D or G, X₅ is Y or A (Chothia); X₁X₂T X₃X₄, wherein X₁ is D or G, X₂ is Y or A, X₃ is V or M, X₄ is S or N (Kabat) GX₁X₂FX₃X₄X₅TX₆X₇, wherein X₁ is Y or F, X₂ is S or T, X₃ is T or S, X₄ is D or G, X₅ is Y or A, X₆ is V or M, X₇ is S or N (Extended)	63 64 65	NPYX₁GG, wherein is X₁ N or T (Chothia) LINPYX₁GGIRYNQKFKG, wherein X₁ is N or T (Kabat)	66 67	IGGYDYX₁MD Y, wherein X₁ is S or T	68

In some embodiments, the antibody comprises three light chain CDRs from Table 3 and three heavy chain CDRs from Table 4.

In some embodiments, the antibody comprises a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 24, 25, 26, 32, 33, 34, 35, 39, 40, 41, 115, 116, 117, 63, 64, or 65, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 27, 28, 44, 45, 66, or 67, and a VH CDR3 comprising the amino acid sequence shown

in SEQ ID NO: 29, 46, or 68; and/or a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 21, 30, 36, 42, 113, or 31, a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 22, 37, 43, 114, or 61, and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 23, 38, or 62.

5 In some embodiments, the antibody comprises a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 50, 51, 52, 56, or 57, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 53, 54, 58, or 59, and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 55, 60, or 124; and/or a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 47, a VL CDR2 comprising
10 the amino acid sequence shown in SEQ ID NO: 48, and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 49.

In some embodiments, the antibody comprises a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 32, 76, 77, 84, 85, or 86, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 78 or 79, and a VH CDR3 comprising the
15 amino acid sequence shown in SEQ ID NO: 80 or 87; and/or a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 73 or 81, a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 74 or 82, and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 75 or 83.

The invention also provides methods of generating, selecting, and making anti-
20 GITR antibodies. The antibodies of this invention can be made by procedures known in the art. In some embodiments, antibodies may be made recombinantly and expressed using any method known in the art.

In some embodiments, antibodies may be prepared and selected by phage display technology. See, for example, U.S. Patent Nos. 5,565,332; 5,580,717; 25 5,733,743; and 6,265,150; and Winter et al., Annu. Rev. Immunol. 12:433-455, 1994. Alternatively, the phage display technology (McCafferty et al., Nature 348:552-553, 1990) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a
30 major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in

selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats; for review see, e.g., Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571, 1993. Several sources of V-gene segments can be used for phage display. Clackson et al., *Nature* 352:624-628, 1991, isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Mark et al., *J. Mol. Biol.* 222:581-597, 1991, or Griffith et al., *EMBO J.* 12:725-734, 1993. In a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling." (Marks et al., *Bio/Technol.* 10:779-783, 1992). In this method, the affinity of "primary" human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors. This technique allows the production of antibodies and antibody fragments with affinities in the pM-nM range. A strategy for making very large phage antibody repertoires (also known as "the mother-of-all libraries") has been described by Waterhouse et al., *Nucl. Acids Res.* 21:2265-2266, 1993. Gene shuffling can also be used to derive human antibodies from rodent antibodies, where the human antibody has similar affinities and specificities to the starting rodent antibody. According to this method, which is also referred to as "epitope imprinting", the heavy or light chain V domain gene of rodent antibodies obtained by phage display technique is replaced with a repertoire of human V domain genes, creating rodent-human chimeras. Selection on antigen results in isolation of human variable regions capable of restoring a functional antigen-binding site, i.e., the epitope governs (imprints) the choice of partner. When the process is repeated in order to replace the remaining rodent V domain, a human antibody is obtained (see PCT Publication No. WO 93/06213). Unlike traditional humanization of rodent antibodies by

CDR grafting, this technique provides completely human antibodies, which have no framework or CDR residues of rodent origin.

In some embodiments, antibodies may be made using hybridoma technology. It is contemplated that any mammalian subject including humans or antibody producing cells therefrom can be manipulated to serve as the basis for production of mammalian, including human, hybridoma cell lines. The route and schedule of immunization of the host animal are generally in keeping with established and conventional techniques for antibody stimulation and production, as further described herein. Typically, the host animal is inoculated intraperitoneally, intramuscularly, orally, subcutaneously, intraplantar, and/or intradermally with an amount of immunogen, including as described herein.

Hybridomas can be prepared from the lymphocytes and immortalized myeloma cells using the general somatic cell hybridization technique of Kohler, B. and Milstein, C., 1975, *Nature* 256:495-497 or as modified by Buck, D. W., et al., *In Vitro*, 18:377-381, 1982. Available myeloma lines, including but not limited to X63-Ag8.653 and those from the Salk Institute, Cell Distribution Center, San Diego, Calif., USA, may be used in the hybridization. Generally, the technique involves fusing myeloma cells and lymphoid cells using a fusogen such as polyethylene glycol, or by electrical means well known to those skilled in the art. After the fusion, the cells are separated from the fusion medium and grown in a selective growth medium, such as hypoxanthine-aminopterin-thymidine (HAT) medium, to eliminate unhybridized parent cells. Any of the media described herein, supplemented with or without serum, can be used for culturing hybridomas that secrete monoclonal antibodies. As another alternative to the cell fusion technique, EBV immortalized B cells may be used to produce the G1TR monoclonal antibodies of the subject invention. The hybridomas or other immortalized B-cells are expanded and subcloned, if desired, and supernatants are assayed for anti-immunogen activity by conventional immunoassay procedures (e.g., radioimmunoassay, enzyme immunoassay, or fluorescence immunoassay).

Hybridomas that may be used as source of antibodies encompass all derivatives, progeny cells of the parent hybridomas that produce monoclonal antibodies specific for G1TR, or a portion thereof.

Hybridomas that produce such antibodies may be grown in vitro or in vivo using known procedures. The monoclonal antibodies may be isolated from the culture media

or body fluids, by conventional immunoglobulin purification procedures such as ammonium sulfate precipitation, gel electrophoresis, dialysis, chromatography, and ultrafiltration, if desired. Undesired activity, if present, can be removed, for example, by running the preparation over adsorbents made of the immunogen attached to a solid phase and eluting or releasing the desired antibodies off the immunogen. Immunization of a host animal with a GTR polypeptide, or a fragment containing the target amino acid sequence conjugated to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl_2 , or $\text{R}^1\text{N}=\text{C}=\text{NR}$, where R and R^1 are different alkyl groups, can yield a population of antibodies (e.g., monoclonal antibodies).

If desired, the anti-GTR antibody (monoclonal or polyclonal) of interest may be sequenced and the polynucleotide sequence may then be cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in vector in a host cell and the host cell can then be expanded and frozen for future use. Production of recombinant monoclonal antibodies in cell culture can be carried out through cloning of antibody genes from B cells by means known in the art. See, e.g. Tiller et al., 2008, J. Immunol. Methods 329, 112; US Patent No. 7,314,622.

In some embodiments, the polynucleotide sequence may be used for genetic manipulation to "humanize" the antibody or to improve the affinity, or other characteristics of the antibody. Antibodies may also be customized for use, for example, in dogs, cats, primate, equines and bovines.

In some embodiments, fully human antibodies may be obtained by using commercially available mice that have been engineered to express specific human immunoglobulin proteins. Transgenic animals that are designed to produce a more desirable (e.g., fully human antibodies) or more robust immune response may also be used for generation of humanized or human antibodies. Examples of such technology are Xenomouse™ from Abgenix, Inc. (Fremont, CA) and HuMAb-Mouse® and TC Mouse™ from Medarex, Inc. (Princeton, NJ).

Antibodies may be made recombinantly by first isolating the antibodies and antibody producing cells from host animals, obtaining the gene sequence, and using the

gene sequence to express the antibody recombinantly in host cells (e.g., CHO cells). Another method which may be employed is to express the antibody sequence in plants (e.g., tobacco) or transgenic milk. Methods for expressing antibodies recombinantly in plants or milk have been disclosed. See, for example, Peeters, et al. Vaccine 19:2756, 2001; Lonberg, N. and D. Huszar Int. Rev. Immunol 13:65, 1995; and Pollock, et al., J Immunol Methods 231:147, 1999. Methods for making derivatives of antibodies, e.g., domain, single chain, etc. are known in the art.

Immunoassays and flow cytometry sorting techniques such as fluorescence activated cell sorting (FACS) can also be employed to isolate antibodies that are specific for GTR.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors (such as expression vectors disclosed in PCT Publication No. WO 87/04462), which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. See, e.g., PCT Publication No. WO 87/04462. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences, Morrison et al., Proc. Nat. Acad. Sci. 81:6851, 1984, or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that manner, "chimeric" or "hybrid" antibodies are prepared that have the binding specificity of a GTR monoclonal antibody herein.

Antibody fragments can be produced by proteolytic or other degradation of the antibodies, by recombinant methods (i.e., single or fusion polypeptides) as described above or by chemical synthesis. Polypeptides of the antibodies, especially shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical synthesis are known in the art and are commercially available. For example, an antibody could be produced by an automated polypeptide synthesizer

employing the solid phase method. See also, U.S. Patent Nos. 5,807,715; 4,816,567; and 6,331,415.

In some embodiments, a polynucleotide comprises a sequence encoding the heavy chain and/or the light chain variable regions of antibody m3G7, h3G7VL1.1, h3G7H1, h3G7H2, h3G7AM, h3G7R5, h3G7LF, h3G7N9, m10H2, h10H2HU, h10H2AM, h10H2N13, h10H2N14, r18H12, and r21B6. The sequence encoding the antibody of interest may be maintained in a vector in a host cell and the host cell can then be expanded and frozen for future use. Vectors (including expression vectors) and host cells are further described herein.

The invention includes affinity matured embodiments. For example, affinity matured antibodies can be produced by procedures known in the art (Marks et al., 1992, *Bio/Technology*, 10:779-783; Barbas et al., 1994, *Proc Nat. Acad. Sci, USA* 91:3809-3813; Schier et al., 1995, *Gene*, 169:147-155; Yelton et al., 1995, *J. Immunol.*, 155:1994-2004; Jackson et al., 1995, *J. Immunol.*, 154(7):3310-9; Hawkins et al., 1992, *J. Mol. Biol.*, 226:889-896; and PCT Publication No. WO2004/058184).

The following methods may be used for adjusting the affinity of an antibody and for characterizing a CDR. One way of characterizing a CDR of an antibody and/or altering (such as improving) the binding affinity of a polypeptide, such as an antibody, termed "library scanning mutagenesis". Generally, library scanning mutagenesis works as follows. One or more amino acid positions in the CDR are replaced with two or more (such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids using art recognized methods. This generates small libraries of clones (in some embodiments, one for every amino acid position that is analyzed), each with a complexity of two or more members (if two or more amino acids are substituted at every position). Generally, the library also includes a clone comprising the native (unsubstituted) amino acid. A small number of clones, e.g., about 20-80 clones (depending on the complexity of the library), from each library are screened for binding affinity to the target polypeptide (or other binding target), and candidates with increased, the same, decreased, or no binding are identified. Methods for determining binding affinity are well-known in the art. Binding affinity may be determined using, for example, Biacore™ surface plasmon resonance analysis, which detects differences in binding affinity of about 2-fold or greater, Kinexa® Biosensor, scintillation proximity assays, ELISA, ORIGEN® immunoassay, fluorescence quenching, fluorescence

transfer, and/or yeast display. Binding affinity may also be screened using a suitable bioassay. Biacore™ is particularly useful when the starting antibody already binds with a relatively high affinity, for example a K_D of about 10 nM or lower.

In some embodiments, every amino acid position in a CDR is replaced (in some
5 embodiments, one at a time) with all 20 natural amino acids using art recognized mutagenesis methods (some of which are described herein). This generates small libraries of clones (in some embodiments, one for every amino acid position that is analyzed), each with a complexity of 20 members (if all 20 amino acids are substituted at every position).

10 In some embodiments, the library to be screened comprises substitutions in two or more positions, which may be in the same CDR or in two or more CDRs. Thus, the library may comprise substitutions in two or more positions in one CDR. The library may comprise substitution in two or more positions in two or more CDRs. The library may comprise substitution in 3, 4, 5, or more positions, said positions found in two, three,
15 four, five or six CDRs. The substitution may be prepared using low redundancy codons. See, e.g., Table 2 of Balint et al., 1993, Gene 137(1):109-18.

The CDR may be heavy chain variable region (VH) CDR3 and/or light chain variable region (VL) CDR3. The CDR may be one or more of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3. The CDR may be a Kabat CDR, a
20 Chothia CDR, an extended CDR, an AbM CDR, a contact CDR, or a conformational CDR.

Candidates with improved binding may be sequenced, thereby identifying a CDR substitution mutant which results in improved affinity (also termed an "improved" substitution). Candidates that bind may also be sequenced, thereby identifying a CDR
25 substitution which retains binding.

Multiple rounds of screening may be conducted. For example, candidates (each comprising an amino acid substitution at one or more position of one or more CDR) with improved binding are also useful for the design of a second library containing at least the original and substituted amino acid at each improved CDR position (i.e., amino acid
30 position in the CDR at which a substitution mutant showed improved binding). Preparation, and screening or selection of this library is discussed further below.

Library scanning mutagenesis also provides a means for characterizing a CDR, in so far as the frequency of clones with improved binding, the same binding, decreased

binding or no binding also provide information relating to the importance of each amino acid position for the stability of the antibody-antigen complex. For example, if a position of the CDR retains binding when changed to all 20 amino acids, that position is identified as a position that is unlikely to be required for antigen binding. Conversely, if a position of CDR retains binding in only a small percentage of substitutions, that position is identified as a position that is important to CDR function. Thus, the library scanning mutagenesis methods generate information regarding positions in the CDRs that can be changed to many different amino acids (including all 20 amino acids), and positions in the CDRs which cannot be changed or which can only be changed to a few amino acids.

Candidates with improved affinity may be combined in a second library, which includes the improved amino acid, the original amino acid at that position, and may further include additional substitutions at that position, depending on the complexity of the library that is desired, or permitted using the desired screening or selection method.

In addition, if desired, adjacent amino acid position can be randomized to at least two or more amino acids. Randomization of adjacent amino acids may permit additional conformational flexibility in the mutant CDR, which may in turn, permit or facilitate the introduction of a larger number of improving mutations. The library may also comprise substitution at positions that did not show improved affinity in the first round of screening.

The second library is screened or selected for library members with improved and/or altered binding affinity using any method known in the art, including screening using Kinexa™ biosensor analysis, and selection using any method known in the art for selection, including phage display, yeast display, and ribosome display.

To express the anti-GITR antibodies of the present invention, DNA fragments encoding VH and VL regions can first be obtained using any of the methods described above. Various modifications, e.g. mutations, deletions, and/or additions can also be introduced into the DNA sequences using standard methods known to those of skill in the art. For example, mutagenesis can be carried out using standard methods, such as PCR-mediated mutagenesis, in which the mutated nucleotides are incorporated into the PCR primers such that the PCR product contains the desired mutations or site-directed mutagenesis.

The invention encompasses modifications to the variable regions shown in Table 1 and the CDRs shown in Tables 2, 3 or 4. For example, the invention includes antibodies comprising functionally equivalent variable regions and CDRs which do not significantly affect their properties as well as variants which have enhanced or decreased activity and/or affinity. For example, the amino acid sequence may be mutated to obtain an antibody with the desired binding affinity to GTR. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or which mature (enhance) the affinity of the polypeptide for its ligand, or use of chemical analogs.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to an epitope tag. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody of an enzyme or a polypeptide which increases the half-life of the antibody in the blood circulation.

Substitution variants have at least one amino acid residue in the antibody molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but framework alterations are also contemplated. Conservative substitutions are shown in Table 5 under the heading of "conservative substitutions." If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 5, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 5: Amino Acid Substitutions

Original Residue	Conservative Substitutions	Exemplary Substitutions
Ala (A)	Val	Val; Leu; Ile
Arg (R)	Lys	Lys; Gln; Asn

Original Residue	Conservative Substitutions	Exemplary Substitutions
Asn (N)	Gln	Gln; His; Asp, Lys; Arg
Asp (D)	Glu	Glu; Asn
Cys (C)	Ser	Ser; Ala
Gln (Q)	Asn	Asn; Glu
Glu (E)	Asp	Asp; Gln
Gly (G)	Ala	Ala
His (H)	Arg	Asn; Gln; Lys; Arg
Ile (I)	Leu	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Ile	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg	Arg; Gln; Asn
Met (M)	Leu	Leu; Phe; Ile
Phe (F)	Tyr	Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr; Phe
Tyr (Y)	Phe	Trp; Phe; Thr; Ser
Val (V)	Leu	Ile; Leu; Met; Phe; Ala; Norleucine

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a β -sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

- (1) Non-polar: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) Polar without charge: Cys, Ser, Thr, Asn, Gln;
- (3) Acidic (negatively charged): Asp, Glu;
- (4) Basic (positively charged): Lys, Arg;
- (5) Residues that influence chain orientation: Gly, Pro; and
- (6) Aromatic: Trp, Tyr, Phe, His.

Non-conservative substitutions are made by exchanging a member of one of these classes for another class.

One type of substitution, for example, that may be made is to change one or more cysteines in the antibody, which may be chemically reactive, to another residue, such as, without limitation, alanine or serine. For example, there can be a substitution of a non-canonical cysteine. The substitution can be made in a CDR or framework region of a variable domain or in the constant region of an antibody. In some embodiments, the cysteine is canonical. Any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant cross-linking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability, particularly where the antibody is an antibody fragment such as an Fv fragment.

The antibodies may also be modified, e.g. in the variable domains of the heavy and/or light chains, e.g., to alter a binding property of the antibody. Changes in the variable region can alter binding affinity and/or specificity. In some embodiments, no more than one to five conservative amino acid substitutions are made within a CDR domain. In other embodiments, no more than one to three conservative amino acid substitutions are made within a CDR domain. For example, a mutation may be made in one or more of the CDR regions to increase or decrease the K_D of the antibody for GITR, to increase or decrease k_{off} , or to alter the binding specificity of the antibody. Techniques in site-directed mutagenesis are well-known in the art. See, e.g., Sambrook et al. and Ausubel et al., *supra*.

A modification or mutation may also be made in a framework region or constant region to increase the half-life of an anti-GITR antibody. See, e.g., PCT Publication No. WO 00/09560. A mutation in a framework region or constant region can also be made to alter the immunogenicity of the antibody, to provide a site for covalent or non-covalent binding to another molecule, or to alter such properties as complement fixation, FcR binding and antibody-dependent cell-mediated cytotoxicity. In some embodiments, no more than one to five conservative amino acid substitutions are made within the framework region or constant region. In other embodiments, no more than one to three conservative amino acid substitutions are made within the framework region or constant region. According to the invention, a single antibody may have

mutations in any one or more of the CDRs or framework regions of the variable domain or in the constant region.

Modifications also include glycosylated and nonglycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Antibodies are glycosylated at conserved positions in their constant regions (Jefferis and Lund, 1997, *Chem. Immunol.* 65:111-128; Wright and Morrison, 1997, *TibTECH* 15:26-32). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd et al., 1996, *Mol. Immunol.* 32:1311-1318; Wittwe and Howard, 1990, *Biochem.* 29:4175-4180) and the intramolecular interaction between portions of the glycoprotein, which can affect the conformation and presented three-dimensional surface of the glycoprotein (Jefferis and Lund, *supra*; Wyss and Wagner, 1996, *Current Opin. Biotech.* 7:409-416). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, antibodies produced by CHO cells with tetracycline-regulated expression of $\beta(1,4)$ -N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting GlcNAc, was reported to have improved ADCC activity (Umana et al., 1999, *Nature Biotech.* 17:176-180).

Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine, asparagine-X-threonine, and asparagine-X-cysteine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may

also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

The glycosylation pattern of antibodies may also be altered without altering the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins, e.g. antibodies, as potential therapeutics is rarely the native cell, variations in the glycosylation pattern of the antibodies can be expected (see, e.g. Hse et al., 1997, J. Biol. Chem. 272:9062-9070).

In addition to the choice of host cells, factors that affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism including introducing or overexpressing certain enzymes involved in oligosaccharide production (U.S. Patent Nos. 5,047,335; 5,510,261 and 5,278,299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example, using endoglycosidase H (Endo H), N-glycosidase F, endoglycosidase F1, endoglycosidase F2, endoglycosidase F3. In addition, the recombinant host cell can be genetically engineered to be defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay. Modified polypeptides are made using established procedures in the art and can be screened using standard assays known in the art, some of which are described below and in the Examples.

In some embodiments, the Fc can be human IgG₁. In some embodiments, the constant region has the sequence shown in SEQ ID NO: 129.

In some embodiments, the Fc can be human IgG₂ or human IgG₄. In some embodiments, the antibody comprises a constant region of IgG₄ comprising the following mutations (Armour et al., 2003, Molecular Immunology 40 585-593): E233F234L235 to P233V234A235 (IgG_{4Δc}), in which the numbering is with reference to wild type IgG₄. In yet another embodiment, the Fc is human IgG₄ E233F234L235 to P233V234A235 with deletion G236 (IgG_{4Δb}). In some embodiments the Fc is any

human IgG₄ Fc (IgG₄, IgG_{4Δb} or IgG_{4Δc}) containing hinge stabilizing mutation S228 to P228 (Aalberse et al., 2002, Immunology 105, 9-19). In other embodiments, the Fc can be human IgG₂ containing the mutation A330P331 to S330S331 (IgG_{2Δa}), in which the amino acid residues are numbered with reference to the wild type IgG₂ sequence. Eur. J. Immunol., 1999, 29:2613-2624.

In some embodiments, the antibody comprises a modified constant region that has increased or decreased binding affinity to a human Fc gamma receptor, is immunologically inert or partially inert, e.g., does not trigger complement mediated lysis, does not stimulate antibody-dependent cell mediated cytotoxicity (ADCC), or does not activate microglia; or has reduced activities (compared to the unmodified antibody) in any one or more of the following: triggering complement mediated lysis, stimulating ADCC, or activating microglia. Different modifications of the constant region may be used to achieve optimal level and/or combination of effector functions. See, for example, Morgan et al., Immunology 86:319-324, 1995; Lund et al., J. Immunology 157:4963-9 157:4963-4969, 1996; Idusogie et al., J. Immunology 164:4178-4184, 2000; Tao et al., J. Immunology 143: 2595-2601, 1989; and Jefferis et al., Immunological Reviews 163:59-76, 1998. In some embodiments, the constant region is modified as described in Eur. J. Immunol., 1999, 29:2613-2624; PCT Publication No. WO99/058572.

In some embodiments, an antibody constant region can be modified to avoid interaction with Fc gamma receptor and the complement and immune systems. The techniques for preparation of such antibodies are described in WO 99/58572. For example, the constant region may be engineered to more resemble human constant regions to avoid immune response if the antibody is used in clinical trials and treatments in humans. See, e.g., U.S. Pat. Nos. 5,997,867 and 5,866,692.

In still other embodiments, the constant region is aglycosylated for N-linked glycosylation. In some embodiments, the constant region is aglycosylated for N-linked glycosylation by mutating the oligosaccharide attachment residue and/or flanking residues that are part of the N-glycosylation recognition sequence in the constant region. For example, N-glycosylation site N297 may be mutated to, e.g., A, Q, K, or H. See, Tao et al., J. Immunology 143: 2595-2601, 1989; and Jefferis et al., Immunological Reviews 163:59-76, 1998. In some embodiments, the constant region is aglycosylated for N-linked glycosylation. The constant region may be aglycosylated for N-linked

glycosylation enzymatically (such as removing carbohydrate by enzyme PNGase), or by expression in a glycosylation deficient host cell.

Other antibody modifications include antibodies that have been modified as described in PCT Publication No. WO 99/58572. These antibodies comprise, in addition
5 to a binding domain directed at the target molecule, an effector domain having an amino acid sequence substantially homologous to all or part of a constant region of a human immunoglobulin heavy chain. These antibodies are capable of binding the target molecule without triggering significant complement dependent lysis, or cell-mediated destruction of the target. In some embodiments, the effector domain is capable of
10 specifically binding FcRn and/or FcγRIIb. These are typically based on chimeric domains derived from two or more human immunoglobulin heavy chain CH2 domains. Antibodies modified in this manner are particularly suitable for use in chronic antibody therapy, to avoid inflammatory and other adverse reactions to conventional antibody therapy.

15 In some embodiments, the antibody comprises a modified constant region that has increased binding affinity for FcRn and/or an increased serum half-life as compared with the unmodified antibody.

In a process known as "germlining", certain amino acids in the VH and VL sequences can be mutated to match those found naturally in germline VH and VL
20 sequences. In particular, the amino acid sequences of the framework regions in the VH and VL sequences can be mutated to match the germline sequences to reduce the risk of immunogenicity when the antibody is administered. Germline DNA sequences for human VH and VL genes are known in the art (see e.g., the "Vbase" human germline sequence database; see also Kabat, E. A., et al., 1991, Sequences of Proteins of
25 Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Tomlinson et al., 1992, J. Mol. Biol. 227:776-798; and Cox et al., 1994, Eur. J. Immunol. 24:827-836).

Another type of amino acid substitution that may be made is to remove potential proteolytic sites in the antibody. Such sites may occur in a CDR or framework region of
30 a variable domain or in the constant region of an antibody. Substitution of cysteine residues and removal of proteolytic sites may decrease the risk of heterogeneity in the antibody product and thus increase its homogeneity. Another type of amino acid substitution is to eliminate asparagine-glycine pairs, which form potential deamidation

sites, by altering one or both of the residues. In another example, the C-terminal lysine of the heavy chain of an anti-GITR antibody of the invention can be cleaved. In various embodiments of the invention, the heavy and light chains of the anti-GITR antibodies may optionally include a signal sequence.

5 Once DNA fragments encoding the VH and VL segments of the present invention are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes, or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another
10 DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked", as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

 The isolated DNA encoding the VH region can be converted to a full-length
15 heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see e.g., Kabat, E. A., et al., 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA
20 fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG₁, IgG₂, IgG₃, IgG₄, IgA, IgE, IgM or IgD constant region, but most preferably is an IgG₁ or IgG₂ constant region. The IgG constant region sequence can be any of the various alleles or allotypes known to occur among different individuals, such as Gm(1), Gm(2), Gm(3), and Gm(17). These
25 allotypes represent naturally occurring amino acid substitution in the IgG1 constant regions. For a Fab fragment heavy chain gene, the VH-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region. The CH1 heavy chain constant region may be derived from any of the heavy chain genes.

30 The isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (see e.g.,

Kabat, E. A., et al., 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region. The kappa constant region may be any of the various alleles known to occur among different individuals, such as Inv(1), Inv(2), and Inv(3). The lambda constant region may be derived from any of the three lambda genes.

To create a scFv gene, the VH- and VL-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (See e.g., Bird et al., 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; McCafferty et al., 1990, Nature 348:552-554. An example of a linking peptide is (GGGGS)₃ (SEQ ID NO: 130), which bridges approximately 3.5 nm between the carboxy terminus of one variable region and the amino terminus of the other variable region. Linkers of other sequences have been designed and used (Bird et al., 1988, supra). Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single chain antibody may be monovalent, if only a single VH and VL are used, bivalent, if two VH and VL are used, or polyvalent, if more than two VH and VL are used. Bispecific or polyvalent antibodies may be generated that bind specifically to GITR and to another molecule. The single chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as E. coli. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

Other forms of single chain antibodies, such as diabodies, are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding

sites (see e.g., Holliger, P., et al., 1993, Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R. J., et al., 1994, Structure 2:1121-1123).

Heteroconjugate antibodies, comprising two covalently joined antibodies, are also within the scope of the invention. Such antibodies have been used to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (PCT Publication Nos. WO91/00360 and WO92/200373; EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents and techniques are well known in the art, and are described in U.S. Patent No. 4,676,980.

Chimeric or hybrid antibodies also may be prepared in vitro using known methods of synthetic protein chemistry, including those involving cross-linking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

The invention also encompasses fusion proteins comprising one or more fragments or regions from the antibodies disclosed herein. In some embodiments, a fusion antibody may be made that comprises all or a portion of an anti-GITR antibody of the invention linked to another polypeptide. In another embodiment, only the variable domains of the anti-GITR antibody are linked to the polypeptide. In another embodiment, the VH domain of an anti-GITR antibody is linked to a first polypeptide, while the VL domain of an anti-GITR antibody is linked to a second polypeptide that associates with the first polypeptide in a manner such that the VH and VL domains can interact with one another to form an antigen binding site. In another preferred embodiment, the VH domain is separated from the VL domain by a linker such that the VH and VL domains can interact with one another. The VH-linker- VL antibody is then linked to the polypeptide of interest. In addition, fusion antibodies can be created in which two (or more) single-chain antibodies are linked to one another. This is useful if one wants to create a divalent or polyvalent antibody on a single polypeptide chain, or if one wants to create a bispecific antibody.

In some embodiments, a fusion polypeptide is provided that comprises at least 10 contiguous amino acids of the variable light chain region shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 111, 120, 122, 69, or 71, and/or at least 10 amino acids of the variable heavy chain region shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20,

70, 72, 112, 121, or 123. In other embodiments, a fusion polypeptide is provided that comprises at least about 10, at least about 15, at least about 20, at least about 25, or at least about 30 contiguous amino acids of the variable light chain region and/or at least about 10, at least about 15, at least about 20, at least about 25, or at least about 30 contiguous amino acids of the variable heavy chain region. In another embodiment, the fusion polypeptide comprises a light chain variable region and/or a heavy chain variable region, as shown in any of the sequence pairs selected from among SEQ ID NOs: 1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10, 11 and 12, 13 and 14, 111 and 112, 15 and 16, 17 and 18, 19 and 20, 120 and 121, 122 and 123, 69 and 70, or 71 and 72. In another embodiment, the fusion polypeptide comprises one or more CDR(s). In still other embodiments, the fusion polypeptide comprises VH CDR3 and/or VL CDR3. For purposes of this invention, a fusion protein contains one or more antibodies and another amino acid sequence to which it is not attached in the native molecule, for example, a heterologous sequence or a homologous sequence from another region. Exemplary heterologous sequences include, but are not limited to a "tag" such as a FLAG tag or a 6His tag. Tags are well known in the art.

A fusion polypeptide can be created by methods known in the art, for example, synthetically or recombinantly. Typically, the fusion proteins of this invention are made by preparing and expressing a polynucleotide encoding them using recombinant methods described herein, although they may also be prepared by other means known in the art, including, for example, chemical synthesis.

In other embodiments, other modified antibodies may be prepared using anti-GITR antibody encoding nucleic acid molecules. For instance, "Kappa bodies" (Ill et al., 1997, Protein Eng. 10:949-57), "Minibodies" (Martin et al., 1994, EMBO J. 13:5303-9), "Diabodies" (Holliger et al., supra), or "Janusins" (Traunecker et al., 1991, EMBO J. 10:3655-3659 and Traunecker et al., 1992, Int. J. Cancer (Suppl.) 7:51-52) may be prepared using standard molecular biological techniques following the teachings of the specification.

For example, bispecific antibodies, monoclonal antibodies that have binding specificities for at least two different antigens, can be prepared using the antibodies disclosed herein. Methods for making bispecific antibodies are known in the art (see, e.g., Suresh et al., 1986, Methods in Enzymology 121:210). For example, bispecific antibodies or antigen-binding fragments can be produced by fusion of hybridomas or

linking of Fab' fragments. See, e.g., Songsivilai & Lachmann, 1990, Clin. Exp. Immunol. 79:315-321, Kostelny et al., 1992, J. Immunol. 148:1547-1553. Traditionally, the recombinant production of bispecific antibodies was based on the coexpression of two immunoglobulin heavy chain-light chain pairs, with the two heavy chains having different specificities (Millstein and Cuello, 1983, Nature 305, 537-539). In addition, bispecific antibodies may be formed as "diabodies" or "Janusins." In some embodiments, the bispecific antibody binds to two different epitopes of GITR. In some embodiments, the modified antibodies described above are prepared using one or more of the variable domains or CDR regions from an anti-GITR antibody provided herein.

