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(54) Titre : ANTICORPS ANTI-GITR POUR LE DIAGNOSTIC DU CANCER
(54) Title: ANTI-GITR ANTIBODIES FOR CANCER DIAGNOSTICS

(57) **Abrégé/Abstract:**

Provided herein are diagnostic antibodies that bind to glucocorticoid-induced tumor necrosis factor receptor (GITR). Such antibodies are useful for methods of detecting the expression of GITR in biological samples, for example, tumor tissue, and identifying a cancer patient likely to respond to anti-GITR immunotherapy or predicting whether a cancer patient will respond to anti-GITR immunotherapy.

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(54) Title: ANTI-GITR ANTIBODIES FOR CANCER DIAGNOSTICS

(57) Abstract: Provided herein are diagnostic antibodies that bind to glucocorticoid-induced tumor necrosis factor receptor (GITR). Such antibodies are useful for methods of detecting the expression of GITR in biological samples, for example, tumor tissue, and identifying a cancer patient likely to respond to anti-GITR immunotherapy or predicting whether a cancer patient will respond to anti-GITR immunotherapy.

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ANTI-GITR ANTIBODIES FOR CANCER DIAGNOSTICS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to United States Provisional Patent Application serial No. 62/170,579, filed June 3, 2015, the contents of which are specifically incorporated by reference herein.

BACKGROUND

Glucocorticoid-inducible TNF receptor (GITR) is a T cell costimulatory type I cell surface transmembrane glycoprotein expressed by activated effector T (Teff) cells and at low levels on B cells, eosinophils, basophils, and macrophages (Nocentini et al., *Adv Exp Med Biol* 2009;647:156-73). GITR is constitutively expressed on regulatory T (Treg) cells. Signaling through GITR potentiates T cell receptor activation of T cells and renders Teff cells resistant to Treg-mediated suppression (Stephens et al., *J Immunol* 2004;173:5008-20). Determining GITR positivity in tumor tissue may provide an opportunity to target patients that are most likely to benefit from (i.e., be responsive to) anti-GITR immunotherapy, as well as monitoring the status of GITR-positive tumors and efficacy of anti-GITR immunotherapy.

SUMMARY

Provided herein are diagnostic anti-GITR antibodies and methods for detecting GITR protein expression in biological samples, such as tumor tissue samples. Such methods may be useful for, e.g., identifying cancer patients who are likely to respond to anti-GITR immunotherapy and monitoring the status of GITR-positive tumors and efficacy of anti-GITR immunotherapy.

Accordingly, in one aspect, provided herein are methods for detecting human GITR in a biological sample, for example, a human tissue sample (e.g., tumor tissue sample), comprising contacting the sample with an antibody, or antigen-binding fragment thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the

antibody or antigen-binding fragment thereof specifically binds to human GITR, and detecting the binding of the antibody to GITR in the sample.

Also provided herein are methods of determining whether a tumor of a cancer patient is GITR-positive comprising:

(a) contacting a tumor sample from the patient with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR,

(b) detecting the binding of the antibody to GITR in the sample, and

(c) determining the level of GITR protein expression in the sample, wherein a level of GITR protein above a threshold level indicates that the tumor is GITR-positive.

Also provided herein are methods of identifying a cancer patient likely to respond to an anti-GITR immunotherapy comprising:

(a) contacting a tumor sample from the patient with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR,

(b) detecting the binding of the antibody to GITR in the tumor sample,

(c) determining the level of GITR protein expression in the sample, wherein a level of GITR protein above a threshold level indicates that the tumor is GITR-positive tumor and that the patient is likely to or predicted to respond to anti-GITR immunotherapy.

Also provide herein are methods of treating a cancer patient comprising:

(a) determining whether a tumor of a cancer patient is GITR-positive by (i) contacting a tumor sample from the patient with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively,

wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR, (ii) detecting the binding of the antibody to GITR in the tumor sample, and (iii) determining the level of GITR protein expression in the sample, wherein a level of GITR protein above a threshold level indicates that the tumor is GITR-positive, and

(b) if the tumor is determined to be GITR-positive, administering to the patient an agent that modulates GITR signaling. In certain embodiments, the agent that modulates GITR signaling is an agonistic (i.e., agonist) anti-GITR antibody.