According to one approach to making bispecific antibodies, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant region sequences. The fusion preferably is with an immunoglobulin heavy chain constant region, comprising at least part of the hinge, CH2 and CH3 regions. It is preferred to have the first heavy chain constant region (CH1), containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are cotransfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In one approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This asymmetric structure, with an immunoglobulin light chain in only one half of the bispecific molecule, facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations. This approach is described in PCT Publication No. WO 94/04690.

In another approach, the bispecific antibodies are composed of amino acid modification in the first hinge region in one arm, and the substituted/replaced amino

acid in the first hinge region has an opposite charge to the corresponding amino acid in the second hinge region in another arm. This approach is described in International Patent Application No. PCT/US2011/036419 (WO2011/143545). In some embodiments, the formation of the bispecific antibodies is enhanced by altering or engineering an interface between a first and a second Fc region (e.g., a hinge region and a CH3 region). In this approach, the bispecific antibodies may be composed of a CH3 region, wherein the CH3 region comprises a first CH3 polypeptide and a second CH3 polypeptide which interact together to form a CH3 interface, wherein one or more amino acids within the CH3 interface destabilize homodimer (e.g., monospecific antibody) formation. See, e.g., PCT/US2011/036419 (WO2011/143545).

In another approach, the bispecific antibodies can be generated using a glutamine-containing peptide tag engineered to the antibody directed to an epitope (e.g., GTR) in one arm and another peptide tag (e.g., a Lys-containing peptide tag or a reactive endogenous Lys) engineered to a second antibody directed to a second epitope in another arm in the presence of transglutaminase. This approach is described in International Patent Application No. PCT/IB2011/054899 (WO2012/059882).

This invention also provides compositions comprising antibodies conjugated (for example, linked) to an agent that facilitate coupling to a solid support (such as biotin or avidin). For simplicity, reference will be made generally to antibodies with the understanding that these methods apply to any of the GTR binding embodiments described herein. Conjugation generally refers to linking these components as described herein. The linking (which is generally fixing these components in proximate association at least for administration) can be achieved in any number of ways. For example, a direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (e.g., a halide) on the other.

The antibodies can be bound to many different carriers. Carriers can be active and/or inert. Examples of well-known carriers include polypropylene, polystyrene, polyethylene, dextran, nylon, amylases, glass, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either

soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation. In some embodiments, the carrier comprises a moiety that targets the lung, heart, or heart valve.

5 An antibody or polypeptide of this invention may be linked to a labeling agent such as a fluorescent molecule, a radioactive molecule or any others labels known in the art. Labels are known in the art which generally provide (either directly or indirectly) a signal.

10 Polynucleotides, vectors, and host cells

The invention also provides polynucleotides encoding any of the antibodies, including antibody fragments and modified antibodies described herein. In another aspect, the invention provides a method of making any of the polynucleotides described herein. Polynucleotides can be made and expressed by procedures known in the art.

15 Accordingly, in some embodiments, the invention provides polynucleotides (or compositions, including pharmaceutical compositions), comprising polynucleotides encoding any of the following: m3G7, h3G7VL1.1, h3G7H1, h3G7H2, h3G7AM, h3G7R5, h3G7LF, h3G7N9, m10H2, h10H2HU, h10H2AM, h10H2N13, h10H2N14, r18H12, r21B6, or any fragment or part thereof having the ability to bind GTR.

20 In another aspect, the invention provides compositions (such as a pharmaceutical compositions) comprising any of the polynucleotides of the invention. In some embodiments, the composition comprises an expression vector comprising a polynucleotide encoding any of the antibodies described herein. In still other embodiments, the composition comprises either or both of the polynucleotides shown in
25 SEQ ID NOs: 88 and 89; SEQ ID NOs: 90 and 91; SEQ ID NOs: 92 and 93; SEQ ID NOs: 94 and 95; SEQ ID NOs: 96 and 97; SEQ ID NOs: 98 and 99; SEQ ID NOs: 100 and 101; SEQ ID NOs: 102 and 103; SEQ ID NOs: 104 and 105; SEQ ID NOs: 106 and 107; SEQ ID NOs: 118 and 119; SEQ ID NOs: 125 and 126; SEQ ID NOs: 127 and 128.

30 Polynucleotides complementary to any such sequences are also encompassed by the present invention. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a

DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

5 Polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes an antibody or a fragment thereof) or may comprise a variant of such a sequence. Polynucleotide variants contain one or more substitutions, additions, deletions and/or insertions such that the immunoreactivity of the encoded polypeptide is not diminished, relative to a native immunoreactive molecule. The effect on the
10 immunoreactivity of the encoded polypeptide may generally be assessed as described herein. Variants preferably exhibit at least about 70% identity, more preferably, at least about 80% identity, yet more preferably, at least about 90% identity, and most preferably, at least about 95% identity to a polynucleotide sequence that encodes a native antibody or a fragment thereof.

15 Two polynucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison
20 window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, or 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the
25 MegAlign® program in the Lasergene® suite of bioinformatics software (DNASTAR®, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O., 1978, A model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research
30 Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J., 1990, Unified Approach to Alignment and Phylogenies pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., 1989, CABIOS 5:151-153; Myers, E.W. and Muller W., 1988, CABIOS 4:11-17; Robinson, E.D., 1971,

Comb. Theor. 11:105; Santou, N., Nes, M., 1987, Mol. Biol. Evol. 4:406-425; Sneath, P.H.A. and Sokal, R.R., 1973, Numerical Taxonomy the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J., 1983, Proc. Natl. Acad. Sci. USA 80:726-730.

5 Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which
10 does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the
15 results by 100 to yield the percentage of sequence identity.

Variants may also, or alternatively, be substantially homologous to a native gene, or a portion or complement thereof. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA sequence encoding a native antibody (or a complementary sequence).

20 Suitable "moderately stringent conditions" include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1 % SDS.

As used herein, "highly stringent conditions" or "high stringency conditions" are
25 those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium
30 chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium

chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

5 It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present
10 invention. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard
15 techniques (such as hybridization, amplification and/or database sequence comparison).

The polynucleotides of this invention can be obtained using chemical synthesis, recombinant methods, or PCR. Methods of chemical polynucleotide synthesis are well known in the art and need not be described in detail herein. One of skill in the art can
20 use the sequences provided herein and a commercial DNA synthesizer to produce a desired DNA sequence.

For preparing polynucleotides using recombinant methods, a polynucleotide comprising a desired sequence can be inserted into a suitable vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification, as
25 further discussed herein. Polynucleotides may be inserted into host cells by any means known in the art. Cells are transformed by introducing an exogenous polynucleotide by direct uptake, endocytosis, transfection, F-mating or electroporation. Once introduced, the exogenous polynucleotide can be maintained within the cell as a non-integrated vector (such as a plasmid) or integrated into the host cell genome. The polynucleotide
30 so amplified can be isolated from the host cell by methods well known within the art. See, e.g., Sambrook et al., 1989.

Alternatively, PCR allows reproduction of DNA sequences. PCR technology is well known in the art and is described in U.S. Patent Nos. 4,683,195, 4,800,159,

4,754,065 and 4,683,202, as well as PCR: The Polymerase Chain Reaction, Mullis et al. eds., Birkauswer Press, Boston, 1994.

RNA can be obtained by using the isolated DNA in an appropriate vector and inserting it into a suitable host cell. When the cell replicates and the DNA is transcribed
5 into RNA, the RNA can then be isolated using methods well known to those of skill in the art, as set forth in Sambrook et al., 1989, *supra*, for example.

Suitable cloning vectors may be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used,
10 useful cloning vectors will generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, e.g., pUC18, pUC19, Bluescript (e.g., pBS SK+) and its derivatives, mp18, mp19, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and
15 shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Strategene, and Invitrogen.

Expression vectors are further provided. Expression vectors generally are replicable polynucleotide constructs that contain a polynucleotide according to the invention. It is implied that an expression vector must be replicable in the host cells
20 either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, cosmids, and expression vector(s) disclosed in PCT Publication No. WO 87/04462. Vector components may generally include, but are not limited to, one or more of the following: a signal sequence; an origin of replication;
25 one or more marker genes; suitable transcriptional controlling elements (such as promoters, enhancers and terminator). For expression (i.e., translation), one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons.

The vectors containing the polynucleotides of interest can be introduced into the
30 host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection

(e.g., where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

The invention also provides host cells comprising any of the polynucleotides described herein. Any host cells capable of over-expressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of mammalian host cells include but not limited to COS, HeLa, and CHO cells. See also PCT Publication No. WO 87/04462. Suitable non-mammalian host cells include prokaryotes (such as *E. coli* or *B. subtilis*) and yeast (such as *S. cerevisiae*, *S. pombe*; or *K. lactis*). Preferably, the host cells express the cDNAs at a level of about 5 fold higher, more preferably, 10 fold higher, even more preferably, 20 fold higher than that of the corresponding endogenous antibody or protein of interest, if present, in the host cells. Screening the host cells for a specific binding to GTR or a GTR domain is effected by an immunoassay or FACS. A cell overexpressing the antibody or protein of interest can be identified.

An expression vector can be used to direct expression of an anti-GTR antibody. One skilled in the art is familiar with administration of expression vectors to obtain expression of an exogenous protein in vivo. See, e.g., U.S. Patent Nos. 6,436,908; 6,413,942; and 6,376,471. Administration of expression vectors includes local or systemic administration, including injection, oral administration, particle gun or catheterized administration, and topical administration. In another embodiment, the expression vector is administered directly to the sympathetic trunk or ganglion, or into a coronary artery, atrium, ventricle, or pericardium.

Targeted delivery of therapeutic compositions containing an expression vector, or subgenomic polynucleotides can also be used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis et al., Trends Biotechnol., 1993, 11:202; Chiou et al., Gene Therapeutics: Methods And Applications Of Direct Gene Transfer, J.A. Wolff, ed., 1994; Wu et al., J. Biol. Chem., 1988, 263:621; Wu et al., J. Biol. Chem., 1994, 269:542; Zenke et al., Proc. Natl. Acad. Sci. USA, 1990, 87:3655; Wu et al., J. Biol. Chem., 1991, 266:338. Therapeutic compositions containing a polynucleotide are administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. Concentration ranges of about 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA can also be used during a gene therapy protocol. The therapeutic

polynucleotides and polypeptides can be delivered using gene delivery vehicles. The gene delivery vehicle can be of viral or non-viral origin (see generally, Jolly, *Cancer Gene Therapy*, 1994, 1:51; Kimura, *Human Gene Therapy*, 1994, 5:845; Connelly, *Human Gene Therapy*, 1995, 1:185; and Kaplitt, *Nature Genetics*, 1994, 6:148).

- 5 Expression of such coding sequences can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence can be either constitutive or regulated.

Viral-based vectors for delivery of a desired polynucleotide and expression in a desired cell are well known in the art. Exemplary viral-based vehicles include, but are not limited to, recombinant retroviruses (see, e.g., PCT Publication Nos. WO 90/07936; 10 WO 94/03622; WO 93/25698; WO 93/25234; WO 93/11230; WO 93/10218; WO 91/02805; U.S. Patent Nos. 5, 219,740 and 4,777,127; GB Patent No. 2,200,651; and EP Patent No. 0 345 242), alphavirus-based vectors (e.g., Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC 15 VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532)), and adeno-associated virus (AAV) vectors (see, e.g., PCT Publication Nos. WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655). Administration of DNA linked to killed adenovirus as described in Curiel, *Hum. Gene Ther.*, 1992, 3:147 can also be employed.

20 Non-viral delivery vehicles and methods can also be employed, including, but not limited to, polycationic condensed DNA linked or unlinked to killed adenovirus alone (see, e.g., Curiel, *Hum. Gene Ther.*, 1992, 3:147); ligand-linked DNA (see, e.g., Wu, *J. Biol. Chem.*, 1989, 264:16985); eukaryotic cell delivery vehicles cells (see, e.g., U.S. Patent No. 5,814,482; PCT Publication Nos. WO 95/07994; WO 96/17072; WO 25 95/30763; and WO 97/42338) and nucleic charge neutralization or fusion with cell membranes. Naked DNA can also be employed. Exemplary naked DNA introduction methods are described in PCT Publication No. WO 90/11092 and U.S. Patent No. 5,580,859. Liposomes that can act as gene delivery vehicles are described in U.S. Patent No. 5,422,120; PCT Publication Nos. WO 95/13796; WO 94/23697; WO 30 91/14445; and EP 0524968. Additional approaches are described in Philip, *Mol. Cell Biol.*, 1994, 14:2411, and in Woffendin, *Proc. Natl. Acad. Sci.*, 1994, 91:1581.

Compositions

The invention also provides pharmaceutical compositions comprising an effective amount of an anti-GITR antibody described herein. Examples of such compositions, as well as how to formulate, are also described herein. In some embodiments, the composition comprises one or more anti-GITR antibodies. In other embodiments, the anti-GITR antibody recognizes GITR. In other embodiments, the anti-GITR antibody is a human antibody. In other embodiments, the anti-GITR antibody is a humanized antibody. In some embodiments, the anti-GITR antibody comprises a constant region that is capable of triggering a desired immune response, such as antibody-mediated lysis, ADCC, and/or ADCP. In other embodiments, the anti-GITR antibody comprises a constant region that does not trigger an unwanted or undesirable immune response, such as antibody-mediated lysis, ADCC, and/or ADCP. In other embodiments, the anti-GITR antibody comprises one or more CDR(s) of the antibody (such as one, two, three, four, five, or, in some embodiments, all six CDRs).

It is understood that the compositions can comprise more than one anti-GITR antibody (e.g., a mixture of GITR antibodies that recognize different epitopes of GITR). Other exemplary compositions comprise more than one anti-GITR antibody that recognize the same epitope(s), or different species of anti-GITR antibodies that bind to different epitopes of GITR. In some embodiments, the compositions comprise a mixture of anti-GITR antibodies that recognize different variants of GITR.

The composition used in the present invention can further comprise pharmaceutically acceptable carriers, excipients, or stabilizers (Remington: The Science and practice of Pharmacy 20th Ed., 2000, Lippincott Williams and Wilkins, Ed. K. E. Hoover), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations, and may comprise buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine,

arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as
5 TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Pharmaceutically acceptable excipients are further described herein.

The anti-GITR antibody and compositions thereof can also be used in conjunction with, or administered separately, simultaneously, or sequentially with other agents that serve to enhance and/or complement the effectiveness of the agents.

10 The invention also provides compositions, including pharmaceutical compositions, comprising any of the polynucleotides of the invention. In some embodiments, the composition comprises an expression vector comprising a polynucleotide encoding the antibody as described herein. In other embodiment, the composition comprises an expression vector comprising a polynucleotide encoding any
15 of the antibodies described herein.

Methods for preventing or treating conditions mediated by GITR

The antibodies and the antibody conjugates of the present invention are useful in various applications including, but are not limited to, therapeutic treatment methods and
20 diagnostic treatment methods.

In one aspect, the invention provides a method for treating a cancer. In some embodiments, the method of treating a cancer in a subject comprises administering to the subject in need thereof an effective amount of a composition (e.g., pharmaceutical composition) comprising any of the GITR antibodies as described herein. As used
25 herein, cancers include, but are not limited to B-cell related cancer, gastric cancer, small intestine cancer, sarcoma, head and neck cancer (e.g., squamous cell head and neck cancer), thymic cancer, epithelial cancer, salivary cancer, liver cancer, biliary cancer, neuroendocrine tumors, stomach cancer, thyroid cancer, lung cancer, mesothelioma, ovarian cancer, breast cancer, prostate cancer, esophageal cancer,
30 pancreatic cancer, glioma, renal cancer (e.g., renal cell carcinoma), bladder cancer, cervical cancer, uterine cancer, vulvar cancer, penile cancer, testicular cancer, anal cancer, choriocarcinoma, colorectal cancer, oral cancer, skin cancer, Merkel cell carcinoma, glioblastoma, brain tumor, bone cancer, eye cancer, and melanoma.

In some embodiments, the B-cell related cancer is, but not limited to, multiple myeloma, malignant plasma cell neoplasm, Hodgkin's lymphoma, nodular lymphocyte predominant Hodgkin's lymphoma, Kahler's disease and Myelomatosis, plasma cell leukemia, plasmacytoma, B-cell prolymphocytic leukemia, hairy cell leukemia, B-cell non-Hodgkin's lymphoma (NHL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), follicular lymphoma, Burkitt's lymphoma, marginal zone lymphoma, mantle cell lymphoma, large cell lymphoma, precursor B-lymphoblastic lymphoma, myeloid leukemia, Waldenstrom's macroglobulinemia, diffuse large B cell lymphoma, follicular lymphoma, marginal zone lymphoma, mucosa-associated lymphatic tissue lymphoma, small cell lymphocytic lymphoma, mantle cell lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B-cell lymphoma, lymphoplasmacytic lymphoma, Waldenström macroglobulinemia, nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, primary central nervous system lymphoma, primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the elderly, diffuse large B-cell lymphoma associated with inflammation, intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, plasmablastic lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, and other B-cell related lymphoma.

In some embodiments, the cancer is relapsed or refractory.

In some embodiments, the cancer is locally advanced or metastatic melanoma, squamous cell head and neck cancer (SCHNC), ovarian cancer, renal cancer, gastric cancer, or lung cancer (e.g., NSCLC (Non-Small Cell Lung Cancer)).

In some embodiments, provided is a method of inhibiting tumor growth or progression in a subject, comprising administering to the subject in need thereof an effective amount of a composition comprising the anti-GITR antibodies as described herein. In some embodiments, provided is a method of inhibiting metastasis of cancer cells in a subject, comprising administering to the subject in need thereof an effective

amount of a composition comprising any of the anti-GITR antibodies as described herein. In other embodiments, provided is a method of inducing regression of a tumor in a subject, comprising administering to the subject in need thereof an effective amount of a composition comprising any of the anti-GITR antibodies as described herein.

5 In another aspect, provided is a method of detecting, diagnosing, and/or monitoring a cancer. For example, the anti-GITR antibodies as described herein can be labeled with a detectable moiety such as an imaging agent and an enzyme-substrate label. The antibodies as described herein can also be used for *in vivo* diagnostic assays, such as *in vivo* imaging (e.g., PET or SPECT), or a staining reagent.

10 In some embodiments, the methods described herein further comprise a step of treating a subject with an additional form of therapy. In some embodiments, the additional form of therapy is an additional anti-cancer therapy including, but not limited to, chemotherapy, radiation, surgery, hormone therapy, and/or additional immunotherapy.

15 With respect to all methods described herein, reference to anti-GITR antibodies also includes compositions comprising one or more additional agents. These compositions may further comprise suitable excipients, such as pharmaceutically acceptable excipients including buffers, which are well known in the art. The present invention can be used alone or in combination with other methods of treatment.

20 The anti-GITR antibody can be administered to a subject via any suitable route. It should be apparent to a person skilled in the art that the examples described herein are not intended to be limiting but to be illustrative of the techniques available. Accordingly, in some embodiments, the anti-GITR antibody is administered to a subject in accord with known methods, such as intravenous administration, e.g., as a bolus or
25 by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, transdermal, subcutaneous, intra-articular, sublingually, intrasynovial, via insufflation, intrathecal, oral, inhalation or topical routes. Administration can be systemic, e.g., intravenous administration, or localized. Commercially available nebulizers for liquid formulations, including jet nebulizers and
30 ultrasonic nebulizers are useful for administration. Liquid formulations can be directly nebulized and lyophilized powder can be nebulized after reconstitution. Alternatively, anti-GITR antibody can be aerosolized using a fluorocarbon formulation and a metered dose inhaler, or inhaled as a lyophilized and milled powder.

In some embodiments, an anti-GITR antibody is administered via site-specific or targeted local delivery techniques. Examples of site-specific or targeted local delivery techniques include various implantable depot sources of the anti-GITR antibody or local delivery catheters, such as infusion catheters, indwelling catheters, or needle catheters, synthetic grafts, adventitial wraps, shunts and stents or other implantable devices, site specific carriers, direct injection, or direct application. See, e.g., PCT Publication No. WO 00/53211 and U.S. Patent No. 5,981,568.

Various formulations of an anti-GITR antibody may be used for administration. In some embodiments, the anti-GITR antibody may be administered neat. In some embodiments, anti-GITR antibody and a pharmaceutically acceptable excipient may be in various formulations. Pharmaceutically acceptable excipients are known in the art, and are relatively inert substances that facilitate administration of a pharmacologically effective substance. For example, an excipient can give form or consistency, or act as a diluent. Suitable excipients include but are not limited to stabilizing agents, wetting and emulsifying agents, salts for varying osmolarity, encapsulating agents, buffers, and skin penetration enhancers. Excipients as well as formulations for parenteral and nonparenteral drug delivery are set forth in Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing, 2000.

In some embodiments, these agents are formulated for administration by injection (e.g., intraperitoneally, intravenously, subcutaneously, intramuscularly, etc.). Accordingly, these agents can be combined with pharmaceutically acceptable vehicles such as saline, Ringer's solution, dextrose solution, and the like. The particular dosage regimen, i.e., dose, timing and repetition, will depend on the particular individual and that individual's medical history.

An anti-GITR antibody can be administered using any suitable method, including by injection (e.g., intraperitoneally, intravenously, subcutaneously, intramuscularly, etc.). Anti-GITR antibodies can also be administered topically or via inhalation, as described herein. Generally, for administration of anti-GITR antibodies, the candidate dosage can be administered daily, every week, every other week, every three weeks, every four weeks, every five weeks, every six weeks, every seven weeks, every eight weeks, every ten weeks, every twelve weeks, or more than every twelve weeks. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of symptoms occurs or until sufficient

therapeutic levels are achieved, for example, to reduce symptoms associated with cancer. The progress of this therapy is easily monitored by conventional techniques and assays. The dosing regimen (including the anti-GITR antibody used) can vary over time.

5 In some embodiments, the candidate dosage is administered daily with the dosage ranging from about any of 1 $\mu\text{g/kg}$ to 30 $\mu\text{g/kg}$ to 300 $\mu\text{g/kg}$ to 3 mg/kg, to 30 mg/kg, to 100 mg/kg or more, depending on the factors mentioned above. For example, daily dosage of about 0.01 mg/kg, about 0.03 mg/kg, about 0.1 mg/kg, about 0.3 mg/kg, about 1 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 5 mg/kg, about 10 mg/kg, about
10 15 mg/kg, and about 25 mg/kg may be used.

In some embodiments, the candidate dosage is administered every week with the dosage ranging from about any of 1 $\mu\text{g/kg}$ to 30 $\mu\text{g/kg}$ to 300 $\mu\text{g/kg}$ to 3 mg/kg, to 30 mg/kg, to 100 mg/kg or more, depending on the factors mentioned above. For example, a weekly dosage of about 0.01 mg/kg, about 0.03 mg/kg, about 0.1 mg/kg,
15 about 0.3 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 25 mg/kg, and about 30 mg/kg may be used.

In some embodiments, the candidate dosage is administered every two weeks with the dosage ranging from about any of 1 $\mu\text{g/kg}$ to 30 $\mu\text{g/kg}$ to 300 $\mu\text{g/kg}$ to 3 mg/kg,
20 to 30 mg/kg, to 100 mg/kg or more, depending on the factors mentioned above. For example, a bi-weekly dosage of about 0.1 mg/kg, about 0.3 mg/kg, about 1 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 25 mg/kg, and about 30 mg/kg may be used.

In some embodiments, the candidate dosage is administered every three weeks
25 with the dosage ranging from about any of 1 $\mu\text{g/kg}$ to 30 $\mu\text{g/kg}$ to 300 $\mu\text{g/kg}$ to 3 mg/kg, to 30 mg/kg, to 100 mg/kg or more, depending on the factors mentioned above. For example, a tri-weekly dosage of about 0.1 mg/kg, about 0.3 mg/kg, about 1 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg,
30 and about 50 mg/kg may be used.

In some embodiments, the candidate dosage is administered every month or every four weeks with the dosage ranging from about any of 1 $\mu\text{g/kg}$ to 30 $\mu\text{g/kg}$ to 300 $\mu\text{g/kg}$ to 3 mg/kg, to 30 mg/kg, to 100 mg/kg or more, depending on the factors

mentioned above. For example, a monthly dosage of about 0.1 mg/kg, about 0.3 mg/kg, about 1 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, and about 50 mg/kg may be used.

5 In other embodiments, the candidate dosage is administered daily with the dosage ranging from about 0.01 mg to about 1200 mg or more, depending on the factors mentioned above. For example, daily dosage of about 0.01 mg, about 0.1 mg, about 1 mg, about 10 mg, about 50 mg, about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900
10 mg, about 1000 mg, about 1100 mg, or about 1200 mg may be used.

 In other embodiments, the candidate dosage is administered every week with the dosage ranging from about 0.01 mg to about 2000 mg or more, depending on the factors mentioned above. For example, weekly dosage of about 0.01 mg, about 0.1 mg, about 1 mg, about 10 mg, about 50 mg, about 100 mg, about 200 mg, about 300 mg,
15 about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg, about 1400 mg, about 1500 mg, about 1600 mg, about 1700 mg, about 1800 mg, about 1900 mg, or about 2000 mg may be used.

 In other embodiments, the candidate dosage is administered every two weeks
20 with the dosage ranging from about 0.01 mg to about 2000 mg or more, depending on the factors mentioned above. For example, bi-weekly dosage of about 0.01 mg, about 0.1 mg, about 1 mg, about 10 mg, about 50 mg, about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg, about
25 1400 mg, about 1500 mg, about 1600 mg, about 1700 mg, about 1800 mg, about 1900 mg, or about 2000 mg may be used.

 In other embodiments, the candidate dosage is administered every three weeks with the dosage ranging from about 0.01 mg to about 2500 mg or more, depending on the factors mentioned above. For example, tri-weekly dosage of about 0.01 mg, about
30 0.1 mg, about 1 mg, about 10 mg, about 50 mg, about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg, about 1400 mg, about 1500 mg, about 1600 mg, about 1700 mg, about 1800 mg, about 1900

mg, about 2000 mg, about 2100 mg, about 2200 mg, about 2300 mg, about 2400 mg, or about 2500 mg may be used.

In other embodiments, the candidate dosage is administered every four weeks or month with the dosage ranging from about 0.01 mg to about 3000 mg or more, depending on the factors mentioned above. For example, monthly dosage of about 0.01 mg, about 0.1 mg, about 1 mg, about 10 mg, about 50 mg, about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg, about 1400 mg, about 1500 mg, about 1600 mg, about 1700 mg, about 1800 mg, about 1900 mg, about 2000 mg, about 2100 mg, about 2200 mg, about 2300 mg, about 2400 mg, about 2500, about 2600 mg, about 2700 mg, about 2800 mg, about 2900 mg, or about 3000 mg may be used.

For the purpose of the present invention, the appropriate dosage of an anti-GITR antibody will depend on the anti-GITR antibody (or compositions thereof) employed, the type and severity of symptoms to be treated, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the agent, the patient's clearance rate for the administered agent, and the discretion of the attending physician. Typically the clinician will administer an anti-GITR antibody until a dosage is reached that achieves the desired result. Dose and/or frequency can vary over course of treatment. Empirical considerations, such as the half-life, generally will contribute to the determination of the dosage. For example, antibodies that are compatible with the human immune system, such as humanized antibodies or fully human antibodies, may be used to prolong half-life of the antibody and to prevent the antibody being attacked by the host's immune system. Frequency of administration may be determined and adjusted over the course of therapy, and is generally, but not necessarily, based on treatment and/or suppression and/or amelioration and/or delay of symptoms. Alternatively, sustained continuous release formulations of anti-GITR antibodies may be appropriate. Various formulations and devices for achieving sustained release are known in the art.

In one embodiment, dosages for an antibody may be determined empirically in individuals who have been given one or more administration(s) of an antibody. For example, individuals are given incremental dosages of an anti-GITR antibody. To assess efficacy, an indicator of the disease can be followed.

Administration of an anti-GITR antibody as described herein in accordance with the method in the present invention can be continuous or intermittent, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of an anti-GITR antibody may be essentially continuous over a preselected period of time or may be in a series of spaced doses.

In some embodiments, more than one anti-GITR antibody may be present. At least one, at least two, at least three, at least four, at least five different, or more antibodies can be present. Generally, those anti-GITR antibodies may have complementary activities that do not adversely affect each other.

In some embodiments, the anti-GITR antibody may be administered in combination with the administration of one or more additional therapeutic agents. These include, but are not limited to, the administration of a biotherapeutic agent, a chemotherapeutic agent, a vaccine, a CAR-T cell-based therapy, radiotherapy, a cytokine therapy (e.g., IL-2, IL-7, IL-15, IL-12, IFN γ , IFN α , IL-8), a vaccine, an inhibitor of other immunosuppressive pathways, an inhibitors of angiogenesis, a T cell activator, an inhibitor of a metabolic pathway, an mTOR (mechanistic target of rapamycin) inhibitor (e.g., rapamycin, rapamycin derivatives, sirolimus, temsirolimus, everolimus, and deforolimus), an inhibitor of an adenosine pathway, a tyrosine kinase inhibitor including but not limited to inlyta, ALK (anaplastic lymphoma kinase) inhibitors (e.g., crizotinib, ceritinib, alectinib, and sunitinib), a BRAF inhibitor (e.g., vemurafenib and dabrafenib), an epigenetic modifier, an inhibitors or depletor of Treg cells and/or of myeloid-derived suppressor cells, a JAK (Janus Kinase) inhibitor (e.g., ruxolitinib and tofacitinib, varicitinib, filgotinib, gandotinib, lestaurtinib, momelotinib, pacritinib, and upadacitinib), a STAT (Signal Transducers and Activators of Transcription) inhibitor (e.g., STAT1, STAT3, and STAT5 inhibitors such as fludarabine), a cyclin-dependent kinase inhibitor, an immunogenic agent (for example, attenuated cancerous cells, tumor antigens, antigen presenting cells such as dendritic cells pulsed with tumor derived antigen or nucleic acids, immune stimulating cytokines (for example, IL-2, IFN α 2, GM-CSF), a MEK inhibitor (e.g., trametinib, cobimetinib, binimetinib, and selumetinib), a GLS1 inhibitor, a PAP inhibitor, an oncolytic virus, an IDO (Indoleamine-pyrrole 2,3-dioxygenase) inhibitor, and cells transfected with genes encoding immune stimulating cytokines such as but not limited to GM-CSF).

In some embodiments, the biotherapeutic agent is an antibody, including but not limited to, an anti-CTLA-4 antibody, an anti-4-1BB antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-TIM3 antibody, an anti-LAG3 antibody, an anti-TIGIT antibody, an anti-OX40 antibody, an IL-8 antibody, an anti-HVEM antibody, an anti-BTLA antibody, an anti-CD40 antibody, an anti-CD40L antibody, anti-CD47 antibody, an anti-CSF1R antibody, an anti-CSF1 antibody, an anti-MARCO antibody, an anti-CXCR4 antibodies, an anti-VEGFR1 antibody, an anti-VEGFR2 antibody, an anti-TNFR1 antibody, an anti-TNFR2 antibody, an anti-CD3 bispecific antibody, an anti-CD19 antibody, an anti-CD20, an anti-Her2 antibody, an anti-EGFR antibody, an anti-ICOS antibody, an anti-CD22 antibody, an anti-CD 52 antibody, an anti-CCR4 antibody, an anti-CCR8 antibody, an anti-CD200R antibody, an anti-VISG4 antibody, an anti-CCR2 antibody, an anti-LILRb2 antibody, an anti-CXCR4 antibody, an anti-CD206 antibody, an anti-CD163 antibody, an anti-KLRG1 antibody, an anti-FLT3 antibody, an anti-B7-H4 antibody, an anti-B7-H3 antibody, or a second anti-GITR antibody.