Also provided herein are methods of monitoring a GITR-positive tumor in a cancer patient comprising:

(a) detecting GITR protein expression in the GITR-positive tumor at a first time point by using an antibody comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR,

(b) determining the level of GITR protein expression at the first time point,

(c) detecting GITR protein expression in the tumor at a second time point using the same antibody used in step (a),

(d) determining the level of GITR protein expression in the tumor from the second time point;

(e) comparing the levels of GITR protein expression determined at the first and second time points, wherein a higher level at the first time point relative to the second time point is indicative of tumor regression, a lower score at the first time point relative to the second time point is indicative of tumor progression, and a relatively unchanged level at the first time point relative to the second time point is indicative of a stable tumor.

Also provided herein are methods of monitoring the efficacy of anti-GITR immunotherapy in a patient having a GITR-positive tumor comprising:

(a) detecting GITR protein expression in the GITR-positive tumor at a first time point before or after initiating anti-GITR immunotherapy by using an antibody comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising

the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR,

(b) determining the level of GITR protein expression in the tumor from the first time point,

(c) detecting GITR protein expression in the GITR-positive tumor at a second time point after initiating anti-GITR immunotherapy using the same antibody from step (a),

(d) determining the level of GITR protein expression in the tumor from the second time point;

(e) comparing the levels of GITR protein expression determined at the first and second time points, wherein a higher level at the first time point relative to the second time point is indicative of effective anti-GITR immunotherapy, a lower score at the first time point relative to the second time point is indicative of ineffective anti-GITR immunotherapy, and an unchanged score at the first time point relative to the second time point is indicative of anti-GITR immunotherapy being stabilizing. In certain embodiments, the anti-GITR immunotherapy comprises administering to the patient a therapeutically effective amount of an anti-GITR antibody, or antigen binding portion thereof.

In certain embodiments, the tumor sample used in the methods described herein is a formalin-fixed paraffin-embedded tumor sample. In certain embodiments, the tumor sample is a frozen tumor tissue sample. In certain embodiments, the methods described herein are performed by immunohistochemistry. In certain embodiments, the level of GITR expression is determined using a scoring system, e.g., the H score system, the Allred score system, the AQUA system, or an automated scoring system.

In certain embodiments, the antibody used in the methods described herein comprises heavy and light chain variable regions comprising the amino acid sequences set forth in SEQ ID NOs: 11 and 12, respectively. In certain embodiments, the antibody used in the methods described herein comprises heavy and light chains comprising the amino acid sequences set forth in SEQ ID NOs: 13 and 14, respectively. In certain embodiments, the heavy chain lacks the C-terminal lysine. In certain embodiments, the antibody is an IgG1, IgG2, IgG3, or IgG4 antibody. In certain embodiments, the antibody binds to soluble human GITR with a K_D of 10 nM or less, as assessed by Biacore.

In certain embodiments, the antibody is a humanized, human, or chimeric antibody. In certain embodiments, the antibody is a chimeric antibody comprising human variable region sequences and non-human constant region sequences, for example, a mouse heavy and light chain constant region. In certain embodiments, the mouse Fc region is a mouse IgG2a Fc region. In certain embodiments, the chimeric antibody comprises heavy and light chain sequences set forth in SEQ ID NOs: 15 and 16, respectively. In certain embodiments, the heavy chain lacks the C-terminal lysine.

In certain embodiments, the antibody used in the methods described herein comprises a detectable moiety, such as a radiolabel, fluorescent label, an enzymatic label, biotin, chromophore, or an ECL label.

Also provided herein are diagnostic kits comprising an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR, and instructions for use. In certain embodiments, the antibody, or antigen-binding portion thereof, comprises the heavy and light chain variable region sequences set forth in SEQ ID NOs: 11 and 12, respectively. In certain embodiments, the antibody comprises the heavy and light chain sequences set forth in SEQ ID NOs: 13 and 14, respectively. In certain embodiments, the antibody comprises the heavy and light chain sequences set forth in SEQ ID NOs: 15 and 16, respectively. In certain embodiments, the heavy chain lacks the C-terminal lysine.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the nucleotide sequence (SEQ ID NO: 17) and amino acid sequence (SEQ ID NO: 11) of the heavy chain variable region of the 6G10 human monoclonal antibody. The CDR1 (SEQ ID NO: 5), CDR2 (SEQ ID NO: 6) and CDR3 (SEQ ID NO: 7) regions are delineated and the V, D and J germline derivations are indicated.

Figure 2 shows the nucleotide sequence (SEQ ID NO: 18) and amino acid sequence (SEQ ID NO: 12) of the kappa light chain variable region of the 6G10 human monoclonal antibody. The CDR1 (SEQ ID NO: 8), CDR2 (SEQ ID NO: 9) and CDR3 (SEQ ID NO: 10) regions are delineated and the V and J germline derivations are indicated.

Figure 3 shows an alignment of the amino acid sequence of the heavy chain variable regions of 6G10 (SEQ ID NO: 11) with the human germline V_H 3-33, 3-10, and JH6 amino acid sequences.

Figure 4 shows an alignment of the amino acid sequence of the light chain variable region (VK1) of 6G10 (SEQ ID NO: 12) with the human germline V_K L18 and JK2 amino acid sequences.

Figure 5 shows the nucleotide sequence (SEQ ID NO: 21) and amino acid sequence (SEQ ID NO: 15) of the heavy chain of the chimeric 6G10 antibody.

Figure 6 shows the nucleotide sequence (SEQ ID NO: 22) and amino acid sequence (SEQ ID NO: 16) of the light chain of the chimeric 6G10 antibody.

Figure 7 shows staining of CD3, FoxP3, and GITR on adjacent formalin-fixed paraffin embedded (FFPE) sections from cervical squamous cell carcinoma samples by immunohistochemistry using the chimeric 6G10 antibody.

DETAILED DESCRIPTION

Disclosed herein are various methods for detecting GITR expression in biological samples, e.g., tumor specimens, using diagnostic anti-GITR antibodies. Such antibodies may be useful for, e.g., monitoring GITR protein levels in a subject; identifying the specific cells that are GITR positive in a specific tissue, such as a tumor tissue (e.g., identifying the presence of GITR in Treg cells and/or tumor cells); the early detection of cancers; monitoring and identification of existing cancers; and assessing the therapeutic efficacy of anti-GITR immunotherapy.

Definitions

In order that the present description may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

The term "glucocorticoid-inducible TNF receptor" or "GITR" as used herein refers to a receptor that is a member of the TNF-receptor superfamily, which binds to GITR ligand (GITR-L). GITR is also referred to as tumor necrosis factor receptor superfamily, member 18 (TNFRSF18), AITR and CD357. The term "GITR" includes any variants or isoforms of GITR which are naturally expressed by cells. Accordingly, antibodies described herein may cross-react

with GTR from species other than human (e.g., cynomolgus GTR). Alternatively, the antibodies may be specific for human GTR and may not exhibit any cross-reactivity with other species. GTR or any variants and isoforms thereof, may either be isolated from cells or tissues which naturally express them or be recombinantly produced using well-known techniques in the art and/or those described herein.