Accordingly, in some embodiments, an anti-GITR antibody is used in conjunction with, for example, an anti-PD-L1 antagonist antibody, such as BMS-936559 (MDX-1105), MPDL3280A, and avelumab (MSB0010718C); an anti-PD-1 antagonist antibody such as for example, nivolumab (OPDIVO®), pembrolizumab (KEYTRUDA®), and pidilizumab; an anti-CTLA-4 antagonist antibody such as for example ipilimumab (YERVOY®); an anti-LAG-3 antagonist antibody such as BMS-986016 and IMP701; an anti-TIM-3 antagonist antibody; an anti-B7-H3 antagonist antibody such as for example MGA271; an-anti-VISTA antagonist antibody; an anti-TIGIT antagonist antibody; an anti-CD28 antagonist antibody; an anti-CD80 antibody; an anti-CD86 antibody; an anti-B7-H4 antagonist antibody; an anti-ICOS agonist antibody; an anti-CD28 agonist antibody; an innate immune response modulator (e.g., TLRs, KIR, NKG2A), and an IDO inhibitor. In some embodiments, an anti-GITR antibody is used in conjunction with a 4-1BB (CD137) agonist such as, for example, PF-05082566 or urelumab (BMS-663513). In some embodiments, an anti-GITR antibody is used in conjunction with an OX40 agonist such as, for example, an anti-OX-40 agonist antibody. In some embodiments, an anti-GITR antibody is used in conjunction with another GITR agonist such as, for example, TRX518. In some embodiments, an anti-GITR antibody is used in conjunction with an IDO inhibitor. In some embodiments, an anti-GITR antibody is used in

conjunction with a cytokine therapy such as, for example without limitation, IL-2, IL-12, IL-7, IL-15, IL-21, IL-33, CSF-1, MCSF-1, etc.

Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; 5 aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramidate, triethylenethiophosphoramidate and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its 10 adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine 15 oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as the enediyne antibiotics (e.g. calicheamicin, especially calicheamicin gamma1 and calicheamicin phil1, see, e.g., Agnew, Chem. Intl. Ed. Engl., 33:183-186 (1994); dynemicin, including 20 dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including 25 morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, and deoxydoxorubicin), pegylated liposomal doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites 30 such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine,

enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiothane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; 5 bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; razoxane; rhizoxin; sizofuran; spirogermanium; 10 tenuazonic acid; triaziquone; 2, 2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel and doxetaxel; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as 15 cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also 20 included are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen, raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the 25 adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, megestrol acetate, exemestane, formestane, fadrozole, vorozole, letrozole, and anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

In some embodiments, an anti-GITR antibody is used in conjunction with one or 30 more other therapeutic agents targeting an immune checkpoint modulator, such as, for example without limitation, an agent targeting PD-1, PD-L1, CTLA-4, LAG-3, B7-H3, B7-H4, B7-DC (PD-L2), B7-H5, B7-H6, B7-H8, B7-H2, B7-1, B7-2, ICOS, ICOS-L, TIGIT, CD2, CD47, CD80, CD86, CD48, CD58, CD226, CD155, CD112, LAIR1, 2B4,

BTLA, CD160, TIM1, TIM-3, TIM4, VISTA (PD-H1), OX40, OX40L, GITRL, CD70, CD27, 4-1BB, 4-BBL, DR3, TL1A, CD40, CD40L, CD30, CD30L, LIGHT, HVEM, SLAM (SLAMF1, CD150), SLAMF2 (CD48), SLAMF3 (CD229), SLAMF4 (2B4, CD244), SLAMF5 (CD84), SLAMF6 (NTB-A), SLAMCF7 (CS1), SLAMF8 (BLAME), SLAMF9 (CD2F), CD28, CEACAM1(CD66a), CEACAM3, CEACAM4, CEACAM5, CEACAM6, CEACAM7, CEACAM8, CEACAM1-3AS CEACAM3C2, CEACAM1-15, PSG1-11, CEACAM1-4C1, CEACAM1-4S, CEACAM1-4L, IDO, TDO, CCR2, CD39-CD73-adenosine pathway (A2AR), BTKs, TIKs, CXCR2, CCR4, CCR8, CCR5, VEGF pathway, CSF-1, or an innate immune response modulator.

In some embodiments, an anti-GITR antibody is used in conjunction with a biotherapeutic agent and a chemotherapeutic agent. For example, provided is a method for treating cancer in a subject in need thereof comprising administering to the subject an effective amount of the anti-GITR antibody as described wherein, an anti-PD-L1 antagonist antibody (e.g., avelumab), and a chemotherapeutic agent (e.g., gemcitabine, methotrexate, or a platinum analog). In some embodiments, provided is a method for treating cancer in a subject in need thereof comprising administering to the subject an effective amount of the anti-GITR antibody as described wherein, an anti-PD-1 antagonist antibody (e.g., nivolumab (OPDIVO®) or pembrolizumab (KEYTRUDA®), and a chemotherapeutic agent (e.g., gemcitabine, methotrexate, or a platinum analog). In some embodiments, provided is a method for treating cancer in a subject in need thereof comprising administering to the subject an effective amount of the anti-GITR antibody as described wherein, an anti-CTLA-4 antagonist antibody (e.g., ipilimumab (YERVOY®)), and a chemotherapeutic agent (e.g., gemcitabine, methotrexate, or a platinum analog).

In some embodiments, the anti-GITR antibody therapy may precede or follow the other agent treatment by intervals ranging from minutes to weeks. In embodiments where the other agents and/or a proteins or polynucleotides are administered separately, one would generally ensure that a significant period of time did not expire between each delivery, such that the agent and the composition of the present invention would still be able to exert an advantageously combined effect on the subject. In such instances, it is contemplated that one may administer both modalities within about 12-24 h of each other and, more preferably, within about 6-12 h of each other. In some situations, it may be desirable to extend the time period for administration

significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

In some embodiments, an anti-GITR antibody composition comprises a second agent selected from crizotinib, palbociclib, gemcitabine, cyclophosphamide, fluorouracil, FOLFOX, folinic acid, oxaliplatin, axitinib, sunitinib malate, tofacitinib, bevacizumab, rituximab, and trastuzumab.

In some embodiments, an anti-GITR antibody composition is combined with a treatment regimen further comprising a traditional therapy selected from the group consisting of: surgery, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy, angiogenesis inhibition and palliative care.

Formulations

Therapeutic formulations of the anti-GITR antibody used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing, 2000), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and may comprise buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEENTM, PLURONICSTM or polyethylene glycol (PEG).

Liposomes containing the anti-GITR antibody are prepared by methods known in the art, such as described in Epstein, et al., Proc. Natl. Acad. Sci. USA 82:3688 (1985); Hwang, et al., Proc. Natl Acad. Sci. USA 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent
5 No. 5,013,556. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter.

10 The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in
15 macroemulsions. Such techniques are disclosed in Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing (2000).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g.
20 films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of
25 lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by, for example, filtration through sterile filtration membranes. Therapeutic anti-GITR antibody compositions are generally placed into a container
30 having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The compositions according to the present invention may be in unit dosage forms such as tablets, pills, capsules, powders, granules, solutions or suspensions, or

suppositories, for oral, parenteral or rectal administration, or administration by inhalation or insufflation.

For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical carrier, e.g. conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and other pharmaceutical diluents, e.g. water, to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention, or a non-toxic pharmaceutically acceptable salt thereof. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation composition is then subdivided into unit dosage forms of the type described above containing from about 0.1 to about 500 mg of the active ingredient of the present invention. The tablets or pills of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer that serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate.

Suitable surface-active agents include, in particular, non-ionic agents, such as polyoxyethylenesorbitans (e.g. TweenTM 20, 40, 60, 80 or 85) and other sorbitans (e.g. SpanTM 20, 40, 60, 80 or 85). Compositions with a surface-active agent will conveniently comprise between 0.05 and 5% surface-active agent, and can be between 0.1 and 2.5%. It will be appreciated that other ingredients may be added, for example mannitol or other pharmaceutically acceptable vehicles, if necessary.

Suitable emulsions may be prepared using commercially available fat emulsions, such as IntralipidTM, LiposynTM, InfonutrolTM, LipofundinTM and LipiphysanTM. The active ingredient may be either dissolved in a pre-mixed emulsion composition or alternatively it may be dissolved in an oil (e.g. soybean oil, safflower oil, cottonseed oil, sesame oil,

corn oil or almond oil) and an emulsion formed upon mixing with a phospholipid (e.g. egg phospholipids, soybean phospholipids or soybean lecithin) and water. It will be appreciated that other ingredients may be added, for example glycerol or glucose, to adjust the tonicity of the emulsion. Suitable emulsions will typically contain up to 20% oil, for example, between 5 and 20%. The fat emulsion can comprise fat droplets between 0.1 and 1.0 μm , particularly 0.1 and 0.5 μm , and have a pH in the range of 5.5 to 8.0.

The emulsion compositions can be those prepared by mixing an anti-GITR antibody with IntralipidTM or the components thereof (soybean oil, egg phospholipids, glycerol and water).

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as set out above. In some embodiments, the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably sterile pharmaceutically acceptable solvents may be nebulised by use of gases. Nebulised solutions may be breathed directly from the nebulising device or the nebulising device may be attached to a face mask, tent or intermittent positive pressure breathing machine. Solution, suspension or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

Kits

The invention also provides kits comprising any or all of the antibodies described herein. Kits of the invention include one or more containers comprising an anti-GITR antibody described herein and instructions for use in accordance with any of the methods of the invention described herein. Generally, these instructions comprise a description of administration of the anti-GITR antibody for the above described therapeutic treatments. In some embodiments, kits are provided for producing a single-dose administration unit. In certain embodiments, the kit can contain both a first container having a dried protein and a second container having an aqueous formulation. In certain embodiments, kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes) are included.

In some embodiments, the antibody is a human antibody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is a monoclonal antibody. The instructions relating to the use of an anti-GITR antibody generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (e.g., multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit), but machine-readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable.

The kits of this invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (e.g., an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-GITR antibody. The container may further comprise a second pharmaceutically active agent.

Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container.

Biological Deposit

Representative materials of the present invention were deposited in the American Type Culture Collection, 10801 University Boulevard, Manassas, Va. 20110-2209, USA, on November 10, 2016. Vector h3G7R5-VH having ATCC Accession No. PTA-123632 is a polynucleotide encoding the h3G7R5 heavy chain variable region, and vector having ATCC Accession No. PTA-123633 is a polynucleotide encoding the h3G7R5 light chain variable region. The deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and Regulations thereunder (Budapest Treaty).

This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Pfizer, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U.S.C. § 122 and the Commissioner's rules pursuant thereto (including 37 C.F.R. § 1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

Examples

Example 1: GITR expression in human peripheral blood T cells.

This example illustrates GITR expression by a minor fraction of T cell subsets in peripheral blood in humans.

The expression of GITR on T cells in the peripheral blood of healthy donors was evaluated. CD4⁺ Foxp3⁺ T regulatory cells (Tregs) contained a significantly higher fraction of GITR-expressing cells (14.35 $\pm$ 1.9%) compared with CD4⁺ Foxp3⁻ T conventional (Tconv) cells (4.75 $\pm$ 1.3% GITR positive cells), or CD8⁺ T cells (1.68 $\pm$ 0.4% GITR positive cells) (Figure 1A). The density of the anti-GITR antibody binding to the GITR positive cells was significantly lower on CD4⁺ Tconv cells compared with Treg or CD8⁺ T cells (Figure 1B). The Treg cells are a minor fraction comprising 1.723 $\pm$

0.61%, whereas CD4+ Tconv comprise 33.88 +/- 3% and CD8+ T cells 20.95 +/- 5.4% of peripheral blood mononuclear cells (Figure 1C).

Example 2: GITR and OX40 Expression in renal cell carcinoma immune cell infiltrate in humans

This example illustrates the expression levels of GITR and OX40 in Renal Cell Carcinoma (RCC)-associated Tregs.

The expression of GITR and OX40 on T cells infiltrating renal cell carcinoma were evaluated. CD4+ Foxp3+ Helios+ Tregs infiltrating RCC contained more GITR and OX40 –expressing cells than did CD8+ T cells, and more OX40-expressing Treg and Tconv cells than CD8+ T cells (Figure 2A). Of Tregs, 67.8 +/- 12.2% expressed GITR and 76.5 +/- 6.5% expressed OX40. Whereas of RCC-associated CD4+ Tconv, 22.8 +/- 7.5% expressed GITR and 32.97 +/- 3.9% expressed OX40 and CD8+ T cells, 12.3 +/- 5% expressed GITR and 9.7 +/- 3.4% expressed OX40 (Figure 2A). GITR and OX40 antibody binding density on RCC-associated T cells was also evaluated. The density of the antibodies binding GITR and OX40 was significantly higher on Tregs than on CD8+ Tcells or CD4+ Tconv cells (Figure 2B). Thus, OX40 and GITR are similarly expressed by tumor-associated T cells, and are differentially higher on Tregs compared with CD8+ Tcells and CD4+ Teff cells.

The Treg cells are a minor fraction of the CD45+ immune cells infiltrating renal cell carcinoma tissue, comprising 3.29 +/- 0.82%, whereas CD4+ Tconv comprise 15.3 +/- 3% and CD8+ T cells 41.29 +/- 5.6% (Figure 2C).

Methods

Enrichment of PBMC from concentrated whole blood

Primary human lymphocytes were isolated from concentrated whole blood provided by the Stanford Blood Bank by first diluting blood 1:4 in Phosphate Buffered Saline (PBS, without Ca²⁺ and Mg²⁺). Peripheral Blood Mononuclear Cells (PBMC) were isolated using a Ficoll gradient underlay (Ficoll-Paque PLUS, GE Healthcare Life Sciences, Pittsburgh, PA). The harvested buffy layer was washed twice with PBS and counted. Cells were re-suspended at 50*10⁶c/mL in PBS+2% Fetal Bovine Serum (FBS) and processed with CD3 negative enrichment kit (Stem Cell Tech Vancouver, BC) according to manufacturer's instructions.

Isolation and analysis of tumor associated lymphocytes:Digestion of human tumor samples

Primary tumor tissue was received whole and processed in house. The tissue
5 was cut into small pieces with a scalpel or razor blade to $\leq 0.5 \text{ cm}^3$. Enzymatic
dissociation was performed using a Human Tumor Dissociation Kit (Miltenyi Biotec, San
Diego, CA). Monosuspension of the tumor was ensured by passing cell suspension
over a 70um strainer. Lysis of RBC was run (if appropriate) using ACK lysis buffer
(Thermo Fisher Scientific, Waltham, MA) for 5 minutes at RT. Processed tumor
10 samples were frozen and banked for future analysis at -80°C in 90% human serum +
10% DMSO. For analysis, frozen dissociated tumor cells were thawed into RPMI
supplemented with 10% HI FBS and rested overnight before FACS analysis. Cells
were washed from media and re-suspended in FACS buffer.

15 Antibody staining and flow cytometric analysis

A control panel was used so background of the antibody could be corrected with
a Florescence Minus One (FMO) which did not include intracellular antibodies or Alexa
647 (A647) antibodies. Cells were stained with a A647-conjugated m3G7 antibody at
1 $\mu\text{g/ml}$ along with antibodies specific for human surface receptors CD45, CD3, CD4,
20 CD8 all from BD (BD Biosciences, San Jose, CA) diluted 1:50 in FACS buffer and a
LIVE/DEAD Fixable Violet Cell Stain (Thermo Fisher Scientific, Waltham, MA). Foxp3
fix perm kit (eBioscience, San Diego, CA.) was used to stain cells with intracellular
markers Helios (22F6, Biolegend) and Foxp3 (259D, Biolegend) per manufacturer's
instructions.

25

Analysis and gating for flow cytometric analysis.

Samples were analyzed post acquisition using Flow Jo software (Treestar, CA).
Live immune cells were identified and gated as live/dead dye negative, CD45hi. CD4+
conventional cells (CD3+CD4+Foxp3-), CD8+ cells (CD3+), and Tregs
30 (CD3+CD4+Foxp3+Helios+/-). Mean fluorescence intensity of A647 was used to
examine relative fluorescent signal and percent expression across samples using FMO
samples to set gating and single color controls to set compensation. Data values were
exported to GraphPad Prism software for graphical representation and statistical

analysis. Data sets were analyzed using paired one-way ANOVA with Geisser-Greenhouse correction and Dunnett's multiple comparison correction, or un-paired students T test as indicated. P value significance is characterized as < 0.05* significant, < 0.01** more significant, < 0.001*** highly significant.

5

Example 3 Determination of kinetics and affinity of GTR/IgG interactions in different species

This example shows the kinetics and affinity of human GTR/IgG, cynomolgus GTR/IgG, and mouse GTR/IgG interaction at 37°C with different anti-GTR antibodies. See Tables 6 and 7.

Table 6

mAb	hGTR kinetics (37°C)				cGTR kinetics (37°C)			
	k_a (1/Ms)	k_d (1/s)	$t_{1/2}$ (min)	K_D (nM)	k_a (1/Ms)	k_d (1/s)	$t_{1/2}$ (min)	K_D (nM)
m3G7				> 100				> 100
h3G7 H1						7.8E-02	0.15	> 100
h3G7 AM	1.1E+06	1.9E-02	0.61	17	6.7E+05	8.8E-03	1.3	13
h3G7 R5	9.0E+05	4.5E-03	2.6	5.0	6.0E+05	3.8E-03	3.0	6.4
h3G7 LF	6.9E+05	1.5E-02	0.76	22	3.3E+05	2.3E-02	0.51	69
m10H2	3.5E+05	7.7E-04	15	2.2	-	7.3E-03	1.6	> 100
h10H2 HU	-	5.63E-02	0.21	> 100	-	-	-	> 100
h10H2 AM	2.33E+05	1.54E-03	7.5	6.6	-	1.1E-02	1.1	> 100

15

Table 7

mAb	mGTR kinetics (37°C)			
	k_a (1/Ms)	k_d (1/s)	$t_{1/2}$ (min)	K_D (nM)
anti-mGTR r18H12 mIgG2a	2.2E+05	4.9E-04	24	2.2
anti-mGTR r21B6 mIgG2a	3.1E+05	6.5E-03	1.8	21

All experiments were performed on a Biacore T200 surface Plasmon resonance biosensor (GE Lifesciences, Piscataway NJ).

a. Human GITR/IgG and cynomolgus GTR/IgG interactions

Sensor Chip Preparation

5 The sensor chip preparation was performed at 25°C with a running buffer of 10 mM HEPES, 150 mM NaCl, 0.05% (v/v) Tween-20, pH 7.4. An anti-human Fc sensor chip was made by activating all flow cells of a Biacore CM4 sensor chip with a 1:1 (v/v) mixture of 400 mM EDC and 100 mM NHS for 7 minutes, at a flow rate of 10 µL/min. An anti-human Fc reagent (Goat Anti-Human IgG Fc, SouthernBiotech Catalog #2081-10 01) was diluted to 30 µg/mL in 10 mM Sodium Acetate pH 4.5 and injected on all flow cells for 7 minutes at 20 µL/min. All flow cells were blocked with 100 mM ethylenediamine in 150 mM Borate buffer pH 8.5 for 7 minutes at 10 µL/min.

Kinetics & Affinity Assay

15 The experiments were performed at 37°C using a running buffer of 10 mM HEPES, 150 mM NaCl, 0.05% (v/v) Tween-20, pH 7.4, 1 mg/mL BSA.

 Antibodies were captured from undiluted supernatants onto downstream flow cells (flow cells 2, 3 and 4) at a flow rate of 10 µL/min for 2 minutes. Different 20 antibodies were captured on each flow cell. Flow cell 1 was used as a reference surface. Following capture of antibodies, analyte (buffer, hGITR, or cyGITR) was injected at 30 µL/min on all flow cells for two minutes. After the analyte injection, dissociation was monitored for 10 minutes followed by regeneration of all flow cells with three 1-minute injections of 75 mM Phosphoric Acid. For each captured antibody, the 25 following analyte injections were performed: buffer, 20 nM hGITR, 200 nM hGITR, 20 nM cyGITR and 200 nM cyGITR. Buffer cycles were collected for each captured antibody for double-referencing purposes (double-referencing as described in Myszk, J. Mol. Recognit. 12:279-284 (1999). The double-referenced sensorgrams were fit globally to a simple *1:1 Langmuir with mass transport* binding model.

30

b. Mouse GTR/IgG interactions

Sensor Chip Preparation

35 The sensor chip preparation was performed at 25°C with a running buffer of 10 mM HEPES, 150 mM NaCl, 0.05% (v/v) Tween-20, pH 7.4. An anti-mouse Fc sensor

chip was made by activating all flow cells of a Biacore CM4 sensor chip with a 1:1 (v/v) mixture of 400 mM EDC and 100 mM NHS for 7 minutes, at a flow rate of 10 μ L/min. An anti-mouse immunoglobulin reagent (Rabbit anti-mouse immunoglobulin antibodies from Biacore Mouse Antibody Capture Kit, GE Lifesciences Catalog# BR-1008-38) was diluted to 60 μ g/mL in 10 mM Sodium Acetate pH 5.0 and injected on all flow cells for 7 minutes at 20 μ L/min. All flow cells were blocked with 100 mM ethylenediamine in 150 mM Borate buffer pH 8.5 for 7 minutes at 10 μ L/min.

Kinetics & Affinity Assay

The experiments were performed at 37°C using a running buffer of 10 mM HEPES, 150 mM NaCl, 0.05% (v/v) Tween-20, pH 7.4, 1 mg/mL BSA.

Purified antibodies were captured at 10 μ g/mL onto downstream flow cells (flow cells 2, 3 and 4) at a flow rate of 10 μ L/min for 2 minutes. Different antibodies were captured on each flow cell. Flow cell 1 was used as a reference surface. Following capture of antibodies, analyte (buffer, or mGITR) was injected at 30 μ L/min on all flow cells for two minutes. After the analyte injection, dissociation was monitored for 10 minutes followed by regeneration of all flow cells with one 3-minute injection of 10 mM Glycine pH 1.7. A 5-membered dilution series of mGITR was analyzed using this method, where the top concentration was 300 nM and the dilution factor was 3-fold.

The 33 nM dilution was run in duplicate. Buffer cycles were collected for each captured antibody for double-referencing purposes (double-referencing as described in Myszk, J. Mol. Recognit. 12:279-284 (1999)). The double-referenced sensorgrams were fit globally to a simple *1:1 Langmuir with mass transport* binding model.

Example 4: GITR Antibody binding to Activated T Cells and Tregs

This example illustrates that different anti-GITR antibodies bind activated Teffectors and Tregs similarly with a dose response.

Anti-GITR antibodies were evaluated for their binding characteristics to T cells and Tregs. To increase GITR expression on freshly isolated peripheral blood T cells, purified T cells and Tregs were activated *in vitro*. GITR antibodies m3G7 and m10H2 showed similar binding to activated T cells as evaluated in mean fluorescence intensity of a secondary antibody binding to the anti-human GITR antibodies (Figure 3A). GITR antibodies R5 (h3G7R5), R9 (h3G7N9) and R13 (10H2N13) had similar binding

patterns to the activated Tregs, and antibody R14 (10H2 N14) had lower density binding compared to the other antibodies (Figure 3B).

Methods

5 T cell enrichment and activation *in vitro*.

Primary human lymphocytes were isolated from concentrated whole blood provided by the Stanford Blood Bank by first diluting blood 1:4 in Phosphate Buffered Saline (PBS, without Ca²⁺ and Mg²⁺). Peripheral Blood Mononuclear Cells (PBMC) were isolated using a Ficoll gradient underlay (Ficoll-Paque PLUS, GE Healthcare Life Sciences, Pittsburgh, PA). The harvested buffy layer was washed twice with PBS and
10 counted. Cells were resuspended at 50*10⁶c/mL in PBS+2% Fetal Bovine Serum (FBS) and processed with CD3 negative enrichment kit (Stem Cell Tech Vancouver, BC) according to manufacturer's instructions. After enrichment, cells were counted and frozen in aliquots of 10-20*10⁶ in 90% FBS 10% DMSO freeze media for future use.
15 For activation, a vial of cells from a donor T cell was thawed and rested overnight in R10c (RPMI 1640+ 10%FBS +1xNEAA +1xSodium Pyruvate +1x Pen-Strep) at 1*10⁶ cells/mL in a T75 flask. Cells were counted and stimulated for 48 hours with Dynabeads Human CD3/28 T cells activating beads at a 1:1 bead to cell ratio(Thermo Fisher). After 48 hours the cells were removed from media and washed with 1x PBS. Cells were
20 titrated into a 96 well plate where they were stained with unlabeled mouse anti-human GITR antibodies (100- 0.001 ug/mL) in PBS and incubated for 30 minutes at 37°C. Unbound antibody was washed from the cells and an APC labeled Fc gamma (Fc γ) Fragment Specific goat-anti-mouse affiniPure F(ab')₂ IgG (Jackson ImmunoResearch Laboratories Inc, West Grove, PA) was used to detect primary bound antibody.
25 Following secondary stain the plate was washed with 1x PBS and spun at 335xg (1200rpm). Wells were re-suspended in PBS+2% PBS and acquired on HTS.

Sorting and Expansion of Primary Tregs

Treg isolation and expansion was based on the protocol by Putnam *et al.*
30 (Diabetes. 58(3):652-62 (2009)). Specifically, primary human lymphocytes were isolated from concentrated whole blood provided by the Stanford Blood Bank by first diluting blood 1:4 in Phosphate Buffered Saline (PBS, without Ca²⁺ and Mg²⁺). Peripheral Blood Mononuclear Cells (PBMC) were isolated using a Ficoll gradient underlay (Ficoll-

Paque PLUS, GE Healthcare Life Sciences, Pittsburgh, PA). The harvested buffy layer was washed twice with PBS and counted. Cells were re-suspended at 50×10^6 c/mL in PBS+2% Fetal Bovine Serum (FBS) and processed with CD4 negative enrichment kit (Stem Cell Tech Vancouver, BC) according to manufacturer's instructions. After
5 enrichment, cells were counted and prepared for FACS sorting.

Treg staining buffer was prepared as PBS with 2% Normal Mouse Serum (Jackson ImmunoResearch Laboratories Inc, West Grove, PA) and 2% Human AB Serum (Corning CellGro, Manassas, VA). CD4 negative enriched cells were stained for 30 minutes at RT in Treg staining buffer with CD3, CD4, CD8, CD25, and CD127
10 diluted 1:50 except CD25 which was stained diluted at 1:12.5. All antibodies provided by BD. (CD3-APC (UCHT1), CD4:PacBlue (RFP-T4, BD26986), CD8:BV786 (HIT8a, BD555635), CD127:PECy7 (M21, BD650822), CD25:PE (2A3, BD340938). After stain cells were washed and resuspended at 50×10^6 c/mL in Treg buffer. FACS sorting was run on the Aria platform (BD Biosciences, San Jose, CA). Tregs, identified by their
15 phenotypic signature of CD3+CD4+CD25^{hi}CD127^{low}, were FACS sorted and checked for 98%+ purity by acquiring a small fraction of the sorted sample. If appropriate cells were run through a second sort to maximize purity.

Cells from the FACS sort were counted and resuspended at 0.25×10^6 c/mL in Treg expansion media composed of Roswell Park Memorial Institute (RPMI)-1640
20 supplemented with 5% Human AB Serum and 1 x MEM-NEAA, Sodium Pyruvate, and Penicillin-Streptomycin (Life Technologies, San Diego, CA) with 1:1 Treg expander beads (Life Technology, San Diego, CA). After two days the media volume was doubled and recombinant human IL-2 was added at 300 IU/mL. On day 5, 7, and 9 cells were counted and subcultured to 0.25×10^6 c/mL with 300 IU/mL IL2. In addition to the
25 subculture on day 9, 1:1 Treg expander beads were added to the culture for a "re-stim" of the Tregs. On day 13 cells were harvested for binding and functional assays.

Phenotype of primary expanded Tregs

Cultured Tregs would be tracked throughout expansion to check purity. Cells
30 were re-suspended in FACS buffer, PBS with 2% FBS for analysis using fluorophore conjugated CD markers. FACS antibodies against GITR and other CD lymphocyte markers were prepared in FACS buffer at the appropriate concentration and incubated for 30 minutes at RT. Treg purity staining utilizes intracellular targets and were

processed with Foxp3 fix/perm kit (Affymetrix/eBioscience, San Diego, CA). Stained cells were resuspended in fresh FACS buffer and analyzed on a FACS analyzer LSRII or Fortessa (BD Biosciences, San Jose, CA). To generate kinetic and binding data unconjugated antibody was titrated from 10-0.001ug/mL (1:10 in serial dilutions, 5 triplicate wells per concentration) and incubated at 4c for 60 minutes with 1*10e5 expanded day 12 Tregs per well. Unbound antibody was washed from cells and an A647 labeled Fc gamma (Fc γ) Fragment Specific goat-anti-human affiniPure F(ab')₂ IgG was used to detect bound antibody (Jackson ImmunoResearch Laboratories Inc, West Grove, PA). Analysis of stained cells completed with FlowJo (Treestar). Geometric 10 mean of APC was used to calculate EC50 by log transforming the molar concentration and generating a non-linear fit curve in GraphPad Prism.

Example 5: GITR Antibodies Mediate Cell Cytotoxicity

This example shows that GITR antibodies mediate cell cytotoxicity in two *in vitro* 15 assays: 1) antibody mediated cell cytotoxicity (ADCC) mediated by un-fractionated PBMC, and 2) antibody mediated cell phagocytosis (ADCP) mediated by macrophages.

hIgG1 anti-GITR antibodies m3G7 and m10H2 exhibited similar levels of ADCC of a GITR+ T cell line compared with isotype control, with maximal target cell killing at 0.01ug/ml of 78.5% for m3G7 and 83.4% for m10H2 (Figure 4A)

20 Tumor associated macrophages (TAM) have been implicated as the cell population mediating antibody directed cytotoxicity of Tregs in mice (Simpson et. al. J. Exp. Med. 210(9): 1695-1710 (2013); Bulliard et. al. J. Exp. Med. 210(9): 1685-93 (2013)). We evaluated GITR antibody-mediated cytotoxicity of a GITR+ T cell line by human monocyte-derived macrophages. hIgG1 GITR antibodies enabled macrophages 25 to mediate a dose dependent killing of GITR+ T cells (Figure 4B). The GITR antibodies were not equivalent; antibody h3G7 R5 ("R5"), h3G7 R9 ("R9"), h10H2 R13 ("R13"), and h10H2 R14 ("R14"), had an EC50 of 919.7, 692.2, 4450. 2013 pM, respectively. Macrophage-mediated cytotoxicity was dependent on the Fc of the GITR antibody. Cytotoxic activity was lost with N297A mutation that reduced binding to FcR (Chao *et al.* 30 Immunol. Invest. 38(1): 76-92 (2009)). (Figure 4C).

Methods

ADCC assay with Effector PBMC and GITR+ target

Primary human lymphocytes were isolated from concentrated whole blood provided by the Stanford Blood Bank by first diluting blood 1:4 in Phosphate Buffered Saline (PBS, without Ca^{2+} and Mg^{2+}). Peripheral Blood Mononuclear Cells (PBMC) were isolated using a Ficoll gradient underlay (Ficoll-Paque PLUS, GE Healthcare Life Sciences, Pittsburgh, PA). The harvested buffy layer was washed twice with PBS and counted. PBMC were re-suspended at 3×10^6 cells/mL in RPMI supplemented with 10% FBS and 1 x MEM-NEAA, Sodium Pyruvate, and Penicillin-Streptomycin (Life Technologies, San Diego, CA). GITR+ Jurkat T cell line were stained in PBS with Cell Trace Violet (Life Technologies, San Diego, CA). After labeling, Jurkat's were re-suspended at 3×10^5 cells/mL. A dilutions series of the anti-GITR antibodies were made by titrating 1:10 a 40ug/mL solution to .0004ug/mL (6 concentrations). 50uL of titrated antibody was plated per well of a 96-well U-bottom plate along with 50uL of labeled Jurkats and 100uL of PBMC to create a 20:1 effector to target ratio with final antibody [10-.0001ug/mL]. The plates were incubated overnight (~18hours) and the next day the plates were harvested for analysis.

The U-well plates were spun at 1200rpm to remove media from cells and washed with PBS. PI (Life Technologies, San Diego, CA) at 1:500 in PBS was added in 30uL per well for 10 minutes at room temp. To the PI approximately 100uL of PBS supplemented with 2% FBS (to quench PI) was added bringing the volume to 100-150uL per well with before HTS analysis. Just prior to acquisition, 1×10^4 Count Bright counting beads (Life Technologies, San Diego, CA) were added to each well. The ratio of viable Treg target count to bead count would be used to normalize across the plate and be compared to ratio of control IgG1 to generate the percent cytotoxicity.