Three isoforms of human GTR have been identified, all of which share the same extracellular domain, except for its C-terminal portion. Variant 1 (Accession No. NP_004186; SEQ ID NO: 1) consists of 241 amino acids and represents the longest transcript. It contains an extra coding segment that leads to a frame shift, compared to variant 2. The resulting protein (isoform 1) contains a distinct and shorter C-terminus, as compared to isoform 2. Variant 2 (Accession No. NP_683699; SEQ ID NO: 2) encodes the longest protein (isoform 2), consisting of 255 amino acids, and is soluble. Variant 3 (Accession No. NP_683700; SEQ ID NO: 3) contains an extra coding segment that leads to a frame shift, compared to variant 2. The resulting protein (isoform 3) contains a distinct and shorter C-terminus, as compared to isoform 2, and consists of 234 amino acids. The amino acid sequences of human GTR isoforms 1, 2, and 3 are set forth in SEQ ID NOs: 1, 2, and 3, respectively, and the extracellular domain of mature GTR has the amino acid sequence set forth in SEQ ID NO: 4.

The term “antibody” as used to herein includes whole antibodies and any antigen binding fragments (*i.e.*, “antigen-binding portions”) or single chains thereof. An “antibody” refers, in one embodiment, to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. In certain naturally occurring antibodies, the heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. In certain naturally occurring antibodies, each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding

domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

Antibodies typically bind specifically to their cognate antigen with high affinity, reflected by a dissociation constant (K_D) of 10^{-5} to 10^{-11} M or less. Any K_D greater than about 10^{-4} M is generally considered to indicate nonspecific binding. As used herein, an antibody that "binds specifically" to an antigen refers to an antibody that binds to the antigen and substantially identical antigens with high affinity, which means having a K_D of 10^{-7} M or less, preferably 10^{-8} M or less, even more preferably 5×10^{-9} M or less, and most preferably between 10^{-8} M and 10^{-10} M or less, but does not bind with high affinity to unrelated antigens.

An immunoglobulin may be from any of the commonly known isotypes, including but not limited to IgA, secretory IgA, IgG and IgM. The IgG isotype is divided in subclasses in certain species: IgG1, IgG2, IgG3 and IgG4 in humans, and IgG1, IgG2a, IgG2b and IgG3 in mice. In certain embodiments, the anti-GITR antibodies described herein are of the IgG1 or IgG2 subtype. Immunoglobulins, *e.g.*, IgG1, exist in several allotypes, which differ from each other in at most a few amino acids. "Antibody" includes, by way of example, both naturally occurring and non-naturally occurring antibodies; monoclonal and polyclonal antibodies; chimeric and humanized antibodies; human and nonhuman antibodies; wholly synthetic antibodies; and single chain antibodies.

The term "antigen-binding portion" of an antibody, as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, human GITR). Such "fragments" are, for example between about 8 and about 1500 amino acids in length, suitably between about 8 and about 745 amino acids in length, suitably about 8 to about 300, for example about 8 to about 200 amino acids, or about 10 to about 50 or 100 amino acids in length. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody, *e.g.*, an anti-GITR antibody described herein, include (i) a Fab fragment, a monovalent fragment consisting of the V_L , V_H , CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and CH1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb

fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR) or (vii) a combination of two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term “antigen-binding portion” of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. Antigen-binding portions can be produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins.

The term “monoclonal antibody,” as used herein, refers to an antibody that displays a single binding specificity and affinity for a particular epitope or a composition of antibodies in which all antibodies display a single binding specificity and affinity for a particular epitope. Accordingly, the term “human monoclonal antibody” refers to an antibody or antibody composition that display(s) a single binding specificity and which has variable and optional constant regions derived from human germline immunoglobulin sequences. In one embodiment, human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

The term “recombinant human antibody,” as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, (b) antibodies isolated from a host cell transformed to express the antibody, *e.g.*, from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies comprise variable and constant regions that utilize particular human germline immunoglobulin sequences are encoded

by the germline genes, but include subsequent rearrangements and mutations which occur, for example, during antibody maturation. As known in the art (see, *e.g.*, Lonberg, *Nature Biotech* 2005;23:1117-25), the variable region contains the antigen binding domain, which is encoded by various genes that rearrange to form an antibody specific for a foreign antigen. In addition to rearrangement, the variable region can be further modified by multiple single amino acid changes (referred to as somatic mutation or hypermutation) to increase the affinity of the antibody to the foreign antigen. The constant region will change in further response to an antigen (*i.e.*, isotype switch). Therefore, the rearranged and somatically mutated nucleic acid molecules that encode the light chain and heavy chain immunoglobulin polypeptides in response to an antigen may not have sequence identity with the original nucleic acid molecules, but instead will be substantially identical or similar (*i.e.*, have at least 80% identity).