ADCP assay: Monocytes derived macrophage effectors and expanded Treg target

PBMC were prepared as described above. Using the EasySepTM CD14+ negative enrichment kit (Stem Cell Tech Vancouver, BC) monocytes were positively enriched and resuspended in R10c supplemented with 20 ng/mL MCSF (R&D systems, Minneapolis, MN) at 1×10^6 cells/mL and plated in a T175 with 55-65mL cell suspension per flask. Monocytes strongly adhered to the tissue culture flask and were not removed when the media was refreshed with new R10c supplemented with 20

ng/mL MCSF on day 2 and on day 5 of culture. The media on day 5 was also supplemented with 10ng/mL IL10 (R&D systems, Minneapolis, MN). After a week of the culture the cells were harvested and plated at 1×10^5 cells/well in 96 well flat plates in activation media consisting of 1mg/mL PGE2 (Sigma, St Louis, MO), 10ng/mL each
5 TNF, IL1B, and IL6 (all R&D systems, Minneapolis, MN). The following day the plated monocytes were washed out of activation media and 5×10^4 Cell Trace Violet labeled Jurkat bulk GITR+ cells were added per well (for a final effector to target ratio of 2:1) along with either control IgG1 antibody or titrated GITR antibodies. The plates were incubated overnight (~18hours) and the next day the plates were harvested for
10 analysis. Cells from the 96-well flat plates were thoroughly triturated and transferred to 96-well U-well plate. The plate was spun at 1200rpm to remove media from cells and washed with PBS. The remainder of the procedure follows section ADCC Acquisition and Analysis beginning with the PI stain.

15 **Example 6: T Cell Cytokine Production Enhancement by the GITR Antibodies**

The example shows that the anti-GITR antibodies of the present invention enhance T cell cytokine production *in vitro*.

GITR is a co-stimulatory receptor on T cells. Ligation of GITR with its ligand causes enhanced proliferation of differentiated T helper cells (Tone et al. Proc. Natl.
20 Acad. Sci. 100(25): 15059-15064 (2003)). Boosted T cell activation and pro-inflammatory cytokine production, such as IFN γ translates to tumor regression in mouse models (Ko et al. J. Exp. Med. 2005. 202(7): 885-891 (2005)). In this example, the GITR antibodies for T cell activation by secretion of IFN γ and TNF α was evaluated. A T
25 cell hybridoma (3A9 ; Dustin et al. J. Immunol. 157(5):2014-2021 (1996)) expressing human GITR was activated with an anti-CD3 antibody (Clone 2C11, Ebioscience) and increasing concentrations of plate immobilized the anti-GITR antibodies (h3G7 R5 ("R5"), h3G7 R9 ("R9"), h10H2 R13 ("R13"), and h10H2 R14 ("R14")). The four GITR
antibodies caused enhanced secretion of TNF α in a dose dependent manner until 0.01 - 0.1 μ g/ml. At higher GITR antibody concentrations, the TNF α secretion dropped
30 (Figure 5A). A similar experiment was conducted with the GITR expressing 3A9 cells and CD3 antibodies 2C11 in the presence of a B cell lymphoma line (LK35.2) to provide FcRs necessary for clustering and signaling of GITR (Zhou et al. Proc. Natl. Acad. Sci. 105(14): 5465-5470 (2008)). GITR antibodies R5, R9, R13 and R14 were added in

solution and all four enhanced secretion of $\text{TNF}\alpha$ between concentrations of 0.01 and 10 $\mu\text{g/ml}$ (Figure 5B).

The effect of GITR antibodies on cytokine secretion of activated primary T cells was evaluated *in vitro*. GITR antibodies R5, R9, R13 and R14 caused dose dependent increases in secreted $\text{IFN}\gamma$ and $\text{TNF}\alpha$ from CD4^+ and CD8^+ T cells peaking between 0.01 and 0.1 $\mu\text{g/ml}$, and then dropping off at higher concentrations (Figure 5C and Figure 5D). Accordingly, this example shows that the GITR antibodies have T cell activating attributes that are dose-dependent.

10 Methods

GITR antibody T cell agonist assay

To prepare immobilized GITR antibody culture plates, sterile Nunc maxisorp 96-flat well plates were coated overnight at 4c with 100 μL of 2 $\mu\text{g/mL}$ Anti-Mouse and Anti-Human Fc fragment specific protein diluted in PBS. After washing 2x with 1x PBS, 100 μL of Mouse CD3 (2C11) at 10 ng/mL was incubated at 37°C for 60-90 minutes. GITR antibodies R5, R9, R13 and R12 were titrated from 10-0.0001 $\mu\text{g/mL}$ in PBS and 100 μL was added to each well with triplicates for each concentration. The plate was incubated at 37°C for 60-90 minutes and washed before adding cells

Using the prepared immobilized GITR plates, 50,000 human GITR+ 3A9 cells were plated per well in 200 μL of DMEM+10% FBS media. After 48hrs of culture the supernatants were collected for cytokine analysis using the Mouse Soluble Protein Assay (Becton-Dinkinson).

The human GITR+ 3A9 cells were also tested in a soluble GITR antibody assay utilizing LK35.2, a mouse B cell lymphoma line. In this assay a 2:1 ratio of 3A9 to LK35.2 was plated along with mouse anti-CD3 antibody (2C11) at 0.1 $\mu\text{g/mL}$ and GITR antibodies titrated from 10-0.0001 $\mu\text{g/mL}$.

Human cytokine secretion was also assayed using freshly isolated human pan T cells ($\text{CD4}^+\text{CD8}^+$) and plating them onto immobilized plates prepared as described above. 100,000 cells were plated per well in RPMI+ 10% FBS and supernatants were collected after 4 days of incubation.

Example 7: GITR Immunohistochemistry Staining

This example describes the optimization of an anti-GITR antibody as a immunohistochemistry (IHC) reagent that specifically stains formalin fixed paraffin embedded (FFPE) tissues.

- 5 The anti-human GITR antibody m10H2 was evaluated and showed correlated density of staining of T cell lines expressing no, low and high human GITR (Figure 6 A, Figure 6 B, and Figure 6C). Confirmation of the minimal, low and high GITR expression is shown by flow cytometry staining surface protein with the m10H2 clone (Figure 6D) and quantitative PCR and normalization to actin B expression (Example 6E).
- 10 Furthermore, FFPE tissues isolated from NSCLC tumors were stained with m10H2 (Figure 6F) or isotype control antibody (Figure 6G). The m10H2 staining was confined to the membrane of cells within the mononuclear infiltrate in the tumor tissue (Figure 6F). Accordingly, this example demonstrates that m10H2 is a validated anti-GITR antibody that can be used on FFPE tissues to quantify GITR expression.

15

Methods

Surface staining on GITR overexpressing Jurkat cell lines:

- Prior to the IHC analysis, the three Jurkat lines were analyzed for surface GITR expression by FACS using an A647 labeled m10H2 antibody. These lines included a
- 20 parent Jurkat cell line as well as two lines generated by transfection of a GITR plasmid into the Jurkat parent cells. Approximately 5×10^6 cells from each line was stained with labeled antibody at a concentration of 1 μ g/mL. Following stain cells were washed from unbound antibody using 1x PBS and analyzed by FACS.

Results

- 25 By FACS the Jurkat parent cells had no detectable GITR expression while the two transduced lines had positive staining. There was approximately a log and a half fold difference between the median GITR expression on the two lines leading us to distinguish them as GITR+ low expresser and GITR+ high expresser.

Quantitative GITR Expression analysis by qPCR:

- 30 Quantitative analysis of GITR expression on Jurkat parent, GITR+ low, and GITR+ high was run by qPCR. RNA was isolated from $5-10 \times 10^6$ cells from each line using the RNeasy Mini Kit (Quiagen). A cDNA template was made using 200ng of the

isolated RNA using the High Capacity cDNA Reverse Transcription kit (Thermo Fisher). TaqMan FAST reagents were used to run the qPCR along with the cDNA template. Primers for both the housekeeping gene Actin B (Hs99999903_m1) and a custom specific GTR primer were run in each reaction. The custom GTR primer was generated by ABI using the vector sequence of the GTR transfected plasmid (sequence not disclosed by ABI). The qPCR was run on a Viia7 system according to manufacturer's instruction and TaqMan FAST thermal cycling specifications. The delta Ct was calculated using the formula: $2^{-(\text{Ct}(\text{GTR}) - \text{Ct}(\text{ActB}))}$.

10 Results

The qPCR analysis confirms the expression ranking of the three lines with the GTR+ low having 2.7 logs higher expression than parent and the GTR+ high line having 2 logs higher GTR expression than the GTR+ low line.

15 IHC Cell Processing:

With GTR expression levels assayed by FACS and qPCR, cells from the Jurkat parent (GTR negative), GTR+ low, and GTR+ high lines were prepared and processed for sectioning as follows. Between 20 and 30*10e6 cells were collected in a 50mL conical and spun at ~750xg for 10 minutes. After removing the supernatant the pellet was transferred to a 15mL conical in 10% neutral buffered formalin and spun at ~750xg for 10 minutes. HistoGel prepared at 50°C was mixed with the cell pellet and allowed to chill by refrigeration at 4°C. The HistoGel cell pellet was then transferred into 10% neutral buffered formalin for fixation for >12 hours and transferred to a cassette for paraffin embedding. Sectioning of slides was performed to generate slices of pellet at 4µm thickness. For IHC staining the UltraVision One Detection System from Thermo Scientific was used. Briefly, slides were prepared by baking at 60° C for 1 hour and deparaffinized in three rounds of xylene wash for three minutes each. The sectioned pellet was rehydrated by sequential incubations for two minutes each in 100% ETOH (2x), 95% ETOH (1x), 70% ETOH (1x), and PBS (1x). Antigen retrieval was performed by processing slides for 5 minutes in DIVA buffer and Decloaking chamber (both from Biocare Medical) heated to 110°C and pressurized to 5 PSI followed by two washes in PBS. Slides were blocked in 3% H₂O₂ (Peroxidase) for 10 min and washed in PBS. Primary antibody, m10H2, was diluted to 1µg/mL in blocking solution and slides were

incubated overnight at 4°C. After washing in PBS (3x) for 3 min slides were incubated in HRP Polymer for 30 minutes at Room Temperature and washed in PBS (3x). DAB reagent was prepared and slides were exposed for 5 minutes. A counterstain was run with Hematoxylin and, after dehydration, mounted slides were complete. The slides
5 were analyzed for GITR levels.

Staining of NSCLC samples

Prepared slides of Non-Small Cell Lung Carcinoma (NSCLC) samples were received from an internal bank. The UltraVision One Detection System from Thermo
10 Scientific was used as described above. As with the staining of the cell pellets 1ug/mL of m10H2 anti-GITR antibody was assayed along with a mouse IgG1 control antibody at 5ug/mL.

Example 8: Anti-tumor Activity of GITR Antibodies

15 This example shows that the anti-mouse GITR antibodies with a mIgG2a isotype have tumor growth reduction activity in a therapeutic treatment model of CT26 colon carcinoma.

The mIgG2a most closely reflects the human IgG1 isotype that can mediate ADCC activity while the mIgG1 isotype most closely resembles hIgG2a that has
20 minimal ADCC activity (Nimmerjahn and Ravetch, Science, 310: 1510-1512 (2005)).

The anti-GITR 21B6 mIgG2a isotype antibody had anti-tumor activity whereas the mIgG2a isotype control antibody did not (Figure 7A-Figure 7D). The 21B6 mIgG2a exhibited 95%, 94%, 102% tumor growth inhibition at 0.2, 1 and 5 mpk (mg/kg) respectively (Table 8, Figure 7A – Figure 7D). The mIgG1 isotype induced 33%, 9%,
25 and 22% tumor growth inhibition at 0.2, 1 and 5 mg/kg respectively (Table 9, Figure 7E – Figure 7H). At the end of study (43 days post initial dose), 80% (8/10) of mice survived in the 5mpk mIgG2a group, while 44.4% (4/9) survived in the 1 mg/kg mIgG2a group, and 40% (4/10) survived in the 0.2 mg/kg mIgG2a group (Table 8 and Table 9, Figure 7I). The mIgG1 treatment resulted in no survival; all needed to be sacrificed due
30 to tumor burden prior to the end of study (43 days) (Table 8 and Table 9, Figure 7I).

Table 8

Dose	TGI	% Tumor Free
5mpk	102.25	80%
1mpk	94.17	44.44%
0.2mpk	95.30	40%

Table 9

Dose	TGI	% Tumor Free
5mpk	21.76	0
1mpk	8.70	0
0.2mpk	33.19	0

5 The inhibition of tumor growth mediated by 21B6 IgG2a correlated with an increase in the ratio of CD8:Treg infiltrating the tumor (Figure 7J). The 1 mg/kg dose of 21B6 IgG2a resulted in the highest CD8:Treg ratio (10.9 X IgG2a isotype control, $p=0.049$). Treatment with 21B6 IgG2a also resulted in an increase in the ratio of CD4 Teff:Treg infiltrating the tumor (Figure 7K) at all doses with the 0.2 mg/kg 21B6 IgG2a dose providing the highest mean ratio (4.7 X higher than IgG2a isotype control). Statistical significance was observed between 5 mg/kg, 1 mg/kg, and 0.2 mg/kg compared to isotype IgG2a control with p values of 0.043, 0.003, and <0.0001 , respectively. These changes were not observed in the mice treated with the 21B6 IgG1 where the CD8 T cell:Treg and CD4 Teff cell: Treg showed no statistical significance compared to IgG1 isotype control (Figure 7J and Figure 7K).

15 Tumor growth inhibition corresponded with a loss of intratumoral Tregs in the 21B6 IgG2a treated mice (Figure 7L). All dose levels of 21B6 IgG2a reduced the number of Treg cells per gram associated with the primary tumor although these changes were not statistically significant. There were reductions of CD8+ T cells at 20 5mg/kg (Figure 7M), and CD4+ T effector (Teff) cells at 5 mg/kg and 1 mg/kg (Figure 7N), although these changes were not statistically significant. Similarly, all doses of 21B6 mIgG2a reduced the proportion of Tregs from 2.56% within the CD45+ immune

cells infiltrating the tumors to 0.69% ($p = 0.0018$) with the 0.2mg/kg dose, to 0.42% ($p = 0.0005$) with the 1mg/kg dose, and to 1.29% ($p = 0.0287$) with the 5mg/kg dose (Figure 7O). The proportion of Treg in the immune infiltrate was not affected by 21B6 mIgG1 treatment (Figure 7O). Changes in the proportion of CD8+ T cells and CD4+ Teff cells in the tumor infiltrate were non-significant (Figure 7P and Figure 7Q). Together, these analyses suggest that reduced tumor Tregs account for the enhanced Teffector cell : Treg ratio, anti-tumor efficacy and mouse survival following GITR antibody administration, which was dependent on a mIgG2a and not a mIgG1 Fc isotype.

T cell subset frequencies were evaluated in a peripheral immune compartment; the spleens of the tumor bearing, 21B6 treated mice. The 0.2 mg/kg dose had the most effect, reducing Tregs from 2.46% of CD45+ cells in control antibody treated mice to 1.11% ($p = 0.0006$) in mIgG2a treated mice, and reducing CD4+ Teff from 15.06% of CD45+ cells in control antibody treated mice to 11.22% ($p = 0.0121$) in mIgG2a treated mice (Figure 7R and 7T). Changes at other doses were non-significant. CD8+ T cells were unaffected at any dose (Figure 7S).

Methods

Cloning and Expression of m21B6 IgG2a

Anti-mGITR 21B6 heavy and light chain variable domain DNA sequences were obtained from cDNA generated using RT-PCR from a Lewis Rat hybridoma fusion. The resulting translated amino acid sequence was codon optimized for Human Epithelial Kidney 293 expression using proprietary algorithms from Thermo Fisher Scientific Geneart. Synthesized codon optimized variable domains were then cloned into the mammalian expression vectors pARC mIg2a and pARC mKappa using BssHII/BstEII and ApaLI/PacI respectively. Correct clones for light and heavy chain constructs were sequence confirmed and correspondingly named h3G7R5-VL and h3G7R5-VH.

Antibody Expression and Purification

A 1:1 mass ratio of plasmids h3G7R5-VL and h3G7R5-VH were co-transfected into Expi293 cells using a PEI-based transfection protocol (0.5 mg each plasmid per 2 million cells). Five days post transfection, the transfected cell culture was harvested, centrifuged, and filtered using a 0.2uM membrane. Filtered supernatant was then purified using Protein A affinity chromatography (MabSelect Sure, GE Healthcare Life

Sciences, Pittsburgh, PA) and then sized to remove aggregated protein (Superdex S200 26/60 GE Healthcare Life Sciences, Pittsburgh, PA). SDS-PAGE in conjunction with Biacore analysis identified monomeric active protein fractions.

5 Syngeneic Tumor Model: CT26

Animals

Mice cohorts of 7-week old female BALB/c mice (BALB/cAnNCrI) obtained from Jackson Laboratories (Bar Harbour, ME) and housed in a pathogen free facility at Pfizer (South San Francisco, CA) in accordance with the Institutional Animal Care and Use Committee (IACUC) protocol. The animals were 8-weeks old when initiated into the experiment.

Cell Culture

The mouse colon carcinoma cell lines CT26 (CRL-2638) was obtained from the American Type Culture Collection (ATCC, Manassas, VA). The cells were propagated in recommended basic media containing 10% FBS and 1% penicillin–streptomycin solution (all Thermo Fisher Scientific, Waltham, MA). Cells were harvested with TrypLE Select (Thermo Fisher Scientific, Waltham, MA) and washed with a 2x volume of media containing serum before pelleting the cells. The pellet was re-suspended in basic media without serum and a cell count and viability check was performed. The CT26 cells were brought to a concentration of 2×10^6 cells per mL in basic media for injection.

In Vivo Experimental Protocol

To establish tumors, 100uL of cell suspension was injected SC into the right flank of the mice. The implants were monitored until the tumors reached an average volume of approximately 80mm^3 and were randomized into groups of mice having nearly equal average tumor volumes and SEM. 21B6 antibody was prepared for a dosing at 5, 1, and 0.2 mg/kg with an isotype control IgG1 (clone MOPC-21) and IgG2 isotype control (C1.18.4, Bioxcell, West Lebanon, NH) at 5mg/kg. Treatment began on the day of randomization (Day 0). Mice were weighed and dosed IP every 2-3 days for a total of three doses.

Evaluation of Tumor Growth

Twice weekly the tumor length and width (in millimeter units) would be taken with digital calipers (Mitutoyo, Aurora, IL). Tumor volumes were calculated using the length and width values with the formula: $V = \frac{1}{2} L \times W^2$, where L (length) is defined as the longest diameter of the tumor and W (width) is perpendicular to L. Efficacy measurements were continued to day 20, and mice were continued to be monitored up to 40 days for survival analysis.

Tumor TIL and Splenocyte Analysis

Tumor and spleen were dissected from 4-5 mice per group one day after the last dose. Tumors were dissociated with program IMP-002 on the Miltenyi Octet (Miltenyi Biotec, San Diego, CA) according to manufacturer instructions. Spleens were manually dissociated over a 70uM mesh filter and red blood cells were lysed with ACK buffer (Thermo Fisher Scientific, Waltham, MA). Both cell populations were washed with PBS and stained with the following fluorescence labelled antibodies for FACS analysis: LIVE/DEAD (Thermo Fisher Scientific, Waltham, MA), CD90.2, CD4, and CD8 all from BD Biosciences and GITR and CD45 from eBioscience. Following extracellular staining for 30 minutes at 4°C the cells were washed with PBS+2% FBS and processed with the eBioscience Foxp3 Fix/Per kit (eBioscience, San Diego, CA) per manufacturer's instructions. Cells were stained with intracellular markers Foxp3-eFlour450 (eBioscience, San Diego, CA) and Ki-67-BV605 (BD Biosciences, San Jose, CA). Analysis of T cell subsets was conducted in FlowJo (FlowJo, Ashland, OR) and plotted using GraphPad Prism software.

Example 9 Combination Therapy Using an Anti-GITR Antibody and Other Therapeutic Agent

This example describes the evaluation of the anti-GITR surrogate antibody 21B6 mIgG2a in combination with other immune-targeting antibodies for therapeutic treatment of tumors.

The CT26 colon carcinoma model underwent complete tumor regression with single agent 21B6 IgG2a (Example 8), so the B16F10 melanoma model is evaluated because it is less responsive to single agent immune targeting therapy (See, e.g., Mosley et al., Cancer Immunology Research, 5(1)29-41 (2017)). The treatment protocol

is similar to the studies described in Example 8: B16F10 tumors are implanted and once reach an average size of 80mm³ are randomized and treated with 3 doses of 1mg/kg 21B6 IgG2a and the established efficacious dose of the combination partner IP every 2-3 days for a total of three doses. The doublet combination of the anti-GITR antibody 21B6 IgG2a with anti-PD-L1, anti-4-1BB, anti-OX40 and anti-PD-1 antibodies all show improved tumor growth inhibition in comparison to either single agent treatment alone.

These data show that the anti-GITR antibodies of the present invention can be combined with immune check-point inhibitors or T cell stimulatory antibodies for improved tumor growth inhibitory effects.

Although the disclosed teachings have been described with reference to various applications, methods, kits, and compositions, it will be appreciated that various changes and modifications can be made without departing from the teachings herein and the claimed invention below. The foregoing examples are provided to better illustrate the disclosed teachings and are not intended to limit the scope of the teachings presented herein. While the present teachings have been described in terms of these exemplary embodiments, the skilled artisan will readily understand that numerous variations and modifications of these exemplary embodiments are possible without undue experimentation. All such variations and modifications are within the scope of the current teachings.

All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated by reference in their entirety. In the event that one or more of the incorporated literature and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls.

The foregoing description and Examples detail certain specific embodiments of the invention and describes the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

Claims

It is claimed:

1. An isolated antibody that specifically binds to GITR (Glucocorticoid
5 Induced Tumor Necrosis Factor Receptor Family Related Protein) and comprises:
- a heavy chain variable region (VH) comprising a VH complementarity
determining region one (CDR1), VH CDR2, and VH CDR3 of the VH having an amino
acid sequence selected from the group consisting of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14,
or 112; and/or
- 10 a light chain variable region (VL) comprising a VL CDR1, VL CDR2, and VL
CDR3 of the VL having an amino acid sequence selected from the group consisting of
SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or 111.
2. The isolated antibody of claim 1, wherein the antibody comprises a VH
CDR1 comprising the amino acid sequence of SEQ ID NO: 24, 25, 26, 32, 33, 34, 35,
15 39, 40, 41, 115, 116, 117, 63, 64, or 65, a VH CDR2 comprising the amino acid
sequence of SEQ ID NO: 27, 28, 44, 45, 66, or 67, and a VH CDR3 comprising the
amino acid sequence shown in SEQ ID NO: 29, 46, or 68; and/or a VL CDR1
comprising the amino acid sequence shown in SEQ ID NO: 21, 30, 36, 42, 113, or 31, a
VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 22, 37, 43, 114,
20 or 61, and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 23,
38, or 62.
3. The isolated antibody of claim 1, wherein the antibody comprises a VH
comprising the amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, or
112, or a variant thereof with one or several conservative amino acid substitutions in
25 residues that are not within a CDR.
4. The isolated antibody of claim 3, wherein the antibody comprises a VL
comprising the amino acid sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or 111,
or a variant thereof with one or several amino acid substitutions in amino acids that are
not within a CDR.
- 30 5. The isolated antibody of claim 1, wherein the antibody comprises a VH
comprising the amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, or

112, and a VL comprising the amino acid sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or 111.

6. An isolated antibody that specifically binds to GITR (Glucocorticoid Induced Tumor Necrosis Factor Receptor Family Related Protein) and comprises:

5 a heavy chain variable region (VH) comprising a VH complementarity determining region one (CDR1), VH CDR2, and VH CDR3 of the VH having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 18, 20, 121, and 123; and/or

10 a light chain variable region (VL) comprising a VL CDR1, VL CDR2, and VL CDR3 of the VL having an amino acid sequence selected from the group consisting of SEQ ID NO: 15, 17, 19, 120, and 122.

7. The isolated antibody of claim 6, wherein the antibody comprises a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 50, 51, 52, 56, or 57, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 53, 54, 58, or 59, and a VH
15 CDR3 comprising the amino acid sequence shown in SEQ ID NO: 55, 60, or 124; and/or a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 47, a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 48, and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 49.

8. An isolated antibody that specifically binds to GITR (Glucocorticoid
20 Induced Tumor Necrosis Factor Receptor Family Related Protein) and comprises:

a heavy chain variable region (VH) comprising a VH complementarity determining region one (CDR1), VH CDR2, and VH CDR3 of the VH having an amino acid sequence selected from the group consisting of SEQ ID NO: 70 and 72; and/or

25 a light chain variable region (VL) comprising a VL CDR1, VL CDR2, and VL CDR3 of the VL having an amino acid sequence selected from the group consisting of SEQ ID NO: 69 and 71.

9. The isolated antibody of claim 7, wherein the antibody comprises a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 32, 76, 77, 84, 85, or 86, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 78 or 79, and a VH
30 CDR3 comprising the amino acid sequence shown in SEQ ID NO: 80 or 87; and/or a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 73 or 81, a VL CDR2

comprising the amino acid sequence shown in SEQ ID NO: 74 or 82, and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 75 or 83.

10. The isolated antibody of any one of claims 1-9, wherein the antibody comprises a constant region.

5 11. The isolated antibody of any one of claims 1-10, wherein the antibody has an isotype that is selected from the group consisting of IgG₁, IgG₂, IgG_{2Δa}, IgG₄, IgG_{4Δb}, IgG_{4Δc}, IgG₄ S228P, IgG_{4Δb} S228P and IgG_{4Δc} S228P.

10 12. An isolated antibody that specifically binds to GITR and competes for binding to GITR with, and/or binds to an epitope that is the same as or overlaps with the epitope on GITR recognized by, the antibody of any one of claims 1-2 and 6-9.

13. An isolated anti-GITR antibody, wherein the antibody comprises a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 39, 40, or 41, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 44 or 45, a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 29, a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 42, a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 43, and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 38.

14. The isolated antibody of any one of claims 1-13, wherein the antibody promotes Treg depletion and activates T effector cells as measured in vivo using a syngeneic tumor model in CT26 cells.

15. The isolated antibody of any one of claims 1-14, wherein the antibody increases the release of the inflammatory cytokines IFN γ and TNF α from the T cells.

16. The isolated antibody of any one of claims 1-15, wherein the antibody enhances anti-tumor immune response.

25 17. The isolated antibody of any one of claims 1-7 and 13, wherein the antibody binds human GITR.

18. An isolated cell line that produces the antibody of any one of claims 1-2 and 6-9.

30 19. An isolated nucleic acid encoding the antibody of any one of claims 1-2 and 6-9.

20. A recombinant expression vector comprising the nucleic acid of claim 19.

21. A host cell comprising the expression vector of claim 20.

22. A hybridoma capable of producing the antibody of any one of claims 1-2 and 6-9.

5 23. A method of producing an anti-GITR antibody, the method comprising: culturing a cell line that recombinantly produces the antibody of any one of claims 1-2 and 5-9 under conditions wherein the antibody is produced; and recovering the antibody.

10 24. A method of producing an anti-GITR antibody, the method comprising: culturing a cell line comprising nucleic acid encoding an antibody comprising a heavy chain comprising the amino acid sequence shown in SEQ ID NO: 110 and a light chain comprising the amino acid sequence shown in SEQ ID NO: 109 under conditions wherein the antibody is produced; and recovering the antibody.

15 25. The method of claim 24, wherein the heavy and light chains of the antibody are encoded on separate vectors.

26. The method of claim 24, wherein the heavy and light chains of the antibody are encoded on the same vector.

27. A pharmaceutical composition comprising the antibody of any one of claims 1-2 and 5-9, and a pharmaceutically acceptable carrier.

20 28. A kit for the treatment of cancer comprising the pharmaceutical composition of claim 27.

25 29. A method for treating cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of the anti-GITR antibody of any one of claims 1-2 and 5 such that one or more symptoms associated with the cancer is ameliorated in the subject.

30 30. The method of claim 29, wherein the cancer is selected from the group consisting of B-cell related cancer, gastric cancer, small intestine cancer, sarcoma, head and neck cancer, thymic cancer, epithelial cancer, salivary cancer, liver cancer, biliary cancer, neuroendocrine tumors, stomach cancer, thyroid cancer, lung cancer, mesothelioma, ovarian cancer, breast cancer, prostate cancer, esophageal cancer,

pancreatic cancer, glioma, renal cancer, bladder cancer, cervical cancer, uterine cancer, vulvar cancer, penile cancer, testicular cancer, anal cancer, choriocarcinoma, colorectal cancer, oral cancer, skin cancer, Merkel cell carcinoma, glioblastoma, brain tumor, bone cancer, eye cancer, and melanoma.

5 31. The method of claim 29 or 30, wherein the cancer is relapsed or refractory.

 32. The method of any one of claims 29-31, wherein the cancer is locally advanced or metastatic melanoma, squamous cell head and neck cancer (SCHNC), ovarian cancer, renal cancer, gastric cancer, or lung cancer.

10 33. The method of any one of claims 29-32, wherein the method further comprises administering an effective amount of a second therapeutic agent.

 34. The method of claim 33, wherein the second therapeutic agent is an antibody selecting from the group consisting of an anti-CTLA-4 antibody, an anti-4-1BB antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-TIM3 antibody, an anti-LAG3 antibody, an anti-TIGIT antibody, an anti-OX40 antibody, an IL-8 antibody, an anti-HVEM antibody, an anti-BTLA antibody, an anti-CD40 antibody, an anti-CD40L antibody, anti-CD47 antibody, an anti-CSF1R antibody, an anti-CSF1 antibody, an anti-MARCO antibody, an anti-CXCR4 antibodies, an anti-VEGFR1 antibody, an anti-VEGFR2 antibody, an anti-TNFR1 antibody, an anti-TNFR2 antibody, an anti-CD3
15 bispecific antibody, an anti-CD19 antibody, an anti-CD20, an anti-Her2 antibody, an anti-EGFR antibody, an anti-ICOS antibody, an anti-CD22 antibody, an anti-CD 52 antibody, an anti-CCR4 antibody, an anti-CCR8 antibody, an anti-CD200R antibody, an anti-VISG4 antibody, an anti-CCR2 antibody, an anti-LILRb2 antibody, an anti-CXCR4 antibody, an anti-CD206 antibody, an anti-CD163 antibody, an anti-KLRG1 antibody, an
20 anti-FLT3 antibody, an anti-B7-H4 antibody, an anti-B7-H3 antibody, and a second anti-GITR antibody.

 35. The method of claim 34, wherein the second therapeutic agent is a cytokine, $TNF\alpha$, a PAP inhibitor, an oncolytic virus, a kinase inhibitor, an ALK inhibitor, a MEK inhibitor, an IDO inhibitor, a GLS1 inhibitor, a tyrosine kinase inhibitor, a CART
25 cell or T cell therapy, a TLR agonist, or a tumor vaccine.

Figure 1A

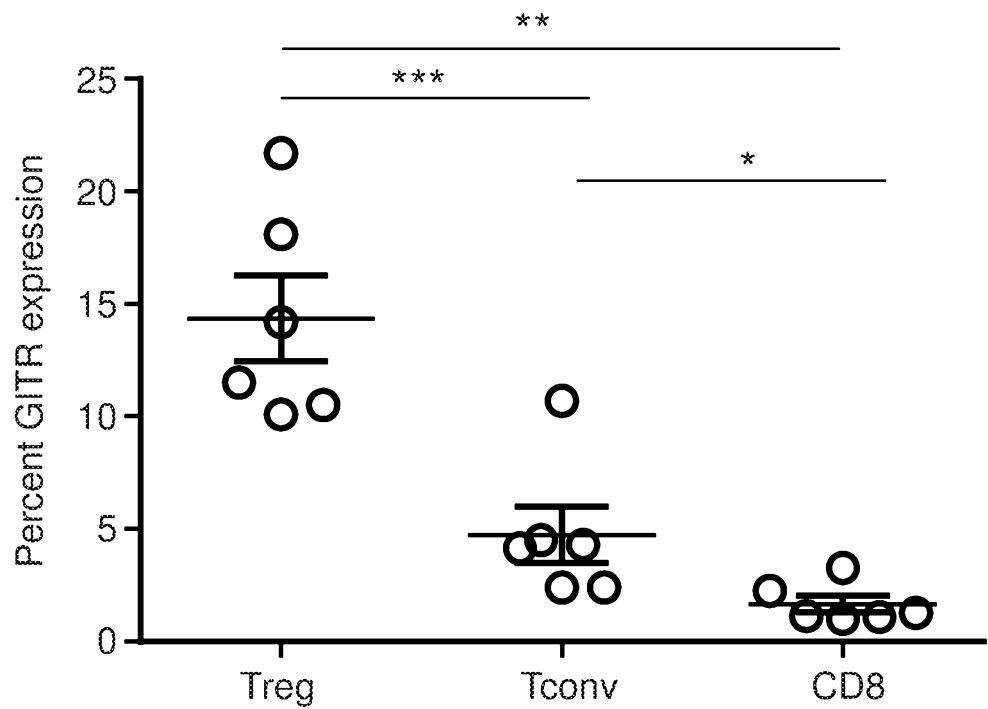


Figure 1B

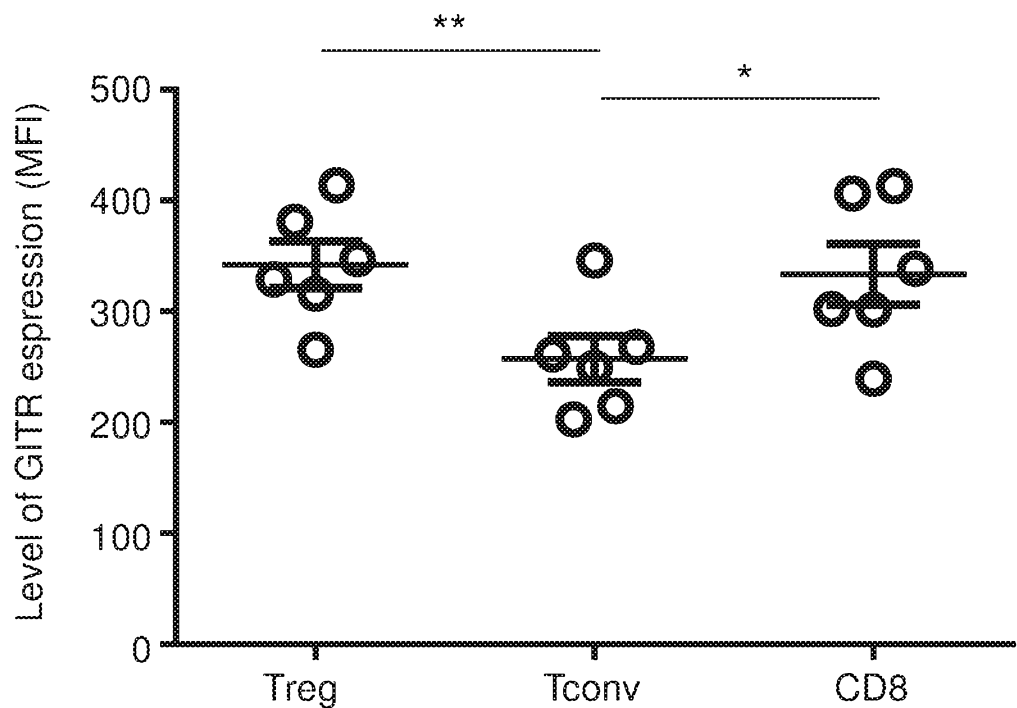


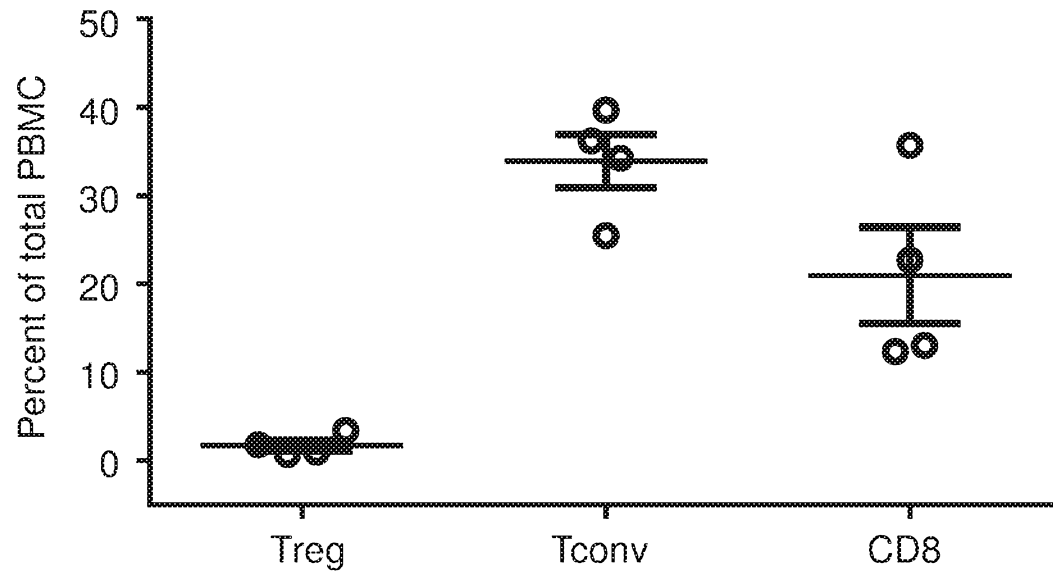
Figure 1C

Figure 2A

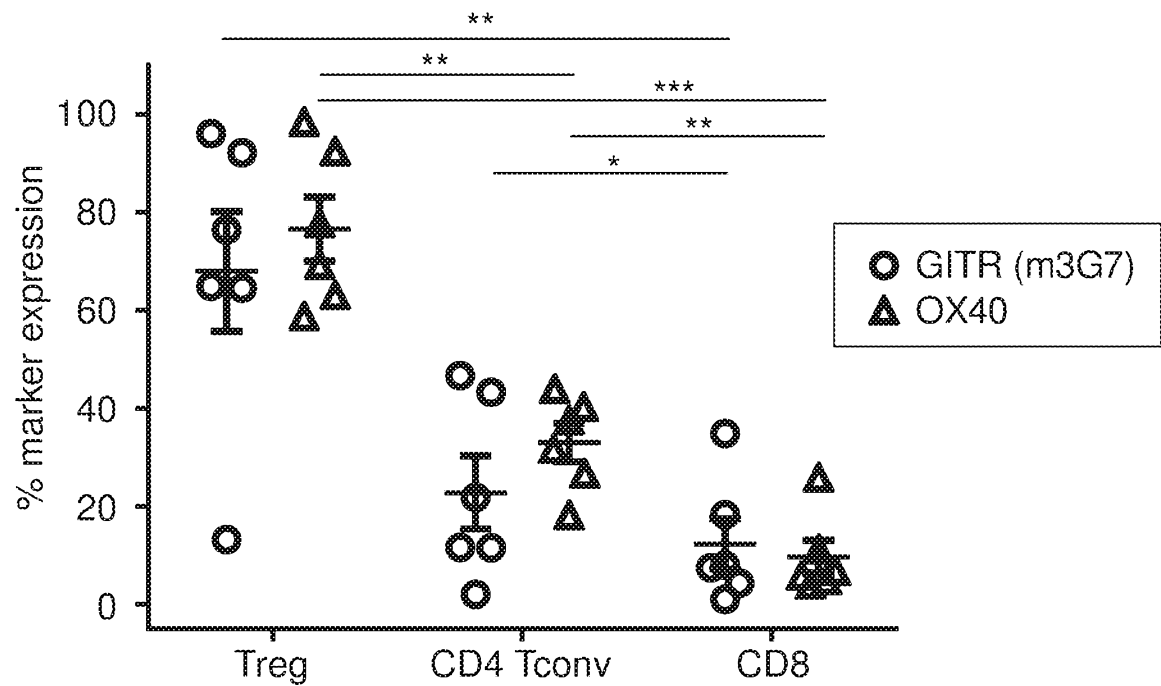


Figure 2B

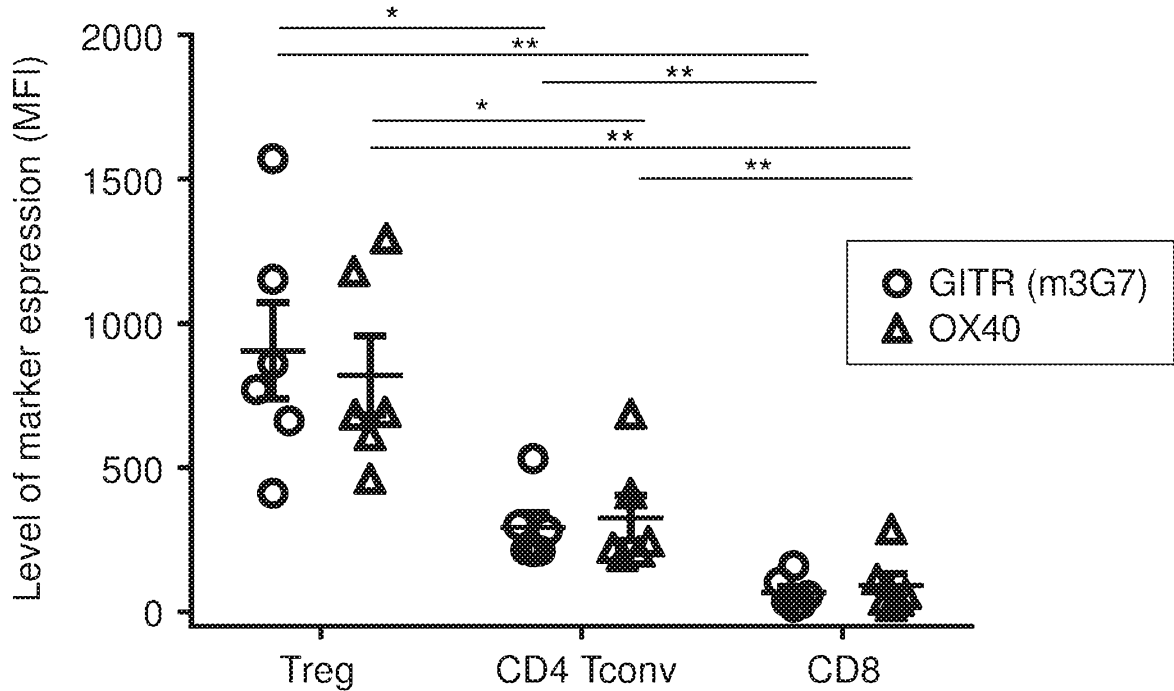


Figure 2C

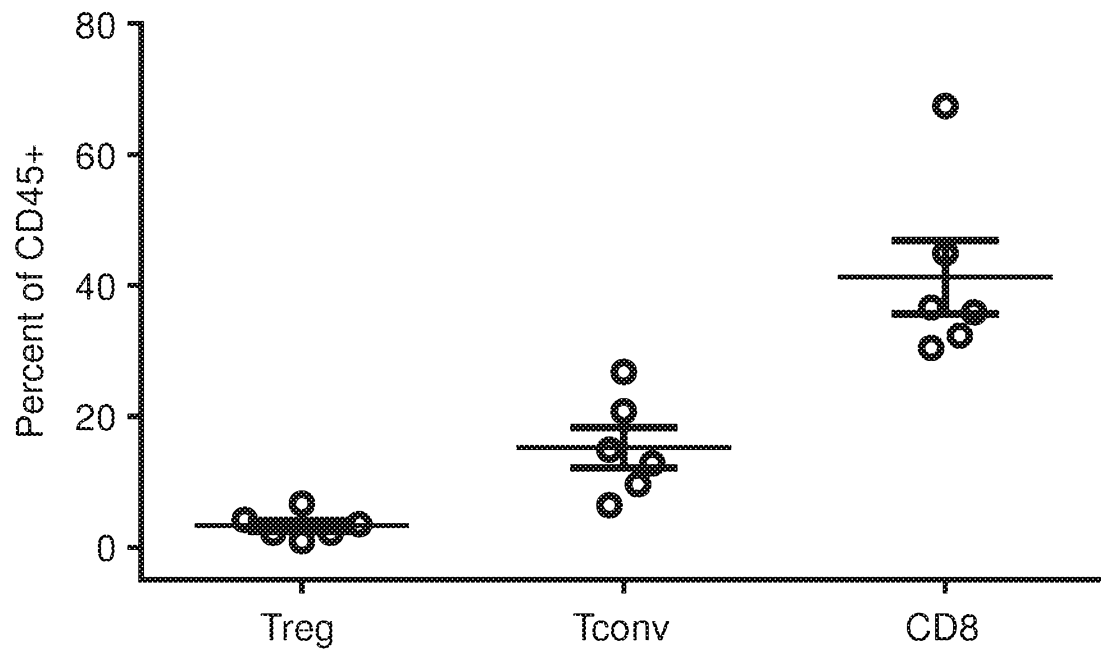


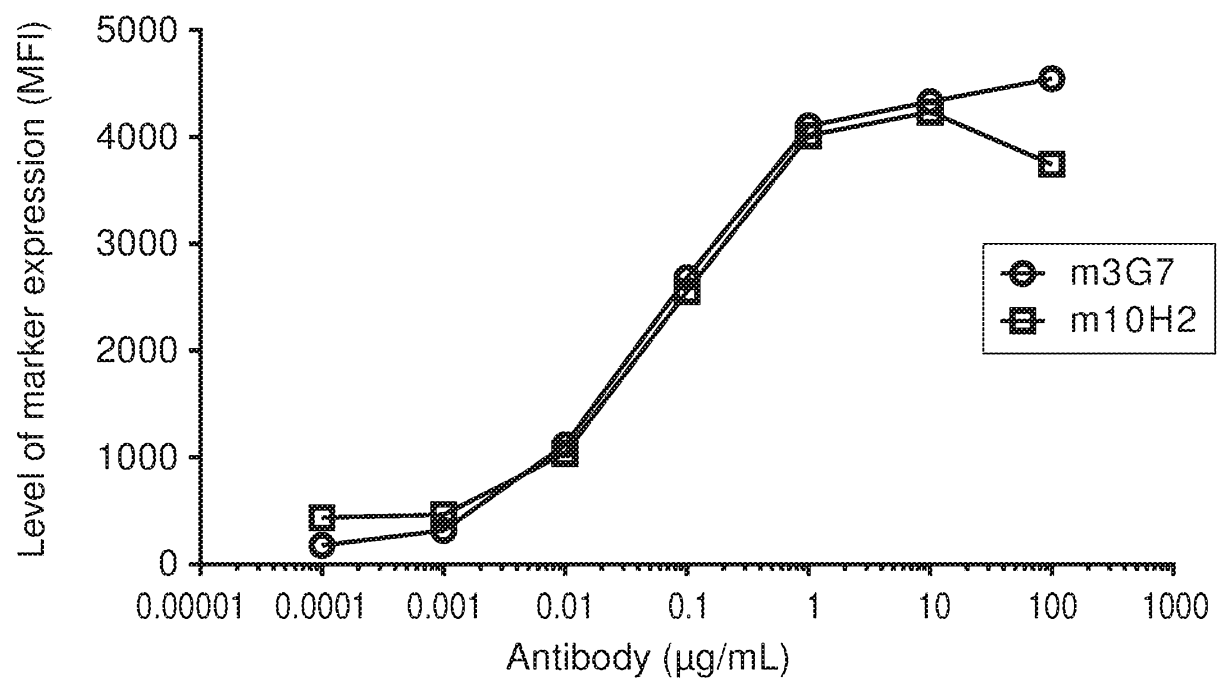
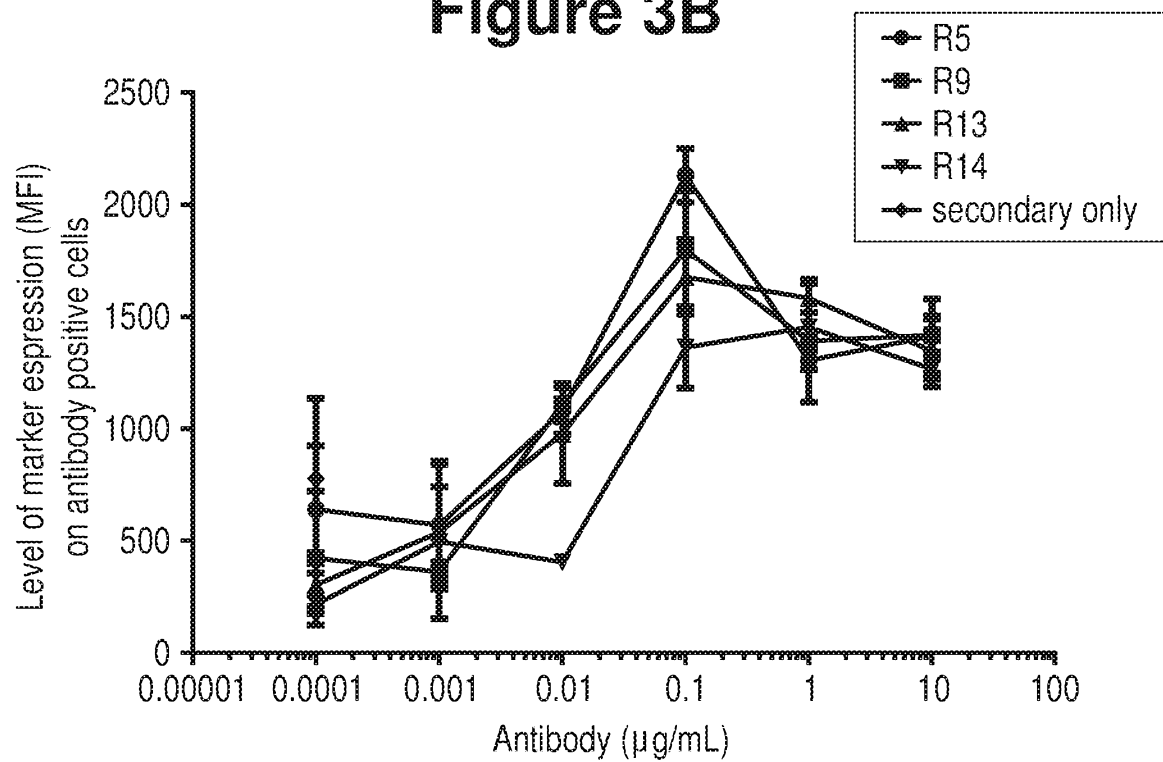
Figure 3A**Figure 3B**

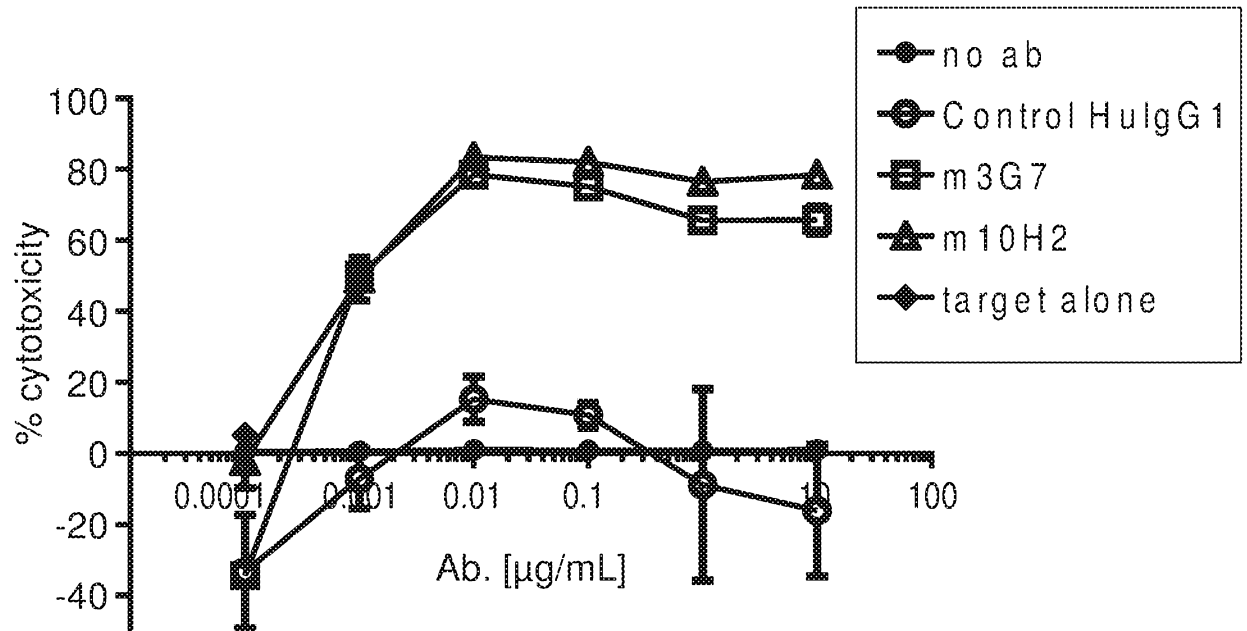
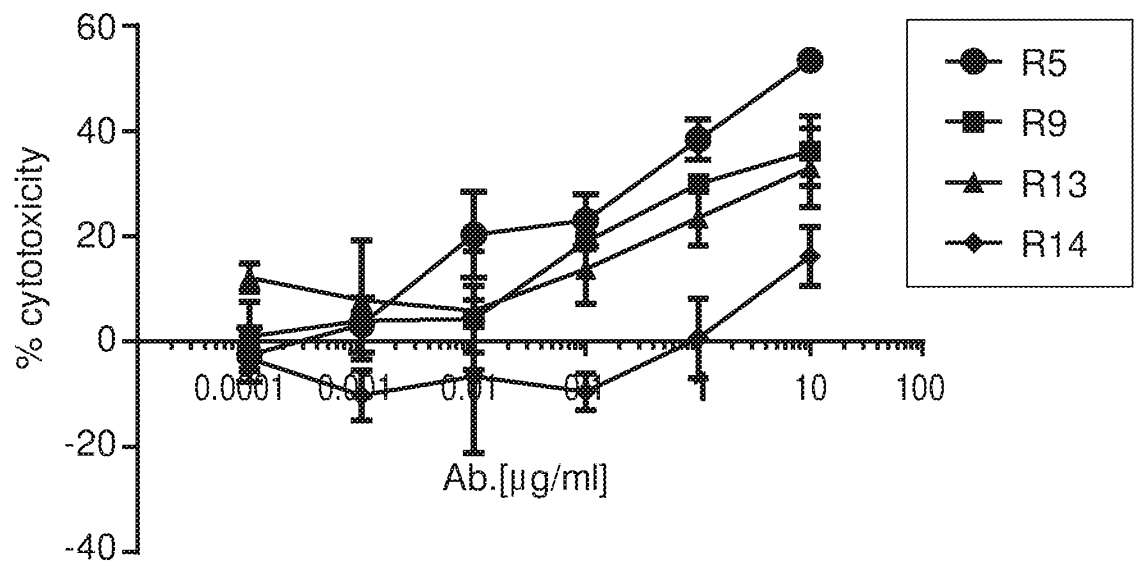
Figure 4A**Figure 4B**

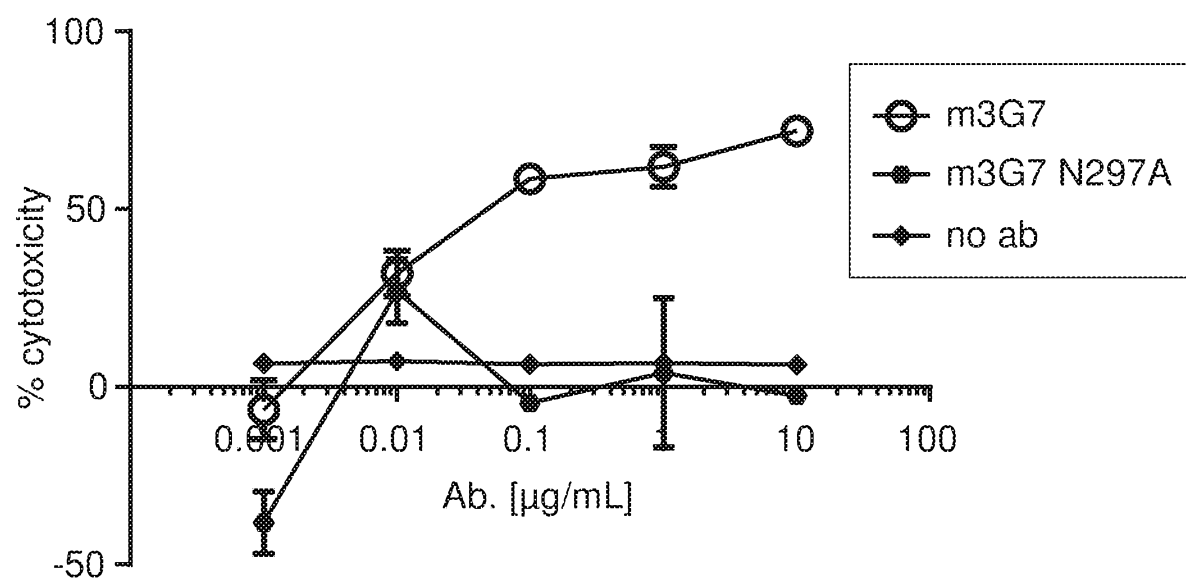
Figure 4C

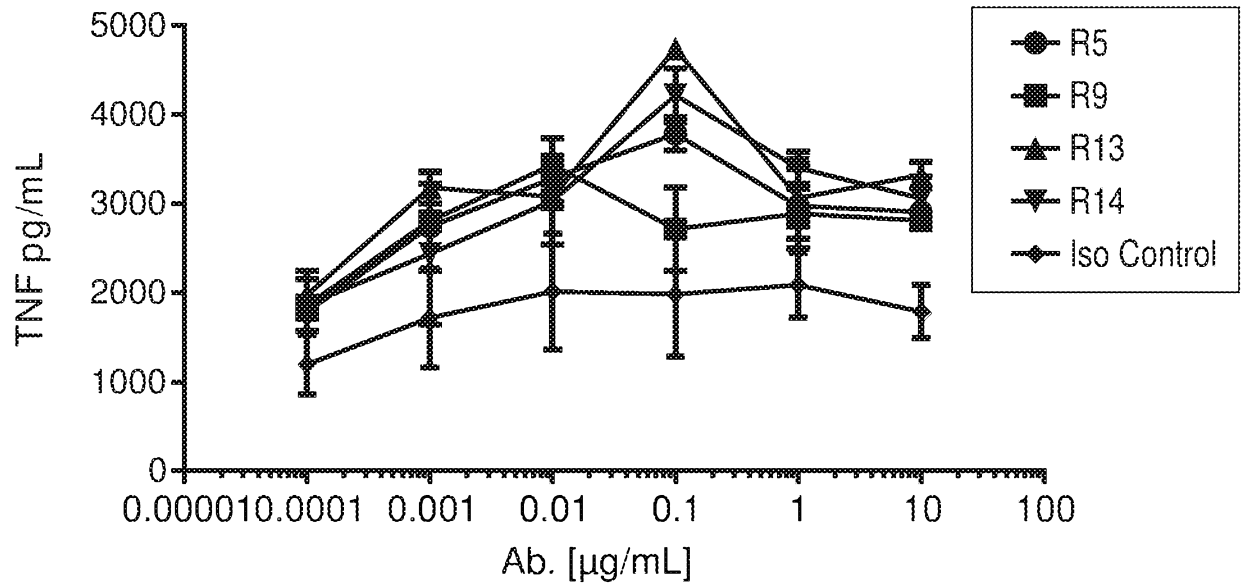
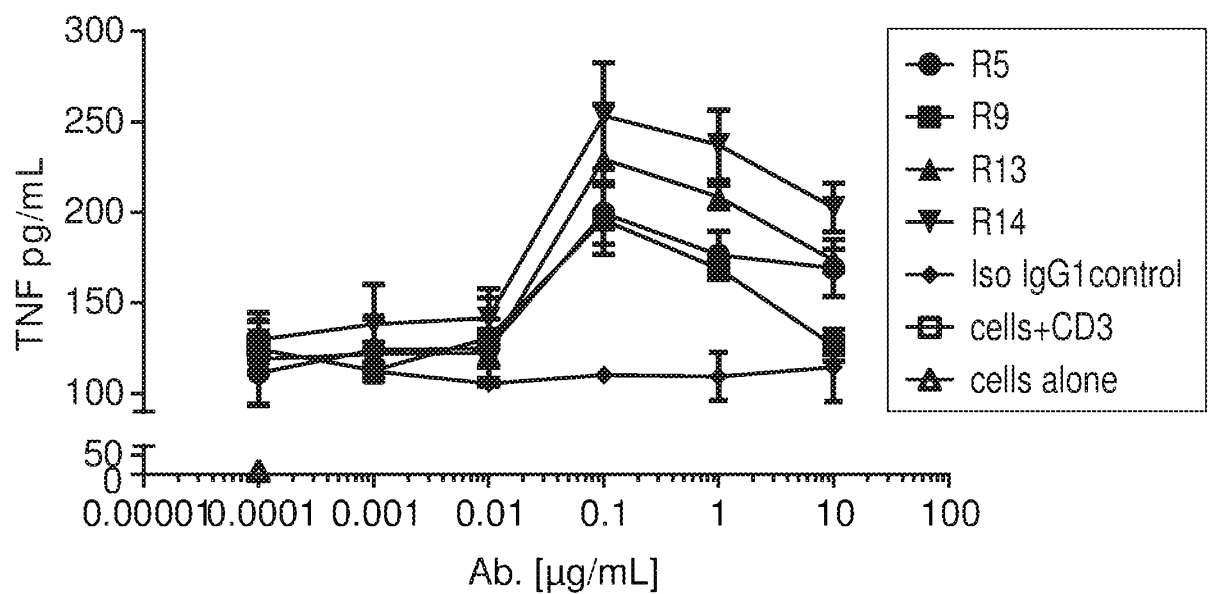
Figure 5A**Figure 5B**