A "human" antibody (HuMAb) refers to an antibody having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The antibodies described herein may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. The terms "human" antibodies and "fully human" antibodies are used synonymously.

A "humanized" antibody refers to an antibody in which some, most or all of the amino acids outside the CDR domains of a non-human antibody are replaced with corresponding amino acids derived from human immunoglobulins. In one embodiment of a humanized form of an antibody, some, most or all of the amino acids outside the CDR domains have been replaced with amino acids from human immunoglobulins, whereas some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they do not abrogate the ability of the antibody to bind to a particular antigen. A "humanized" antibody retains an antigenic specificity similar to that of the original antibody.

A "chimeric antibody" refers to an antibody in which the variable regions are derived

from one species (e.g., human variable regions) and the constant regions are derived from another species (e.g., mouse constant regions), such as an antibody in which the variable regions are derived from a mouse antibody and the constant regions are derived from a human antibody. Constant region sequences for various species are known in the art and could be readily obtained by the skilled artisan to generate chimeric antibodies. An exemplary chimeric anti-GITR antibody comprises human variable region sequences and mouse constant chain sequences. For example, in one embodiment, a chimeric anti-GITR antibody comprises the heavy and light chain sequences set forth in SEQ ID NOs: 15 and 16, respectively. In certain embodiments, the heavy chain of the chimeric antibody lacks the C-terminal lysine.

An “isolated antibody,” as used herein, is intended to refer to an antibody which is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds to GITR is substantially free of antibodies that specifically bind antigens other than GITR). An isolated antibody that specifically binds to an epitope of GITR may, however, have cross-reactivity to other GITR proteins from different species.

As used herein, the terms “specific binding,” “selective binding,” “selectively binds,” and “specifically binds,” refer to antibody binding to an epitope on a predetermined antigen. Typically, the antibody (i) binds with an equilibrium dissociation constant (K_D) of approximately less than 10^{-7} M, such as approximately less than 10^{-8} M, 10^{-9} M or 10^{-10} M or even lower when determined by, e.g., surface plasmon resonance (SPR) technology in a BIACORE 2000 instrument using the predetermined antigen, e.g., recombinant human GITR, as the analyte and the antibody as the ligand.

The term " k_{assoc} " or " k_a ," as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term " k_{dis} " or " k_d ," as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term " K_D ," as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of k_d to k_a (i.e., k_d/k_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. A preferred method for determining the K_D of an antibody is by using surface plasmon resonance, preferably using a biosensor system such as a Biacore® system.

A "polypeptide" refers to a chain comprising at least two consecutively linked amino acid residues, with no upper limit on the length of the chain. One or more amino acid residues in the

protein may contain a modification such as, but not limited to, glycosylation, phosphorylation or disulfide bond formation. A "protein" may comprise one or more polypeptides.

"Immunotherapy" refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response. In this context, "anti-GITR immunotherapy" refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease (e.g., cancer) by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response by administering an agent (e.g., an agonistic anti-GITR antibody) that modulates GITR signaling and results in, e.g., the induction and/or enhancement of T cell activation (e.g., increase in IL-2 and/or IFN- γ production by T cells and/or increase in proliferation of T cells), depletion of regulatory T cells, etc.

As used herein, the term "linked" refers to the association of two or more molecules. The linkage can be covalent or non-covalent. The linkage also can be genetic (*i.e.*, recombinantly fused). Such linkages can be achieved using a wide variety of art recognized techniques, such as chemical conjugation and recombinant protein production.

As used herein, "administering" refers to the physical introduction of a composition comprising a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Preferred routes of administration for antibodies described herein include intravenous, intraperitoneal, intramuscular, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intraperitoneal, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation. Alternatively, an antibody described herein can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

As used herein, "cancer" refers to a broad group of diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division may result in the formation of malignant tumors or cells that invade neighboring tissues and may metastasize to distant parts of the body through the lymphatic system or bloodstream.

As used herein, a "tumor" refers to an abnormal mass of tissue resulting from excessive cell division that is uncontrolled and progressive. Tumors can be benign or malignant.

The term "patient" includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment.

As used herein, the term "subject" includes any human or non-human animal. For example, the methods and compositions described herein can be used to treat a subject having cancer. The term "non-human animal" includes all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, reptiles, *etc.* Various aspects described herein are described in further detail in the following subsections.

As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. The use of "or" or "and" means "and/or" unless stated otherwise. Furthermore, use of the term "including" as well as other forms, such as "include," "includes," and "included," is not limiting.

The term "about" as used herein when referring to a measurable value such as an amount, temporal duration, and the like, encompasses variations of up to $\pm 10\%$ from the specified value. Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, scores in an art-recognized scoring system, *etc.*, used herein are understood as being modified by the term "about."

The term "sample of a subject" refers to cells (*e.g.*, tumor cells, T cells, B cells or other), tissues (*e.g.*, tumor), fluids and/or combination thereof isolated from a subject.

The term "control sample" or "reference sample" as used herein refers to any clinically relevant control or reference sample, including, *e.g.*, a sample from a healthy subject or a sample made at an earlier time from the subject being assessed. For example, a control sample or reference sample can be a sample taken from a subject prior to onset of cancer, an earlier stage of disease, or before administration of treatment.

As used herein, "threshold level" or "cut-off level" in the context of expression levels refers to a level of GITR protein expression above which a patient sample (*e.g.*, a patient tumor

sample) is deemed "GITR-positive" and below which the sample is deemed "GITR-negative." The threshold level may be based on, e.g., one or more compilations of data from "normal" patient samples (e.g., a patient population who do not have GITR-positive tumors) or matching normal tissue from the patient being assessed. The threshold level may be placed in the framework of an art-recognized scoring system (e.g., the H score system). For example, in one embodiment, the tumor of a patient having an H score of 1 in matching normal tissue would be considered GITR-positive if the H score of the tumor is greater than 1. A threshold detection level may be the lowest detection level using a given detection method. For example, a threshold level may be the level below which GITR cannot be detected using immunohistochemistry, e.g., using an immunohistochemistry method described herein.

Diagnostic anti-GITR antibodies

Provided herein are diagnostic anti-GITR antibodies that are useful for detecting GITR protein expression in human tissue, e.g., tumor tissue. Such antibodies are useful, e.g., for identifying/selecting subjects who are likely to benefit from (i.e., be responsive to) treatment with anti-GITR immunotherapy, monitoring the progression of a GITR-positive tumor, and/or monitoring the efficacy of anti-GITR immunotherapy.

Accordingly, in certain embodiments, a diagnostic anti-GITR antibody useful for detecting GITR protein expression in tissue samples, e.g., human tumor tissue samples, comprises the heavy and light chain variable region CDR sequences, heavy and light chain variable region sequences, or full length heavy and light chain sequences of antibody 6G10 (a fully human anti-GITR antibody). A summary of the relevant sequences for 6G10 is provided in Table 2.

In some embodiments, the anti-GITR antibody has the heavy chain variable region CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NOs: 5-7, and the light chain variable region CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NOs: 8-10, and specifically binds to human GITR.

In some embodiments, the anti-GITR antibody has the heavy chain variable region sequence set forth in SEQ ID NO: 11, and the light chain variable region sequence set forth in SEQ ID NO: 12.

In some embodiments, the anti-GITR antibody has heavy chain and light chain sequences set forth in SEQ ID NOs: 13 and 14, respectively. In certain embodiments, the heavy chain lacks the C-terminal lysine.