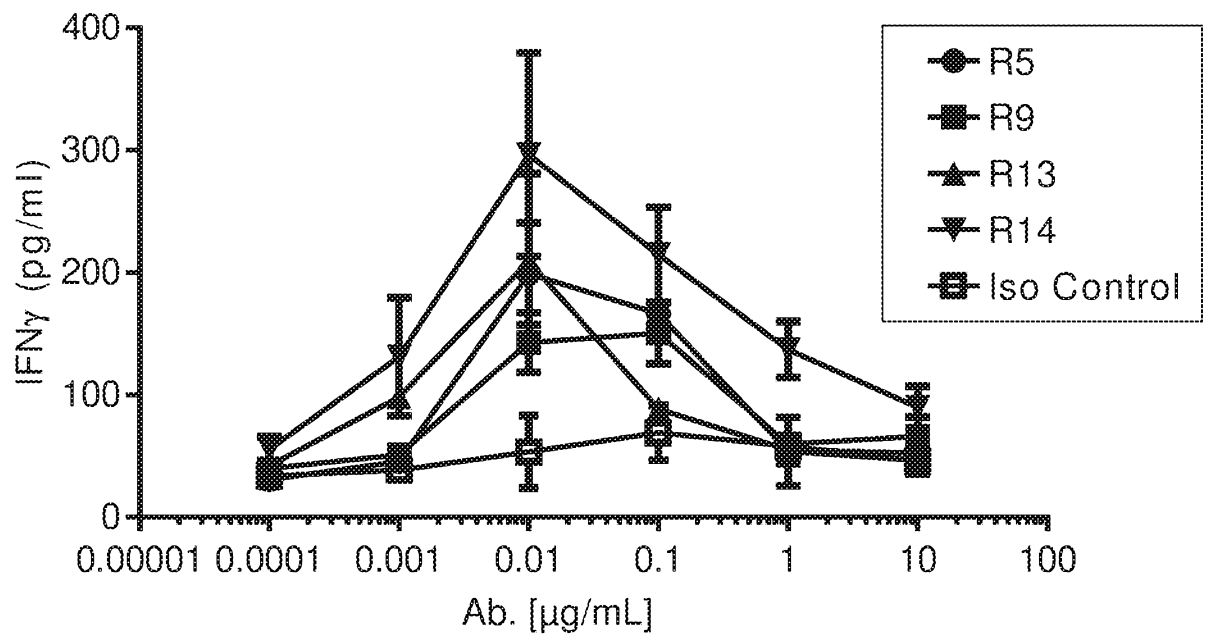
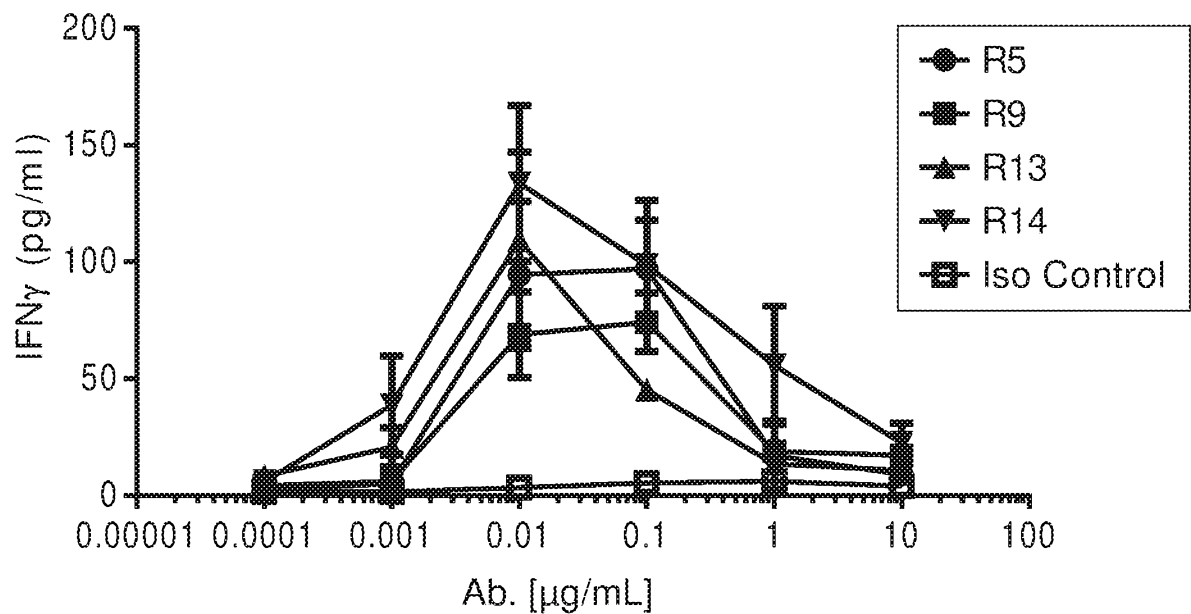
Figure 5C**Figure 5D**

Figure 6A

Parent Jurkat

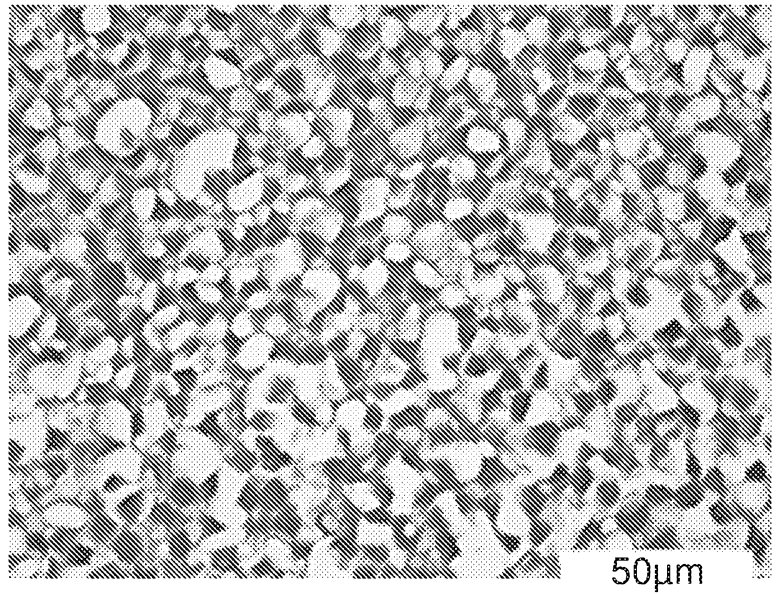


Figure 6B

GITR+ low

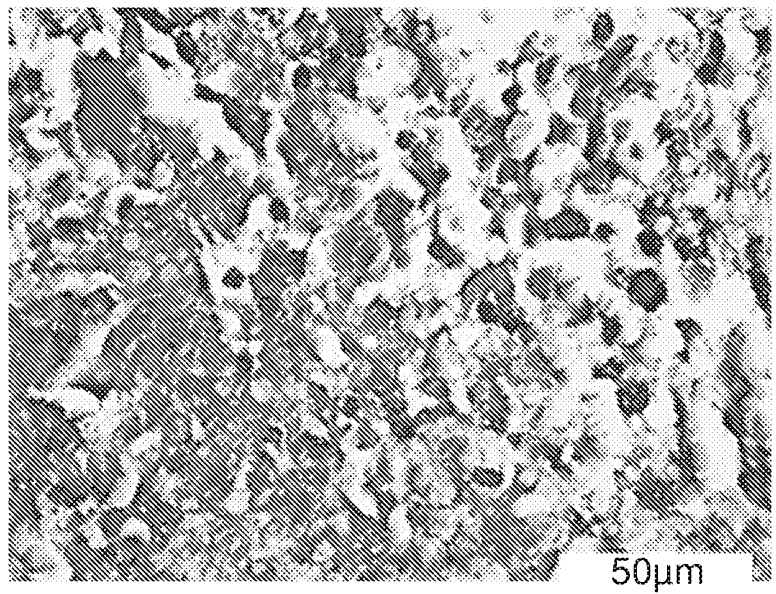


Figure 6C

GITR+ high

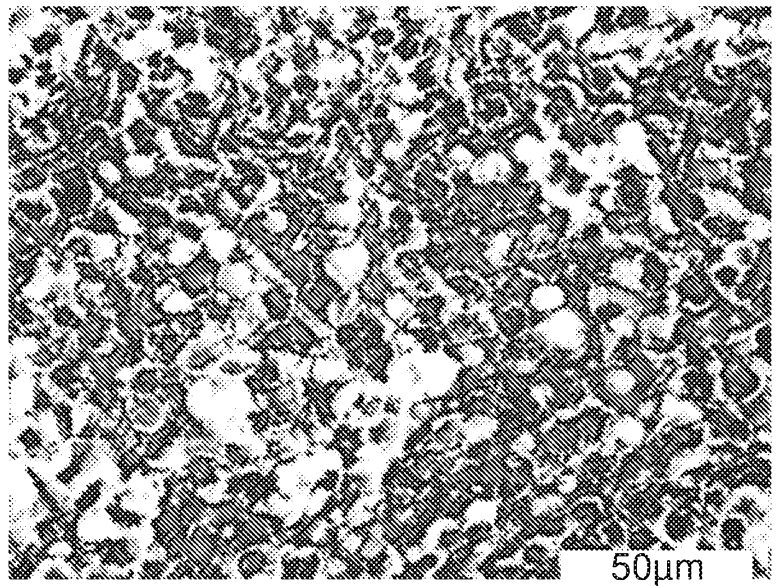


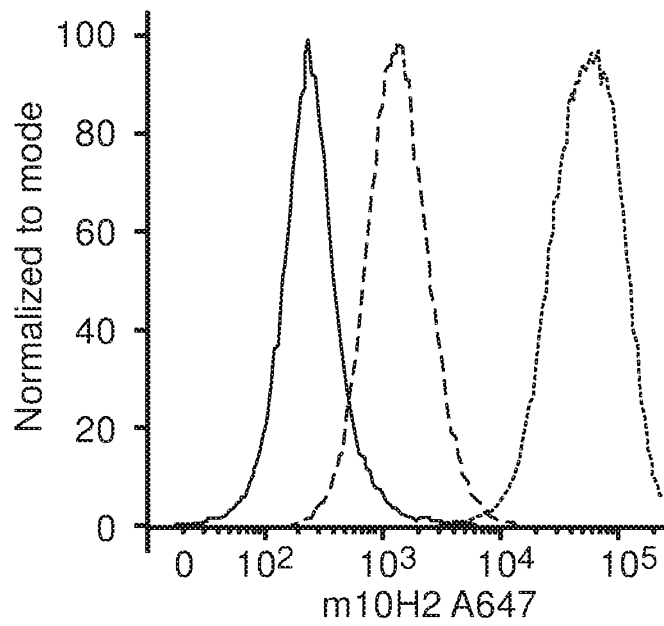
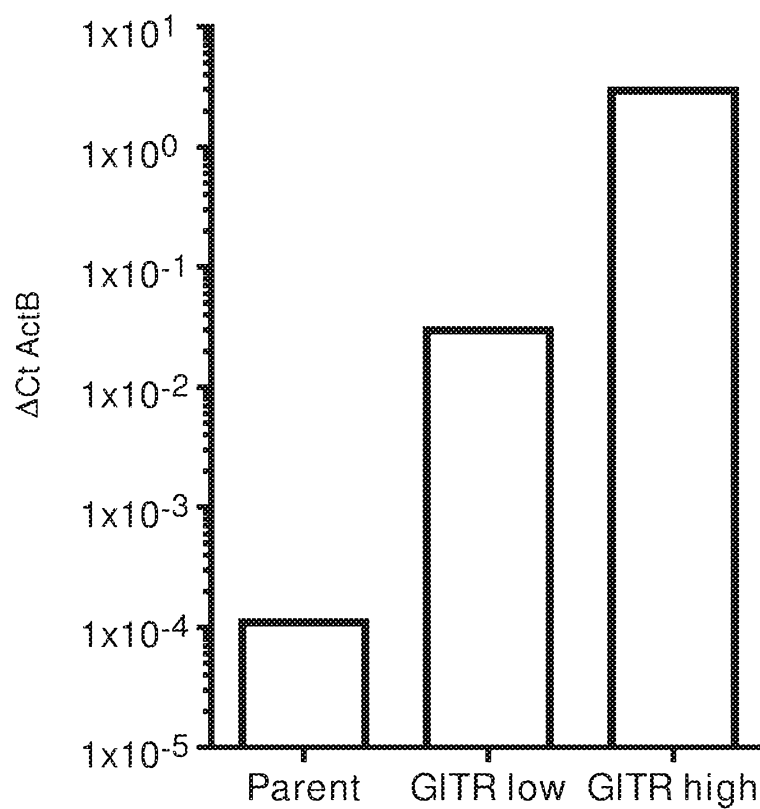
Figure 6D**Figure 6E**

Figure 6F

10H2 1 μ g/ml

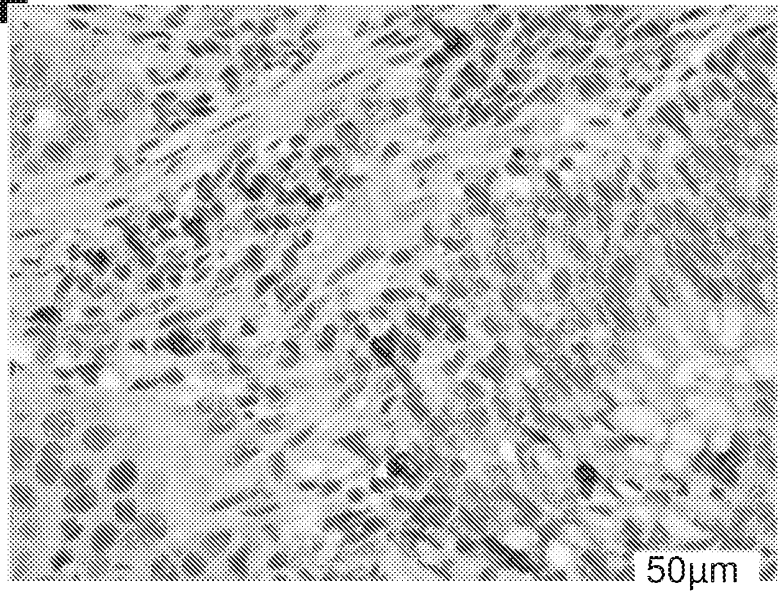


Figure 6G

Mouse IgG 5 μ g/ml

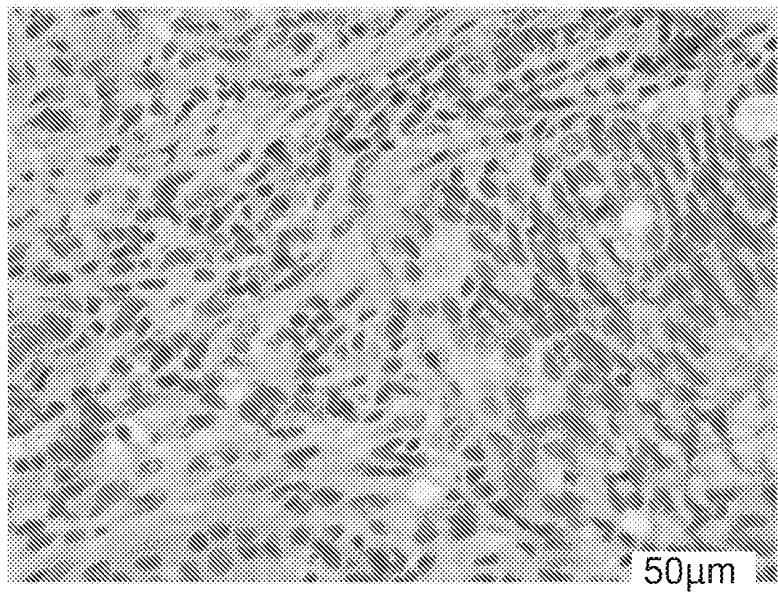


Figure 7A

IgG2a Isotype Control
5mpk

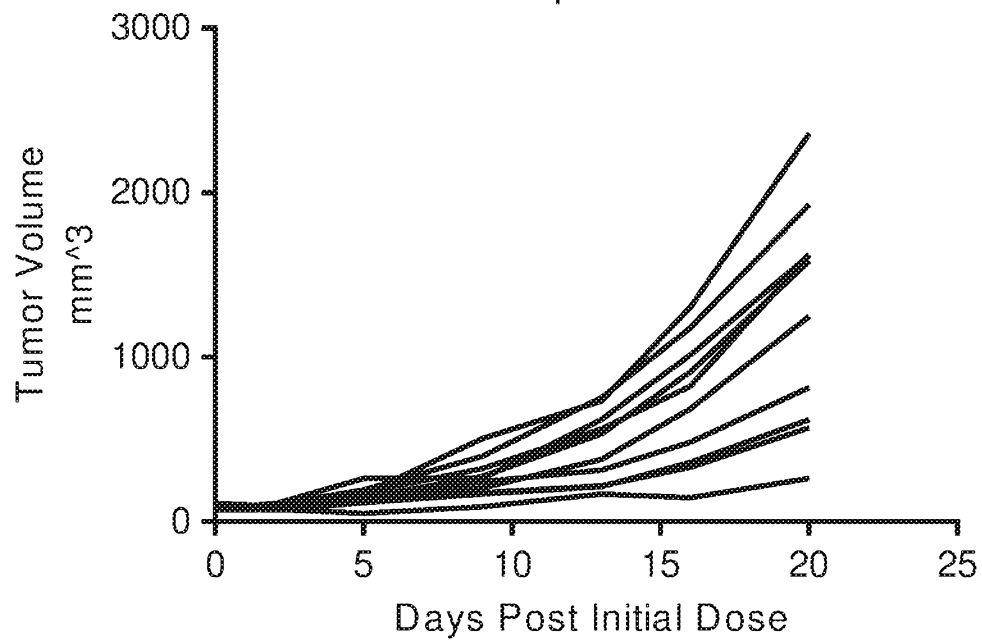


Figure 7B

21B6 IgG2a 0.2mpk

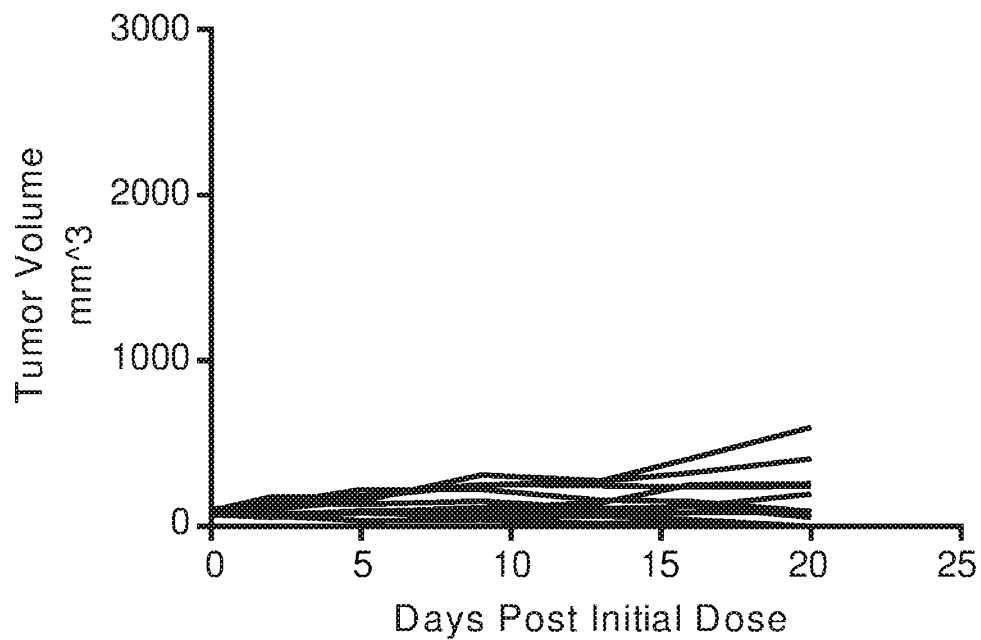


Figure 7C

21B6 IgG2a 1mpk

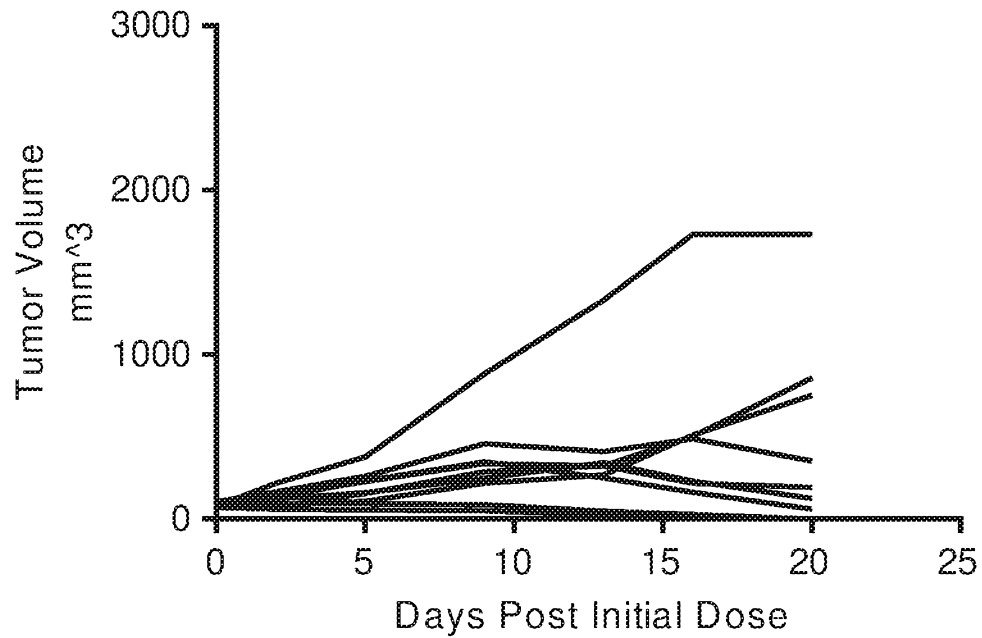


Figure 7D

21B6 IgG2a 5mpk

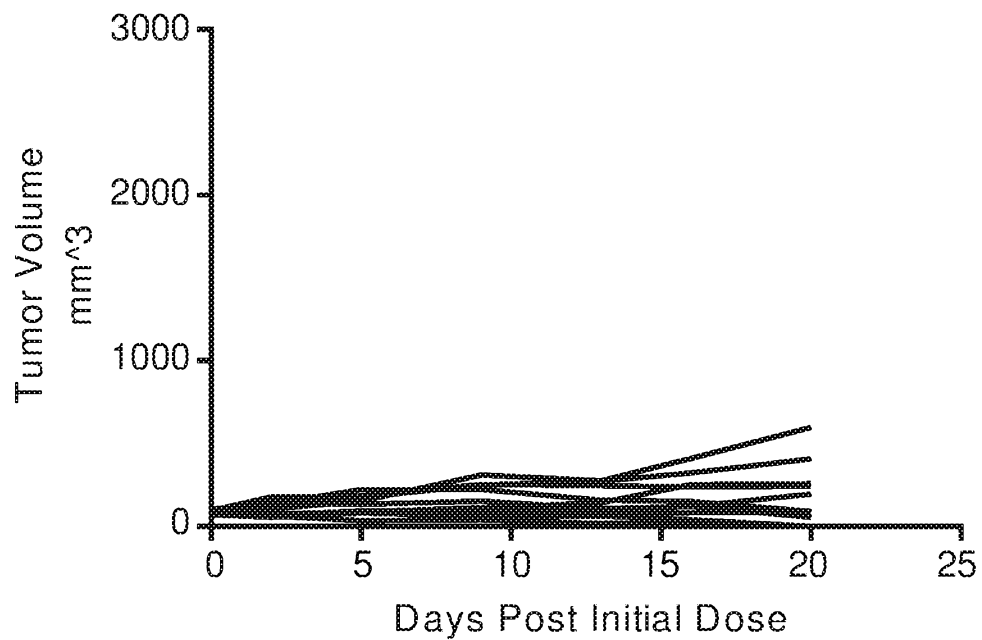


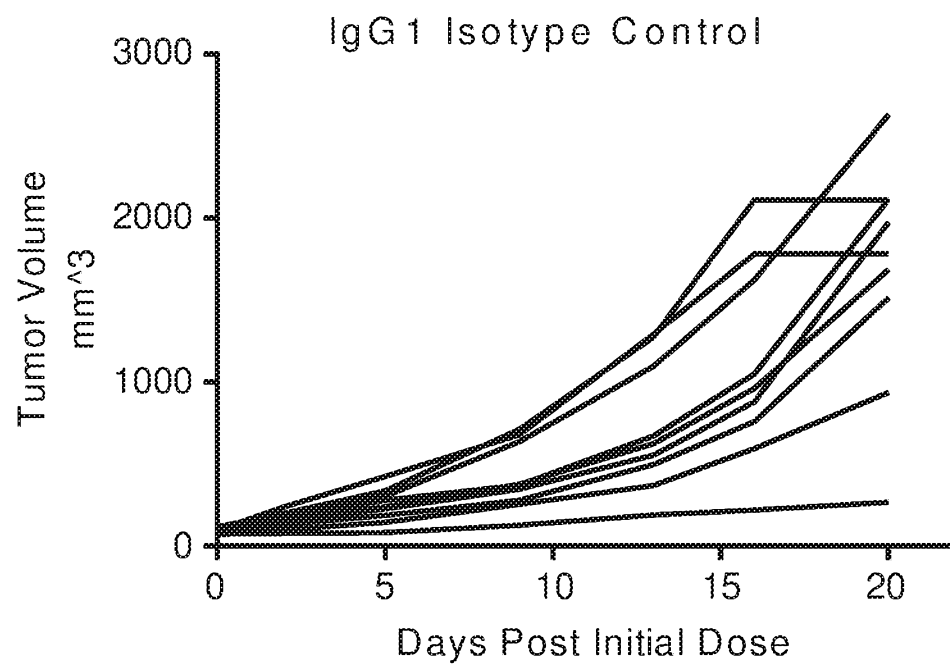
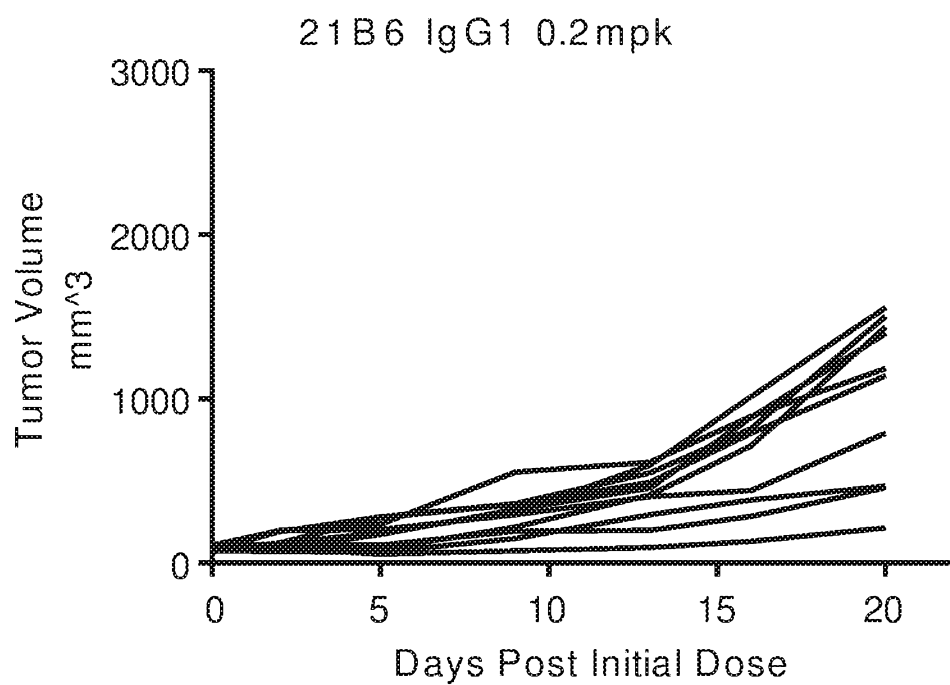
Figure 7E**Figure 7F**

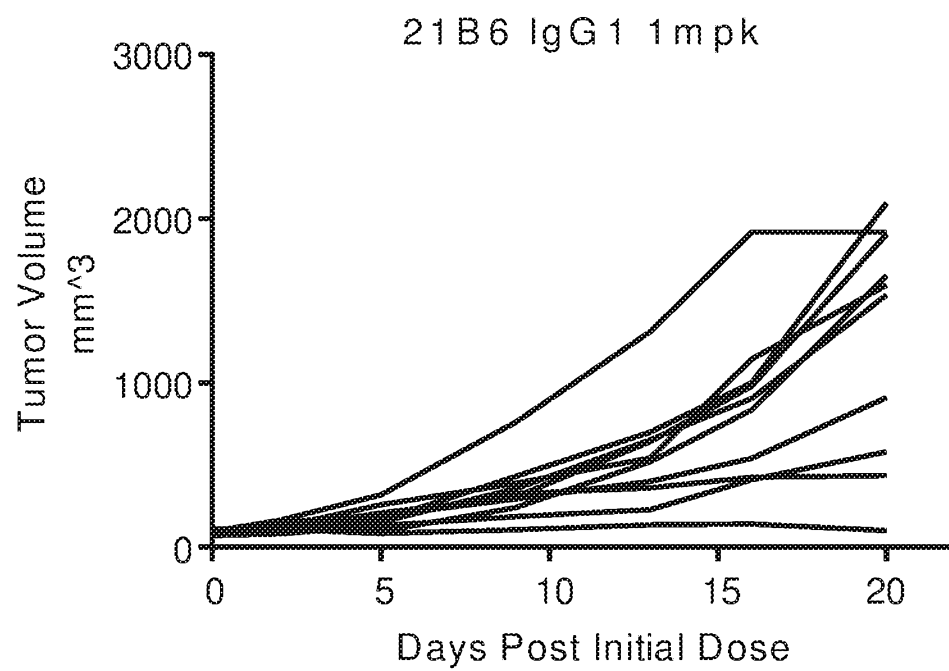
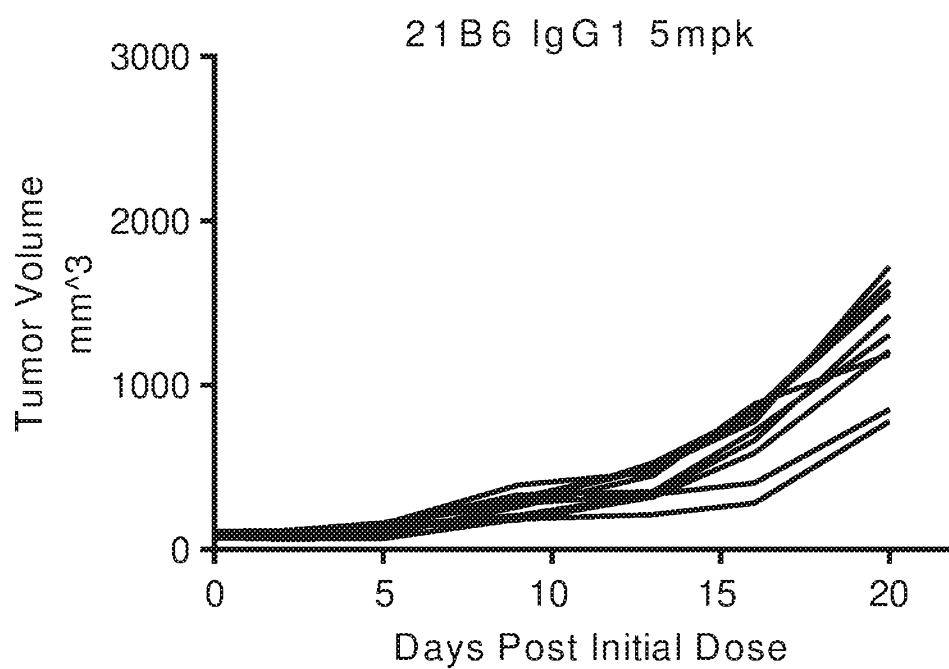
Figure 7G**Figure 7H**

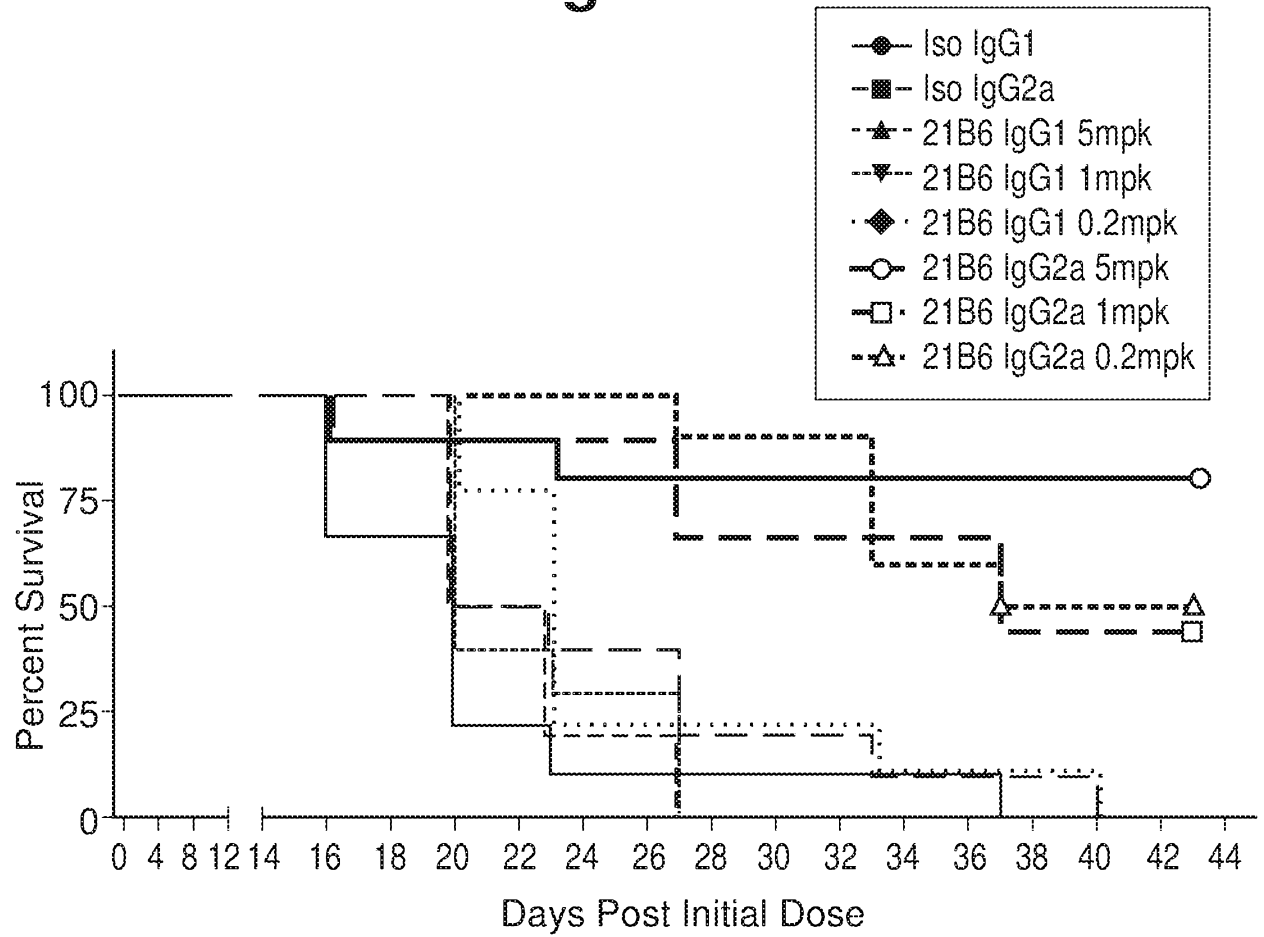
Figure 7I

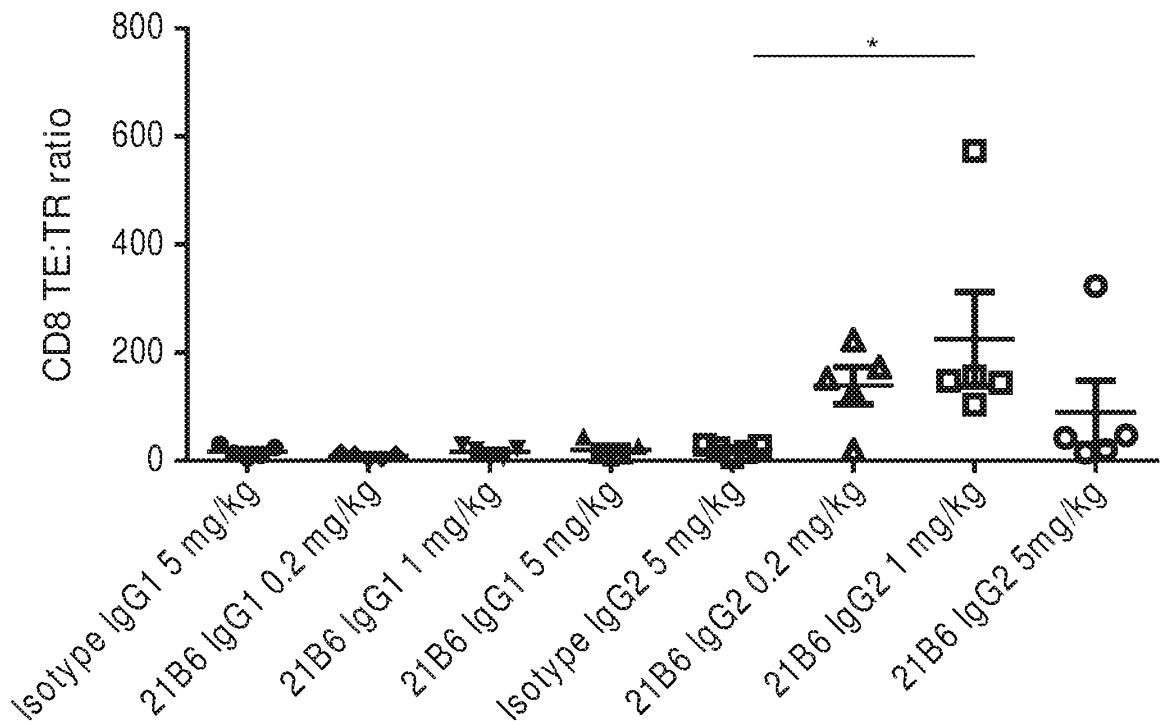
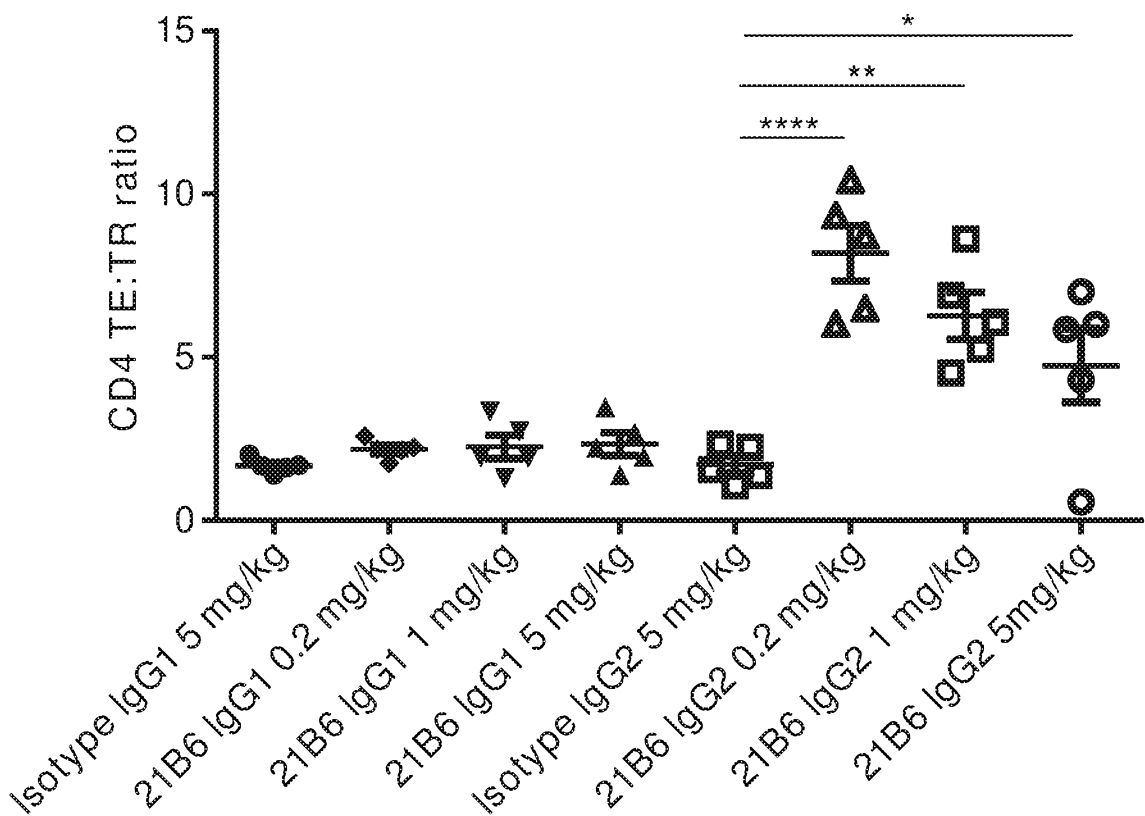
Figure 7J Tumor CD8+Tcell: Treg**Figure 7K** Tumor CD4+ Teff: Treg

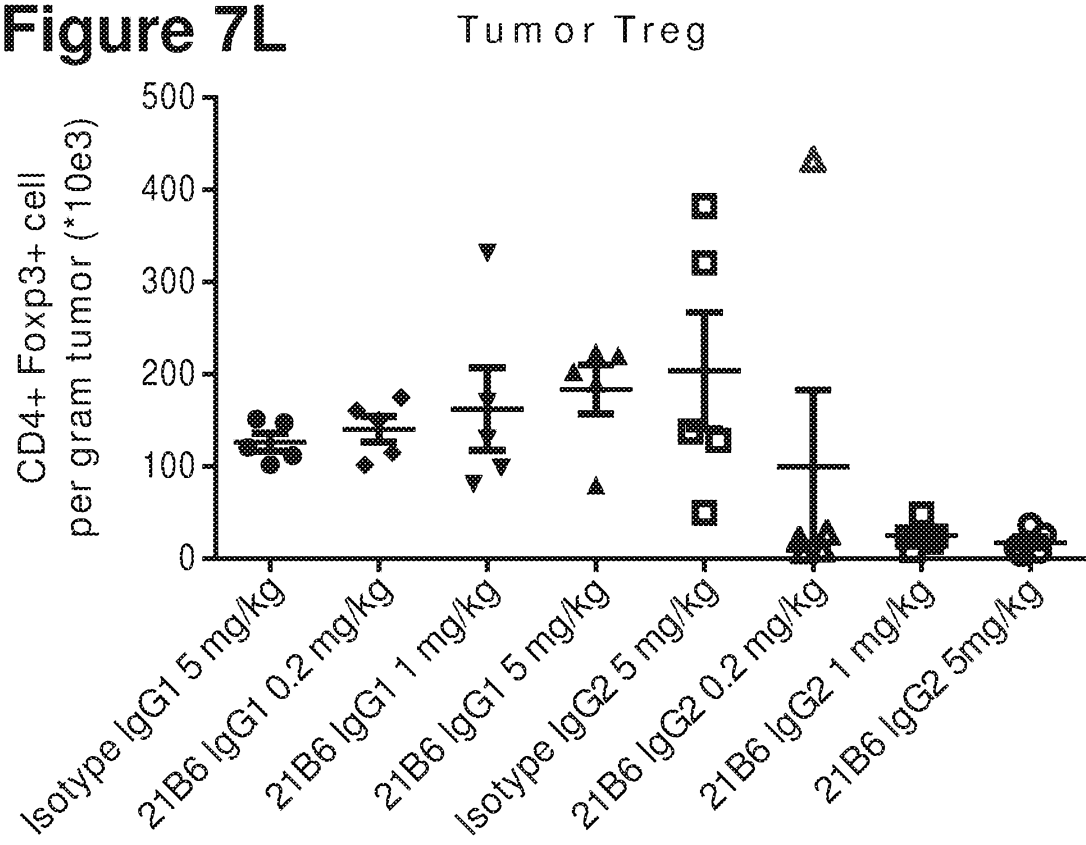
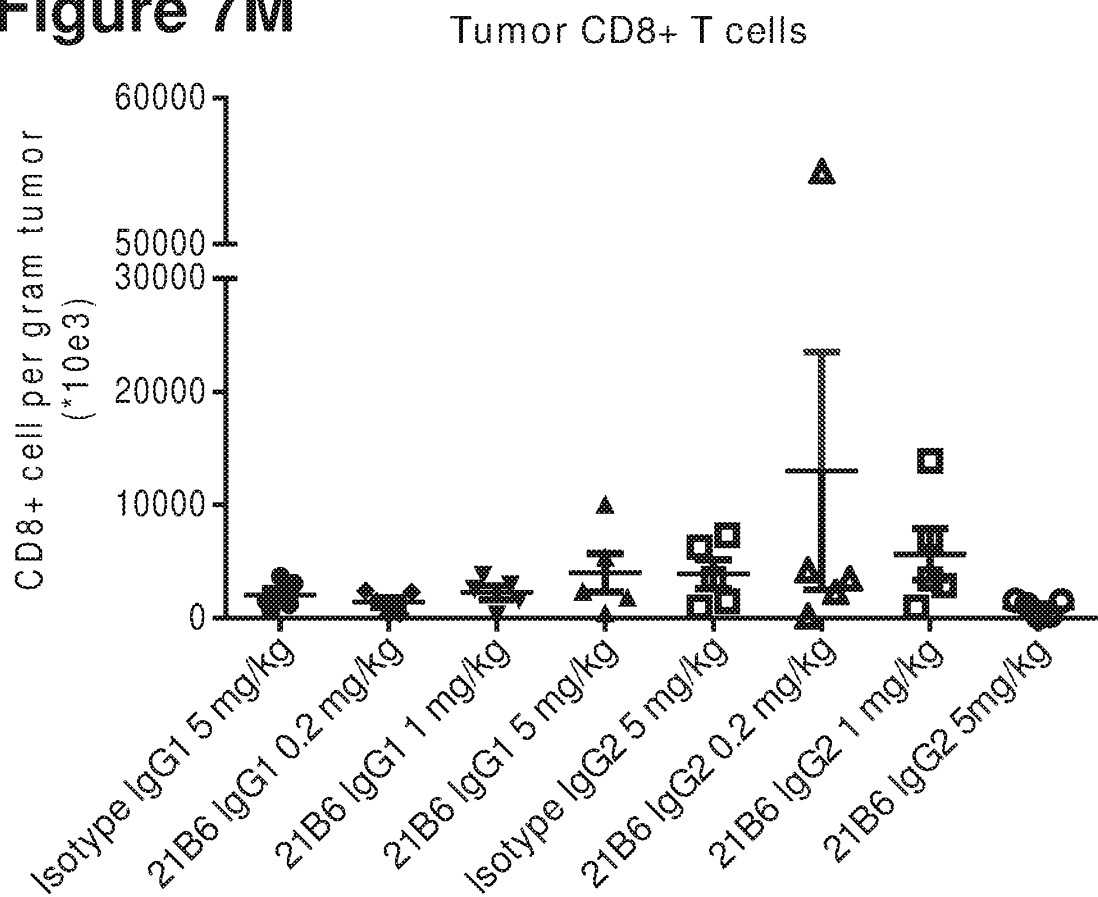
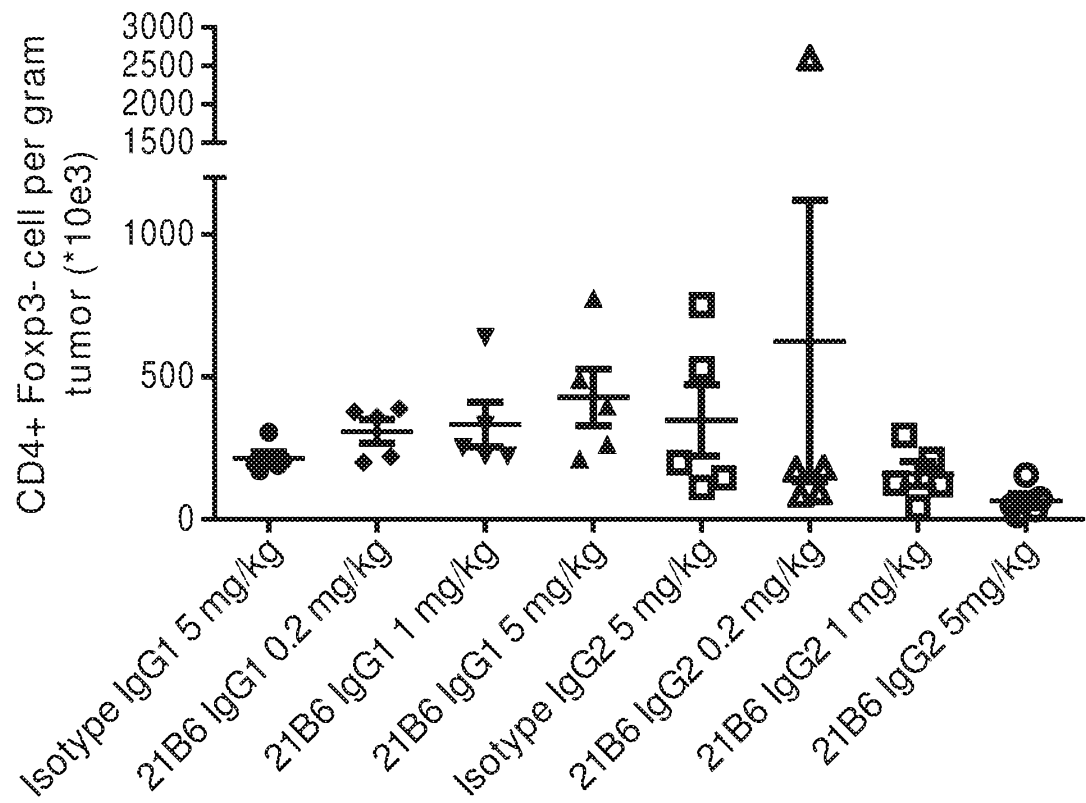
Figure 7L**Figure 7M**

Figure 7N

Tumor CD4+ Teff cell

**Figure 7O**

Tumor Treg

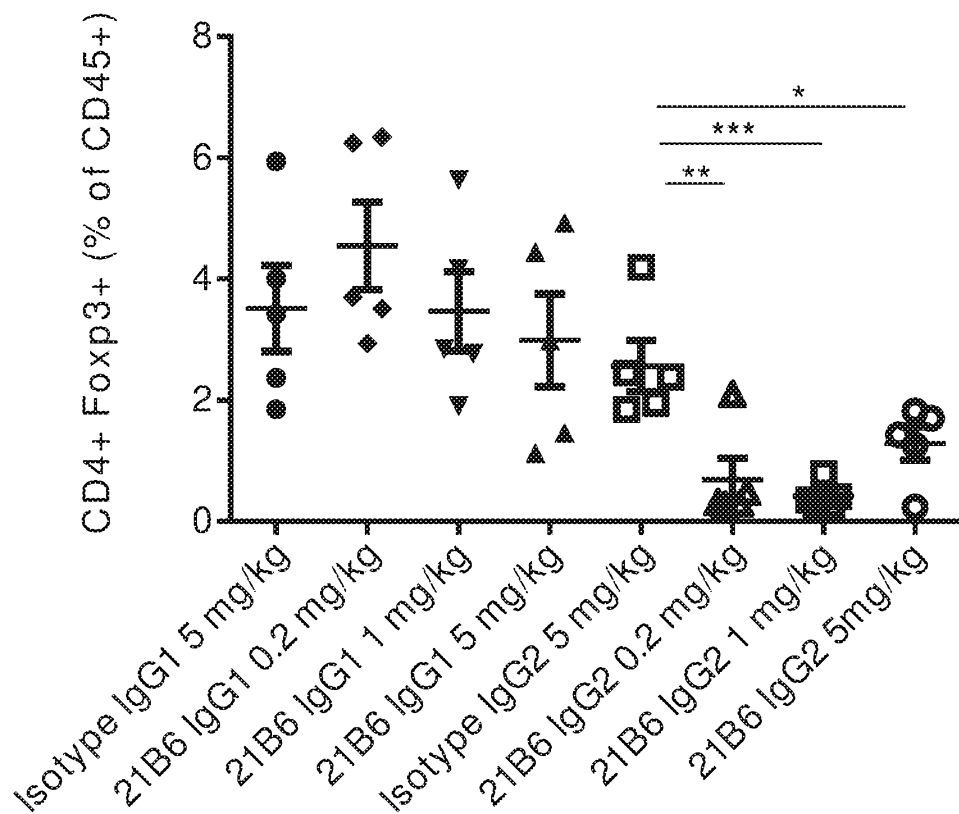
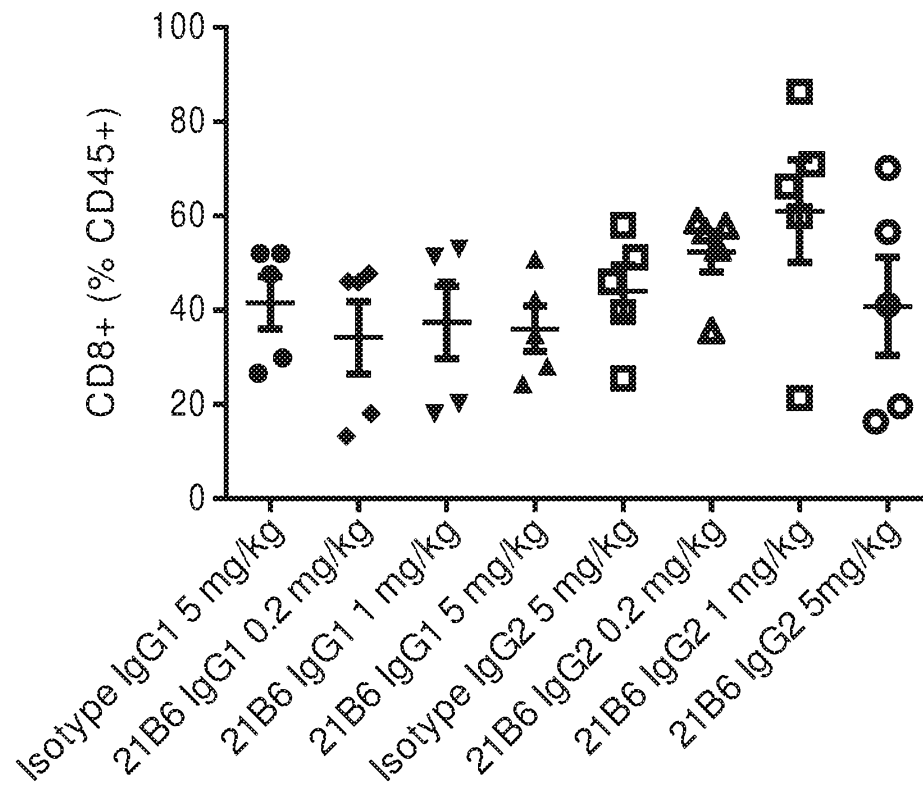


Figure 7P

Tumor CD8+ T cell

**Figure 7Q**

Tumor CD4+ Teff cell

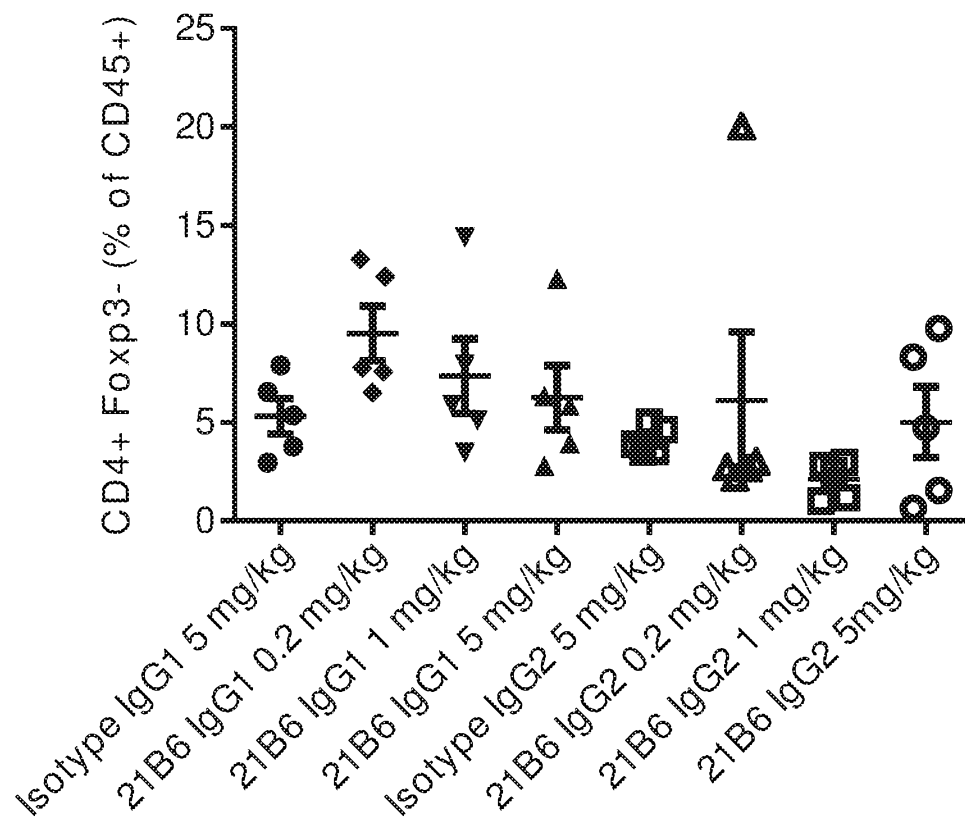


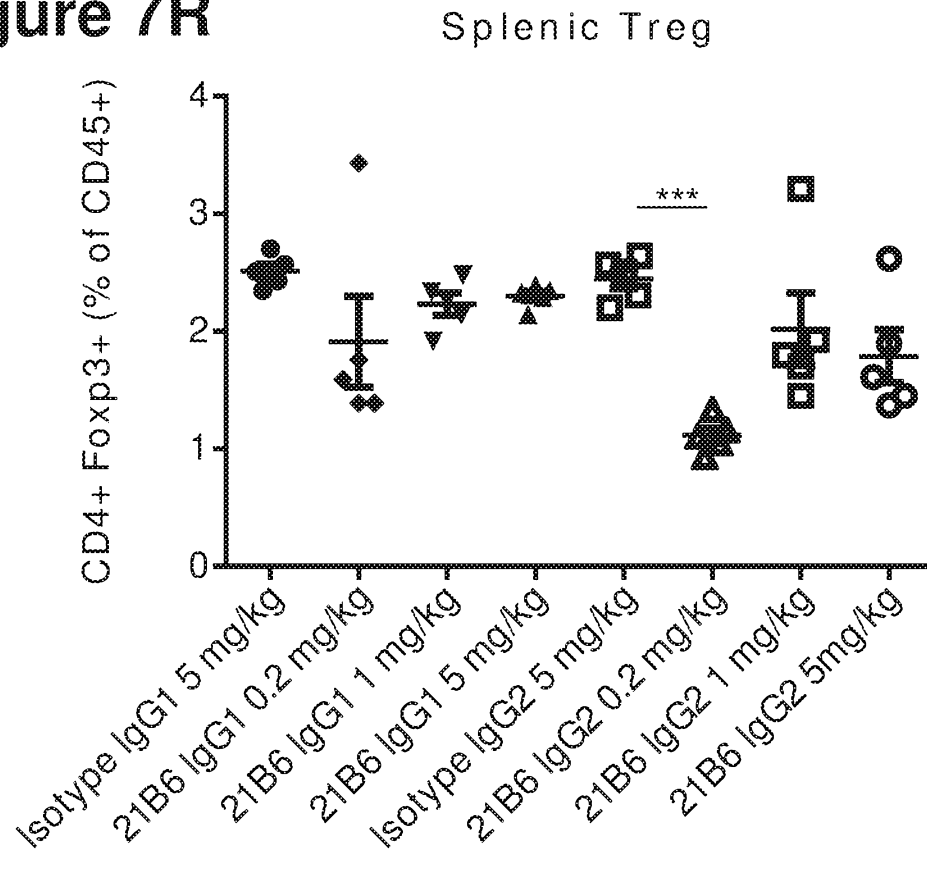
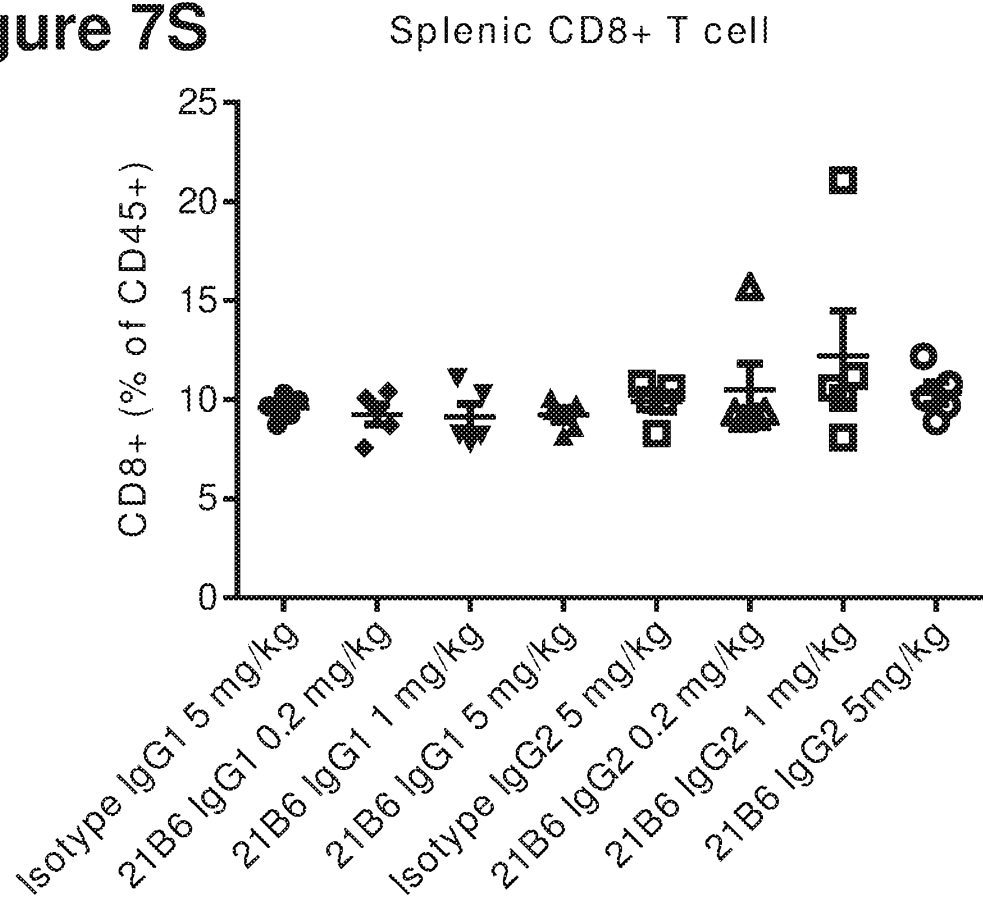
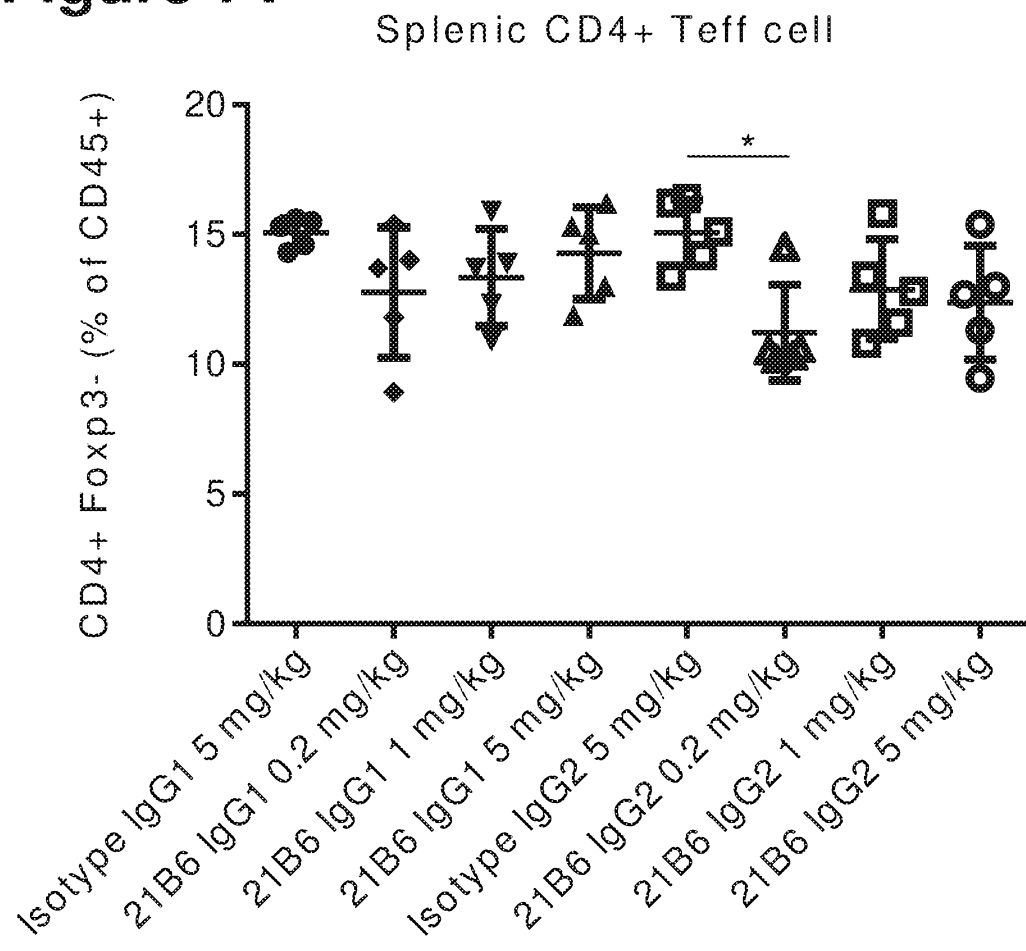
Figure 7R**Figure 7S**