In some embodiments, the anti-GITR antibody is an IgG1, IgG2, IgG3, or IgG4 antibody, or a variant thereof. In other embodiments, the antibody is an IgA, IgD, IgE, or IgM antibody.

In some embodiments, the anti-GITR antibody is a human antibody, a humanized antibody, or a chimeric antibody.

In some embodiments, the anti-GITR antibody binds to soluble GITR with a K_D of 10 nM or less as determined by Biacore.

In certain embodiments, the anti-GITR antibody is a chimeric antibody. In one embodiment, the anti-GITR antibody has the heavy and light chain variable region sequences of the 6G10 antibody (SEQ ID NOs: 11 and 12, respectively), and the constant regions of a non-human species, e.g., a mouse IgG2a constant region (SEQ ID NO: 25). Such chimeric 6G10 antibodies are useful for, e.g., reducing background staining in immunohistochemistry when detecting GITR protein expression in human tissue. Accordingly, in one embodiment, the diagnostic anti-GITR antibody is a chimeric antibody comprising the heavy and light chain sequences set forth in SEQ ID NOs: 15 and 16, respectively. In certain embodiments, the heavy chain of the chimeric antibody lacks the C-terminal lysine.

In certain embodiments, the diagnostic anti-GITR antibodies described herein comprise a detectable moiety to facilitate detection of GITR expression in, for example, tumor samples by, e.g., immunohistochemistry, as described *infra*. Exemplary detection moieties include luminescent labels, fluorescent labels, radiolabels (e.g., ^{99}Tc , ^{45}Ti , ^{112}In , ^{111}In , ^3H , ^{121}I , ^{125}I , ^{131}I , ^{14}C , ^{18}F , ^{36}Cl , ^{55}Co , ^{58}Co , ^{51}Cr , ^{67}Cu , ^{64}Cu , ^{66}Ga , ^{68}Ga , ^{76}Br , ^{89}Zr , ^{35}S , ^{32}P , ^{90}Y , ^{13}N , ^{15}O , ^{211}At , ^{186}Re , ^{188}Re , ^{75}Se), and enzymatic labels (e.g., horseradish peroxidase, alkaline phosphatase, glucose oxidase, urease, acetylcholinesterase, luciferase, and beta-galactosidase), epitope tags, chromophore labels, phosphorescent labels, photoaffinity molecules, ECL labels, dyes, biotin, haptens, and the like. Such labels are well known in the art and can be attached to the anti-GITR antibodies using art-recognized methods. Preferably, attachment of the detectable label does not significantly interfere with binding of the antibody to GITR. The detection means is determined by the chosen label. Appearance of the label or its reaction products can be achieved using the naked eye, in the case where the label is particulate and accumulates at

appropriate levels, or using instruments such as a spectrophotometer, a luminometer, a fluorimeter, and the like, all in accordance with standard practice.

Preferably, methods of conjugating the labels result in linkages which are substantially (or nearly) non-immunogenic, e.g., peptide- (i.e. amide-), sulfide-, (sterically hindered), disulfide-, hydrazone-, and ether linkages. These linkages are nearly non-immunogenic and show reasonable stability within serum (see e.g. Senter et al., *Curr Opin Chem Biol* 2009;13 235-44; WO 2009/059278; WO 95/17886).

Depending on the biochemical nature of the moiety and the antibody, different conjugation strategies can be employed. In case the moiety is naturally occurring or recombinant of between 50 to 500 amino acids, there are standard procedures in text books describing the chemistry for synthesis of protein conjugates, which can be easily followed by the skilled artisan (see e.g. Hackenberger et al., *Angew Chem Int Ed Engl* 2008;47:10030-74). In one embodiment the reaction of a maleinimido moiety with a cysteine residue within the antibody or the moiety is used. This is an especially suited coupling chemistry in case e.g. a Fab or Fab'-fragment of an antibody is used. Alternatively in one embodiment coupling to the C-terminal end of the antibody or moiety is performed. C-terminal modification of a protein, e.g. of a Fab-fragment can e.g. be performed as described (Sunbul et al., *Org Biomol Chem* 2009;7:3361-71).