Figure 7T

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<160> 130
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Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Phe
20 25 30

Gly Ile Asn Phe Met Asn Trp Phe Gln Gln Lys Ser Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asp Ile His
65 70 75 80

Pro Met Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Lys Glu
85 90 95

Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 2
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 <212> PRT
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<220>
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<400> 2

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Arg Val Lys Pro Gly Ala
 1 5 10 15

Ser Met Lys Ile Ser Cys Lys Val Ser Gly Tyr Ser Phe Thr Asp Tyr
 20 25 30

Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu Glu Trp Ile
 35 40 45

Gly Leu Ile Asn Pro Tyr Asn Gly Gly Ile Arg Tyr Asn Gln Lys Phe
 50 55 60

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

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

Ala Arg Ile Gly Gly Tyr Tyr Asp Ser Met Asp Tyr Trp Gly Gln Gly
 100 105 110

Thr Ser Val Thr Val Ser Ser
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<400> 3

PC72321A-seql-000001.txt

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1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Ser Val Asp Asn Phe
20 25 30

Gly Ile Asn Phe Leu Asn Trp Phe Gln Gln Lys Pro Gly Lys Ala Pro
35 40 45

Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Lys
85 90 95

Glu Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 4
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<213> Artificial Sequence

<220>
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<400> 4

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Tyr Ser Phe Thr Asp Tyr
20 25 30

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

Ala Leu Ile Asn Pro Tyr Asn Gly Gly Ile Arg Tyr Asn Gln Lys Phe

50

55

60

Lys Gly Arg Phe Thr Ile Ser Val Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

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

Ala Arg Ile Gly Gly Tyr Tyr Asp Ser Met Asp Tyr Trp Gly Gln Gly
 100 105 110

Thr Leu Val Thr Val Ser Ser
 115

<210> 5

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<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 5

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Ser Val Asp Asn Phe
 20 25 30

Gly Ile Asn Phe Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
 35 40 45

Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly Val Pro Ser
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
 65 70 75 80

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Lys
 85 90 95

Glu Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

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<400> 6

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

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

Ala Leu Ile Asn Pro Tyr Asn Gly Gly Ile Arg Tyr Asn Gln Lys Phe
50 55 60

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

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

Ala Arg Ile Gly Gly Tyr Tyr Asp Ser Met Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 7
<211> 111
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<213> Artificial Sequence

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<223> Synthetic Construct

<400> 7

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Phe
20 25 30

Gly Ile Asn Phe Met Asn Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
35 40 45

Arg Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly Ile Pro Asp
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Lys
85 90 95

Glu Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 8

<211> 119

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 8

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Thr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met

35

40

45

Gly Leu Ile Asn Pro Tyr Asn Gly Gly Ile Arg Tyr Asn Gln Lys Phe
 50 55 60

Lys Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
 65 70 75 80

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

Ala Arg Ile Gly Gly Tyr Tyr Asp Ser Met Asp Tyr Trp Gly Gln Gly
 100 105 110

Thr Leu Val Thr Val Ser Ser
 115

<210> 9

<211> 111

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<400> 9

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Ser Val Asp Pro Arg
 20 25 30

Gly Ile Asn Phe Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
 35 40 45

Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Ala Lys Gly Val Pro Ser
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
 65 70 75 80

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Leu
85 90 95

Glu Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 10
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 10

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Ala
20 25 30

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

Gly Leu Ile Asn Pro Tyr Asn Gly Gly Ile Arg Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65 70 75 80

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

Ala Arg Ile Gly Gly Tyr Tyr Asp Ser Met Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 11

<211> 111
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 <213> Artificial Sequence

<220>
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<400> 11

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Ser Val Glu Pro Arg
 20 25 30

Gly Ile Asn Phe Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
 35 40 45

Lys Leu Leu Ile Tyr Ala Ala Ser Gln Ala Ser Lys Gly Val Pro Ser
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
 65 70 75 80

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Leu
 85 90 95

Glu Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105 110

<210> 12
 <211> 119
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<220>
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<400> 12

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Ala

20

25

30

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

Gly Leu Ile Asn Pro Tyr Thr Gly Gly Ile Arg Tyr Asn Gln Lys Phe
 50 55 60

Lys Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
 65 70 75 80

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

Ala Arg Ile Gly Gly Tyr Tyr Asp Ser Met Asp Tyr Trp Gly Gln Gly
 100 105 110

Thr Leu Val Thr Val Ser Ser
 115

<210> 13

<211> 111

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 13

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Ser Val Glu Pro Arg
 20 25 30

Gly Ile Asn Phe Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
 35 40 45

Lys Leu Leu Ile Tyr Ala Ala Ser Gln Ala Ser Lys Gly Val Pro Ser
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Leu
85 90 95

Glu Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 14

<211> 119

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 14

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Ala
20 25 30

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

Gly Leu Ile Asn Pro Tyr Thr Gly Gly Ile Arg Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65 70 75 80

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

Ala Arg Ile Gly Gly Tyr Tyr Asp Thr Met Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser

115

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 <212> PRT
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<400> 15

Asp Ile Leu Leu Thr Gln Ser Pro Ala Ile Leu Ser Val Ser Pro Gly
 1 5 10 15

Glu Arg Val Ser Phe Ser Cys Arg Ala Ser Gln Ser Ile Gly Thr Ser
 20 25 30

Ile His Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Ile
 35 40 45

Lys Tyr Ala Ser Glu Ser Val Ser Gly Ile Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Phe Ile Asn Ser Val Glu Ser
 65 70 75 80

Glu Asp Ile Ala Asp Tyr Tyr Cys Gln Gln Thr Tyr Ser Trp Pro Ala
 85 90 95

Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
 100 105

<210> 16
 <211> 118
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 16

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Lys Pro Gly Ala

1 5 10 15
 Ser Val Lys Ile Ser Cys Thr Ala Thr Gly Tyr Thr Ile Ser Arg Tyr
 20 25 30
 Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
 35 40 45
 Gly Glu Ile Leu Pro Gly Ser Gly Val Thr Asn Tyr Asn Glu Lys Phe
 50 55 60
 Lys Gly Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
 65 70 75 80
 Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Ser Cys
 85 90 95
 Ala Arg Lys Gly Thr Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
 100 105 110
 Ser Val Thr Val Ser Ser
 115

<210> 17
 <211> 107
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 17

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Ile Gly Thr Ser
 20 25 30
 Ile His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
 35 40 45

Tyr Tyr Ala Ser Glu Ser Val Ser Gly Ile Pro Asp Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Thr Tyr Ser Trp Pro Ala
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 18

<211> 118

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 18

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Arg Tyr
20 25 30

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

Gly Glu Ile Leu Pro Gly Ser Gly Val Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

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

Ala Arg Lys Gly Thr Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr

100

105

110

Ser Val Thr Val Ser Ser
115

<210> 19
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 19

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Ile Gly Thr Ser
20 25 30

Ile His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Tyr Ala Ser Glu Ser Val Ser Gly Ile Pro Asp Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Thr Tyr Ser Trp Pro Ala
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 20
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<213> Artificial Sequence

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<400> 20

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Arg Tyr
20 25 30

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

Gly Glu Ile Leu Pro Gly Ser Gly Val Thr Phe Glu Asn Glu Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

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

Ala Arg Lys Gly Arg Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 21

<211> 15

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<400> 21

Arg Ala Ser Glu Ser Val Asp Asn Phe Gly Ile Asn Phe Met Asn
1 5 10 15

<210> 22

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<400> 22

Ala Ala Ser Asn Gln Gly Ser
1 5

<210> 23

<211> 9

<212> PRT

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<400> 23

Gln Gln Ser Lys Glu Leu Pro Trp Thr
1 5

<210> 24

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<400> 24

Gly Tyr Ser Phe Thr Asp Tyr
1 5

<210> 25

<211> 5

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<400> 25

Asp Tyr Thr Met Asn
1 5

<210> 26
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<400> 26

Gly Tyr Ser Phe Thr Asp Tyr Thr Met Asn
 1 5 10

<210> 27
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<220>
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<400> 27

Asn Pro Tyr Asn Gly Gly
 1 5

<210> 28
 <211> 17
 <212> PRT
 <213> Artificial Sequence

<220>
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<400> 28

Leu Ile Asn Pro Tyr Asn Gly Gly Ile Arg Tyr Asn Gln Lys Phe Lys
 1 5 10 15

Gly

<210> 29
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<400> 29

Ile	Gly	Gly	Tyr	Tyr	Asp	Ser	Met	Asp	Tyr
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<210> 30

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Arg	Ala	Ser	Glu	Ser	Val	Asp	Asn	Phe	Gly	Ile	Asn	Phe	Leu	Asn
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<210> 31

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<223> Xaa is F or R

<220>

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<223> Xaa is M or L

<400> 31

Arg	Ala	Ser	Glu	Ser	Val	Xaa	Xaa	Xaa	Gly	Ile	Asn	Phe	Xaa	Asn
1				5					10					15

<210> 32

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 32

Gly	Phe	Thr	Phe	Ser	Asp	Tyr
1				5		

<210> 33

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 33

Gly	Phe	Thr	Phe	Ser	Asp	Tyr	Thr	Met	Asn
1				5					10

<210> 34

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

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<400> 34

Gly	Tyr	Thr	Phe	Thr	Asp	Tyr
1				5		

<210> 35

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 35

Gly	Tyr	Thr	Phe	Thr	Asp	Tyr	Thr	Met	Asn
1				5				10	

<210> 36

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 36

Arg	Ala	Ser	Glu	Ser	Val	Asp	Pro	Arg	Gly	Ile	Asn	Phe	Leu	Asn
1				5					10				15	

<210> 37

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 37

Ala	Ala	Ser	Asn	Gln	Ala	Lys
1				5		

<210> 38

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 38

Gln	Gln	Ala	Leu	Glu	Leu	Pro	Trp	Thr
1				5				

<210> 39
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 <212> PRT
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<220>
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<400> 39

Gly Tyr Thr Phe Thr Gly Ala
 1 5

<210> 40
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 40

Gly Ala Thr Met Asn
 1 5

<210> 41
 <211> 10
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 <213> Artificial Sequence

<220>
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<400> 41

Gly Tyr Thr Phe Thr Gly Ala Thr Met Asn
 1 5 10

<210> 42
 <211> 15
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<220>
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<400> 42

Arg Ala Ser Glu Ser Val Glu Pro Arg Gly Ile Asn Phe Leu Asn
1 5 10 15

<210> 43
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 43

Ala Ala Ser Gln Ala Ser Lys
1 5

<210> 44
<211> 6
<212> PRT
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<220>
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<400> 44

Asn Pro Tyr Thr Gly Gly
1 5

<210> 45
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 45

Leu Ile Asn Pro Tyr Thr Gly Gly Ile Arg Tyr Asn Gln Lys Phe Lys
1 5 10 15

Gly

<210> 46
<211> 10

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 46

Ile Gly Gly Tyr Tyr Asp Thr Met Asp Tyr
1 5 10

<210> 47
<211> 11
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<220>
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<400> 47

Arg Ala Ser Gln Ser Ile Gly Thr Ser Ile His
1 5 10

<210> 48
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<400> 48

Tyr Ala Ser Glu Ser Val Ser
1 5

<210> 49
<211> 9
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<213> Artificial Sequence

<220>
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<400> 49

Gln Gln Thr Tyr Ser Trp Pro Ala Thr
1 5

<210> 50
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<220>
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<400> 50

Gly Tyr Thr Ile Ser Arg Tyr Trp Ile Glu
 1 5 10

<210> 51
 <211> 7
 <212> PRT
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<220>
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<400> 51

Gly Tyr Thr Ile Ser Arg Tyr
 1 5

<210> 52
 <211> 5
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<220>
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<400> 52

Arg Tyr Trp Ile Glu
 1 5

<210> 53
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<400> 53

Glu Ile Leu Pro Gly Ser Gly Val Thr Asn Tyr Asn Glu Lys Phe Lys
1 5 10 15

Gly

<210> 54

<211> 6

<212> PRT

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<220>

<223> Synthetic Construct

<400> 54

Leu Pro Gly Ser Gly Val
1 5

<210> 55

<211> 9

<212> PRT

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<400> 55

Lys Gly Thr Tyr Tyr Ala Met Asp Tyr
1 5

<210> 56

<211> 10

<212> PRT

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<400> 56

Gly Gly Thr Phe Ser Arg Tyr Trp Ile Glu
1 5 10

<210> 57
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<220>
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<400> 57

Gly Gly Thr Phe Ser Arg Tyr
 1 5

<210> 58
 <211> 17
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<220>
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<400> 58

Glu Ile Leu Pro Gly Ser Gly Val Thr Phe Glu Asn Glu Lys Phe Lys
 1 5 10 15

Gly

<210> 59
 <211> 6
 <212> PRT
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<220>
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<400> 59

Leu Pro Gly Ser Gly Val
 1 5

<210> 60
 <211> 9
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<400> 60

Lys Gly Arg Tyr Tyr Ala Met Asp Tyr
1 5

<210> 61

<211> 7

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<220>

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<222> (4)..(4)

<223> Xaa is N or Q

<220>

<221> MISC_FEATURE

<222> (5)..(5)

<223> Xaa is Q or A

<220>

<221> MISC_FEATURE

<222> (6)..(6)

<223> Xaa is G, A, or S

<220>

<221> MISC_FEATURE

<222> (7)..(7)

<223> Xaa is S or K

<400> 61

Ala Ala Ser Xaa Xaa Xaa Xaa
1 5

<210> 62

<211> 9

<212> PRT

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 <223> Xaa is S or A

<220>
 <221> MISC_FEATURE
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 <223> Xaa is K or L

<400> 62

Gln Gln Xaa Xaa Glu Leu Pro Trp Thr
 1 5

<210> 63
 <211> 7
 <212> PRT
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<220>
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<220>
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 <222> (2)..(2)
 <223> Xaa is Y or F

<220>
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 <222> (3)..(3)
 <223> Xaa is S or T

<220>
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 <222> (5)..(5)
 <223> Xaa is T or S

<220>
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 <222> (6)..(6)
 <223> Xaa is D or G

<220>
 <221> MISC_FEATURE
 <222> (7)..(7)
 <223> Xaa is Y or A

<400> 63

Gly Xaa Xaa Phe Xaa Xaa Xaa
 1 5

<210> 64
 <211> 5
 <212> PRT
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<220>
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<220>
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 <223> Xaa is D or G

<220>
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 <222> (2)..(2)
 <223> Xaa is Y or A

<400> 64

Xaa Xaa Thr Met Asn
 1 5

<210> 65
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<220>
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 <222> (5)..(5)
 <223> Xaa is T or S

<220>
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<220>
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 <222> (7)..(7)
 <223> Xaa is Y or A

<400> 65

Gly	Xaa	Xaa	Phe	Xaa	Xaa	Xaa	Thr	Met	Asn
1			5						10

<210> 66
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 <223> Xaa is N or T

<400> 66

Asn	Pro	Tyr	Xaa	Gly	Gly
1				5	

<210> 67
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 <222> (6)..(6)
 <223> Xaa is N or T

<400> 67

Leu Ile Asn Pro Tyr Xaa Gly Gly Ile Arg Tyr Asn Gln Lys Phe Lys
1 5 10 15

Gly

<210> 68
<211> 10
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<223> Xaa is S or T

<400> 68

Ile Gly Gly Tyr Tyr Asp Xaa Met Asp Tyr
1 5 10

<210> 69
<211> 113
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<220>
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<400> 69

Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
1 5 10 15

Glu Thr Val Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

Gly Asn Gln Lys Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Pro Arg Gln Ser Gly Val

50

55

60

Pro Asp Arg Phe Ile Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 65 70 75 80

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

Tyr Tyr Asp Ala Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
 100 105 110

Lys

<210> 70

<211> 119

<212> PRT

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<400> 70

Glu Val Gln Leu Val Glu Ser Asp Gly Val Leu Val Gln Pro Gly Lys
 1 5 10 15

Ser Leu Thr Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
 20 25 30

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

Ala Thr Ile Ser Tyr Asp Asp Ile Thr Thr Phe Tyr Arg Asp Ser Val
 50 55 60

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

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

Thr Arg Gly Leu Gln Trp Pro Tyr Val Met Asp Ala Trp Gly Gln Gly
100 105 110

Ala Leu Val Thr Val Ser Ser
115

<210> 71
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 71

Glu Ile Val Leu Thr Gln Ser Pro Thr Thr Met Ala Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Leu Asn Tyr Met
20 25 30

His Trp Phe Gln Gln Lys Ser Gly Thr Ser Pro Lys Pro Trp Ile Tyr
35 40 45

Asp Thr Ser Glu Leu Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Leu Gln Lys Asn Gly Tyr Pro Leu Thr
85 90 95

Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 72
<211> 123
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 72

Glu Ala Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Lys Leu Ser Cys Glu Ala Ser Gly Phe Thr Phe Thr Lys Tyr
 20 25 30

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

Ala Ser Ile Ile Asn Ser Gly Gly Asn Thr Tyr Tyr Arg Asp Ser Val
 50 55 60

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

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

Ala Thr Asp Tyr Tyr Asp Gly Ser Tyr His Ser Asp Val Val Asp Ala
 100 105 110

Trp Gly Gln Gly Val Leu Val Thr Val Ser Ser
 115 120

<210> 73

<211> 17

<212> PRT

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<400> 73

Lys Ser Ser Gln Ser Leu Leu Tyr Ser Gly Asn Gln Lys Ser Tyr Leu
 1 5 10 15

Ala

<210> 74
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 <212> PRT
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<220>
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<400> 74

Trp Ala Ser Pro Arg Gln Ser
 1 5

<210> 75
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 <212> PRT
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<220>
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<400> 75

Gln Gln Tyr Tyr Asp Ala Pro Pro Thr
 1 5

<210> 76
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<400> 76

Asp Tyr Tyr Met Ala
 1 5

<210> 77
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<400> 77

Gly Phe Thr Phe Ser Asp Tyr Tyr Met Ala
1 5 10

<210> 78

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<400> 78

Ser Tyr Asp Asp Ile Thr
1 5

<210> 79

<211> 17

<212> PRT

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<220>

<223> Synthetic Construct

<400> 79

Thr Ile Ser Tyr Asp Asp Ile Thr Thr Phe Tyr Arg Asp Ser Val Lys
1 5 10 15

Gly

<210> 80

<211> 10

<212> PRT

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<223> Synthetic Construct

<400> 80

Gly Leu Gln Trp Pro Tyr Val Met Asp Ala
1 5 10

<210> 81
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<400> 81

Arg Ala Ser Ser Ser Leu Asn Tyr Met His
 1 5 10

<210> 82
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<220>
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<400> 82

Asp Thr Ser Glu Leu Ala Ser
 1 5

<210> 83
 <211> 9
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<220>
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<400> 83

Leu Gln Lys Asn Gly Tyr Pro Leu Thr
 1 5

<210> 84
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 <212> PRT
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<220>
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<400> 84

Gly Phe Thr Phe Thr Lys Tyr Gly Met Ala
1 5 10

<210> 85
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 85

Lys Tyr Gly Met Ala
1 5

<210> 86
<211> 7
<212> PRT
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<220>
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<400> 86

Gly Phe Thr Phe Thr Lys Tyr
1 5

<210> 87
<211> 14
<212> PRT
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<400> 87

Asp Tyr Tyr Asp Gly Ser Tyr His Ser Asp Val Val Asp Ala
1 5 10

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<211> 255
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 88

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cagcagaagt ccggacagcc tccgaaactg ttgatctacg ccgcgagcaa ccagggatcg	180
ggagtgccag cccgcttcag cggatcaggc tccggtaccg atttctccct ggacatccac	240
ccgatggagg aggac	255

<210> 89

<211> 255

<212> DNA

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<400> 89

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tcttgcaagg tgagtggata cagctttacc gactacacaa tgaactgggt gaagcagtct	120
cacgggaaga acctggagtg gattggcctg atcaatcctt acaacggcgg aattagatat	180
aaccagaagt ttaaaggaaa ggccctcctg accgttgaca aaagctcaaa taccgcctac	240
atggagctgt tgtct	255

<210> 90

<211> 255

<212> DNA

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<400> 90

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attacctgtc gcgcgagcga atccgtggat aacttcggta ttaactttct gaattggttt	120
caacaaaagc cgggcaaggc gccgaaactg ctgatctacg cagcgtcaaa tcagggtagc	180
ggtgttccgt cgcgcttcag cggtagcggg tccggcacgg actttacgct gacgatcagc	240

agcctgcagc cagag 255

<210> 91
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 <212> DNA
 <213> Artificial Sequence

<220>
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 ccgggttaaag gtctggagtg ggtcgctctg atcaatccgt acaacggcgg catccgctac 180
 aatcaaaagt ttaagggtcg cttcacgatt tcggttgata acgcgaagaa cagcctgtat 240
 ctgcaaatga atagc 255

<210> 92
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 <212> DNA
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<220>
 <223> Synthetic Construct

<400> 92
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 cagcagaaac caggcaaagc cccaaaactg ctgatttatg ccgcaagcaa ccagggcagc 180
 ggtgtgccga gccgcttttag cggtagcggc agcggcacccg attttaccct gaccattagc 240
 agcctgcagc cggaa 255

<210> 93
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 <212> DNA
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<220>
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<400> 93

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ccaggcaaag gcctggaatg ggtggccctg attaaccgtg ataacgggtg cattcgctat	180
aaccagaaat tcaaaggtcg ctttaccatc agtcgagata acgcaaaaaa cagcctgtat	240
ctgcagatga acagc	255

<210> 94
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cagcagaaac caggccaggc accacgcctg ctgatttatg ccgcaagcaa ccagggcagc	180
ggcattccgg atcgctttag cggcagcggt agcggcaccg attttaccct gaccattagt	240
cgcttgaac cggaa	255

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ccaggtcagg gcctggaatg gatgggcctg attaaccgtg ataacgggtg cattcgctat	180
aaccagaaat tcaaaggtcg cgtgaccatg accgcgata ccagcaccag caccgtgtat	240
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 cagcagaagc ccggcaaggc cccaagctg ctgatctacg ccgccagcaa tcaggccaag 180
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 cagggcctgg aatggatggg cctgatcaac ccctacaacg gcggcatccg gtacaaccag 180
 aaattcaagg gcagagtgc catgaccgag gacaccagca cctccaccgt gtacatggaa 240
 ctgagcagcc tgcgg 255

<210> 98
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 <212> DNA
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<220>
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cagcagaagc ctggaaaggc cccgaagctg cttatctatg ccgctccca agcctcaaaa	180
ggagtgccgt cgaggttctc cgggtcggga tccggaaccg acttcaccct gactattagc	240
agcctccagc ccgag	255

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cctggacagg gcctggaatg gatgggcctg atcaaccctt acaccggcgg catccggtac	180
aaccagaaat tcaagggcag agtgaccatg acccgggaca ccagcacctc caccgtgtac	240
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cagcagaagc ctggaaaggc cccgaagctg cttatctatg ccgctccca agcctcaaaa	180
ggagtgccgt cgaggttctc cgggtcggga tccggaaccg acttcaccct gactattagc	240
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<400> 101

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cctggacagg gcctggaatg gatgggcctg atcaaccctt acaccggcgg catccggtac 180

aaccagaaat tcaagggcag agtgaccatg acccgggaca ccagcacctc caccgtgtac 240

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<212> DNA

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<400> 102

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aatggttctc caaggcttct cataaagtat gcttctgagt ctgtctctgg gatcccttcc 180

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gaagatattg cagat 255

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tcctgcacgg ctactggcta cacaatcagt agatattgga tagagtgggt aaagcagagg 120

cctggacatg gccttgagtg gattggagag attttacctg gaagtgggtgt tactaactac 180

aatgagaagt tcaagggcaa ggccacattc actgcagata catcctcaa cacagcctac 240

atgcaactca gcagc 255

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 gggcaggcgc cccggctgct catctattac gcctccgaat ccgtgtccgg catcccggat 180
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 gaggacttcg ctgtg 255

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 cccggacagg gccttgagtg gatgggcgaa attctgccgg gatccggagt gaccaactac 180
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gggcaggcgc cccggctgct catctattac gcctccgaat ccgtgtccgg catcccggat      180
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tcctgcaaag cctccggcgg tacctttagc cgggtactgga tcgaatgggt cagacaggcg      120
cctgggcagg gactggaatg gatgggggaa atcctgccgg gctccggagt gaccttcgag      180
aacgagaagt tcaagggccg cgtgaccatt accgctgacg agtcgacttc aaccgcatat      240
atggaactca gcagc                                     255

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<210> 108
 <211> 137
 <212> PRT
 <213> Homo sapiens

<400> 108

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Gln Arg Pro Thr Gly Gly Pro Gly Cys Gly Pro Gly Arg Leu Leu Leu
1           5           10           15

```

```

Gly Thr Gly Thr Asp Ala Arg Cys Cys Arg Val His Thr Thr Arg Cys
20           25           30

```

```

Cys Arg Asp Tyr Pro Gly Glu Glu Cys Cys Ser Glu Trp Asp Cys Met
35           40           45

```

```

Cys Val Gln Pro Glu Phe His Cys Gly Asp Pro Cys Cys Thr Thr Cys
50           55           60

```

Arg His His Pro Cys Pro Pro Gly Gln Gly Val Gln Ser Gln Gly Lys
65 70 75 80

Phe Ser Phe Gly Phe Gln Cys Ile Asp Cys Ala Ser Gly Thr Phe Ser
85 90 95

Gly Gly His Glu Gly His Cys Lys Pro Trp Thr Asp Cys Thr Gln Phe
100 105 110

Gly Phe Leu Thr Val Phe Pro Gly Asn Lys Thr His Asn Ala Val Cys
115 120 125

Val Pro Gly Ser Pro Pro Ala Glu Pro
130 135

<210> 109
<211> 218
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 109

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Ser Val Glu Pro Arg
20 25 30

Gly Ile Asn Phe Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
35 40 45

Lys Leu Leu Ile Tyr Ala Ala Ser Gln Ala Ser Lys Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

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Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Leu
85 90 95

Glu Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 110
<211> 217
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 110

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Ala
20 25 30

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

Gly Leu Ile Asn Pro Tyr Thr Gly Gly Ile Arg Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65 70 75 80

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

Ala Arg Ile Gly Gly Tyr Tyr Asp Ser Met Asp Tyr Trp Gly Gln Gly
100 105 110

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

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

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

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

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

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

Ser Asn Thr Lys Val Asp Lys Lys Val
210 215

<210> 111

<211> 111

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 111

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Ser Val Gln Pro Arg
 20 25 30

Gly Ile Asn Phe Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
 35 40 45

Lys Leu Leu Ile Tyr Ala Ala Ser Asn Pro Ser Lys Gly Val Pro Ser
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
 65 70 75 80

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Leu
 85 90 95

Glu Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105 110

<210> 112

<211> 119

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 112

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
 20 25 30

Thr Val Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Leu Ile Asn Pro Tyr Thr Gly Gly Ile Arg Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65 70 75 80

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

Ala Arg Ile Gly Gly Tyr Tyr Asp Ser Met Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 113
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 113

Arg Ala Ser Glu Ser Val Gln Pro Arg Gly Ile Asn Phe Leu Asn
1 5 10 15

<210> 114
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 114

Ala Ala Ser Asn Pro Ser Lys
1 5

<210> 115
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 115

Gly	Tyr	Thr	Phe	Thr	Gly	Tyr	Thr	Val	Ser
1				5					10

<210> 116
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 116

Gly	Tyr	Thr	Phe	Thr	Gly	Tyr
1				5		

<210> 117
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 117

Gly	Tyr	Thr	Val	Ser
1				5

<210> 118
 <211> 255
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 118
caggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac caggcgccag cgtgaaggtg 60
tcctgcaagg ccagcggcta cacctttaca ggctacaccg tgtcctgggt gcgccaggct 120
cctggacagg gactggaatg gatgggcctg atcaaccctt acaccggcgg catccggtac 180
aaccagaaat tcaagggcag agtgaccatg acccgggaca ccagcacctc caccgtgtac 240
atggaactga gcagc 255

<210> 119
<211> 255
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 119
gacatccaaa tgaccagtc cccttcctca ctctccgctt ccgtgggcga ccgcgtgacc 60
atcacgtgtc gggcatcgga aagcgtgcaa ccaagaggga ttaacttcct gaactggtac 120
cagcagaagc ctggaaaggc cccgaagctg cttatctatg ccgcgtccaa cccgtcaaaa 180
ggagtgccgt cgaggttctc cgggtcggga tccggaaccg acttcaccct gactattagc 240
agcctccagc ccgag 255

<210> 120
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 120

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Ile Gly Thr Ser
20 25 30

Ile His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Tyr Ala Ser Glu Ser Val Ser Gly Ile Pro Asp Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Thr Tyr Ser Trp Pro Ala
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 121
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 121

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Arg Tyr
20 25 30

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

Gly Glu Ile Leu Pro Gly Ser Gly Val Glu Trp Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

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

Ala Arg Lys Gly Arg Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
 100 105 110

Ser Val Thr Val Ser Ser
 115

<210> 122
 <211> 107
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 122

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Ile Gly Thr Ser
 20 25 30

Ile His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
 35 40 45

Tyr Tyr Ala Ser Glu Ser Val Ser Gly Ile Pro Asp Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro
 65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Thr Tyr Ser Trp Pro Ala
 85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 123
 <211> 118
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 123

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Arg Tyr
 20 25 30

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

Gly Glu Ile Leu Pro Gly Ser Gly Val Thr Phe Glu Asn Glu Lys Phe
 50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
 65 70 75 80

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

Ala Arg Lys Gly Arg Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
 100 105 110

Ser Val Thr Val Ser Ser
 115

<210> 124

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 124

Lys Gly Arg Gly Tyr Ala Met Asp Tyr
 1 5

<210> 125

<211> 255

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 125

gaaatcgtgc tgactcagtc acctggaacc ctctcgttgt cccccgggga aagggccact 60

ctttcctgcc gggcatccca gtcgatcggga acctccattc actggtacca gcagaagccg 120

gggcaggcgc cccggctgct catctattac gcctccgaat ccgtgtccgg catcccggat 180

agattcagcg gaagcggctc aggcaccgac ttaccctga ctatctcgcg cctggagcct 240

gaggacttcg ctgtg 255

<210> 126

<211> 255

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 126

caagtgaac ttgtgcagtc gggagccgaa gtcaagaagc ccggttcctc cgtgaaagtg 60

tcctgcaaag cctccggcgg taccttttagc cggtactgga tcgaatgggt cagacaggcg 120

cctgggcagg gactggaatg gatgggggaa atcctgccgg gctccggagt gaccttcgag 180

aacgagaagt tcaagggccg cgtgaccatt accgctgacg agtcgacttc aaccgcatat 240

atggaactca gcagc 255

<210> 127

<211> 255

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 127

gaaatcgtgc tgactcagtc acctggaacc ctctcgttgt cccccgggga aagggccact 60

ctttcctgcc gggcatccca gtcgatcggga acctccattc actggtacca gcagaagccg 120

gggcaggcgc cccggctgct catctattac gcctccgaat ccgtgtccgg catcccggat 180

agattcagcg gaagcggctc aggcaccgac tttaccctga ctatctcgcg cctggagcct 240
gaggacttcg ctgtg 255

<210> 128
<211> 255
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 128
caagtgcaac ttgtgcagtc gggagccgaa gtcaagaagc ccggttcctc cgtgaaagt 60
tcctgcaaag cctccggcgg taccttttagc cggtactgga tcgaatgggt cagacaggcg 120
cctgggcagg gactggaatg gatgggggaa atcctgccgg gctccggagt gaccttcgag 180
aacgagaagt tcaagggccg cgtgaccatt accgctgacg agtcgacttc aaccgcatat 240
atggaactca gcagc 255

<210> 129
<211> 255
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 129

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

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Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
115 120 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
130 135 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145 150 155 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225 230 235 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
245 250 255

<210> 130
<211> 15
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 130

Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser
1				5					10					15