In general, site specific reaction and covalent coupling is based on transforming a natural amino acid into an amino acid with a reactivity which is orthogonal to the reactivity of the other functional groups present. For example, a specific cysteine within a rare sequence context can be enzymatically converted in an aldehyde (see Frese et al. *ChemBioChem* 2009;10:425-7). It is also possible to obtain a desired amino acid modification by utilizing the specific enzymatic reactivity of certain enzymes with a natural amino acid in a given sequence context (see, e.g., Taki et al., *Prot Eng Des Sel* 2004;17:119-26; Gautier et al. *Chem Biol* 2008;15:128-36; Bordusa et al., *Bioorganic Chemistry* (2004) 389-403).

Site specific reaction and covalent coupling can also be achieved by the selective reaction of terminal amino acids with appropriate modifying reagents.

The reactivity of an N-terminal cysteine with benzonitrils (see Ren et al., *Angew Chem Int Ed Engl* 2009;48:9658-62) can be used to achieve a site-specific covalent coupling.

Native chemical ligation can also rely on C-terminal cysteine residues (Taylor et al., *Nucleic Acids and Molecular Biology* 2009;22:65-96). EP 1 074 563 describes a conjugation

method which is based on the faster reaction of a cysteine within a stretch of negatively charged amino acids with a cysteine located in a stretch of positively charged amino acids.

The moiety may also be a synthetic peptide or peptide mimic. In case a polypeptide is chemically synthesized, amino acids with orthogonal chemical reactivity can be incorporated during such synthesis (see, e.g., de Graaf et al., *Bioconjug Chem* 2009;20:1281-95). Since a great variety of orthogonal functional groups is at stake and can be introduced into a synthetic peptide, conjugation of such peptide to a linker is standard chemistry.

In some embodiments, the detectable label is indirectly conjugated to the antibody. For example, the antibody can be conjugated to biotin, and the label can be conjugated to avidin using methods well known in the art. In another embodiment, an unlabeled antibody is detected using a labeled antibody which binds to the unlabeled antibody. In certain embodiments, the labeled antibody that is used for detection is labeled with any of the labels set forth above. In certain embodiments, the labeling and detection is conducted as described in the Examples, e.g., using the Leica Bond Polymer Refine Detection System (Leica, Buffalo Grove, IL, Catalog DS9800).

Methods of production

The diagnostic anti-GITR antibodies described herein can be produced using art-recognized techniques. In certain embodiments, the antibody can be purified from a hybridoma producing the antibody (e.g., the 6G10 antibody). For example, a hybridoma producing the antibody can be grown in two-liter spinner-flasks for monoclonal antibody purification. Supernatants can be filtered and concentrated before affinity chromatography with protein A-sepharose (Pharmacia, Piscataway, N.J.). Eluted IgG can be checked by gel electrophoresis and high performance liquid chromatography to ensure purity. The buffer solution can be exchanged into PBS, and the concentration can be determined by OD280 using the 1.43 extinction coefficient.

In certain embodiments, the antibodies described herein can be produced recombinantly. For example, DNAs encoding partial or full-length light and heavy chains described herein can be inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational

control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vector or both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector(s) by standard methods (*e.g.*, ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). The light and heavy chain variable regions of the antibodies described herein can be used to create full-length antibody genes of any antibody isotype by inserting them into expression vectors already encoding heavy chain constant and light chain constant regions of the desired isotype such that the V_H segment is operatively linked to the C_H segment(s) within the vector and the V_L segment is operatively linked to the C_L segment within the vector.

In addition to the antibody chain genes, recombinant expression vectors may carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel (*Gene Expression Technology. Methods in Enzymology* 185, Academic Press, San Diego, CA (1990)). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences, may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc.* Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV), Simian Virus 40 (SV40), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP) and polyoma. Alternatively, nonviral regulatory sequences may be used, such as the ubiquitin promoter or β -globin promoter. Still further, regulatory elements composed of sequences from different sources, such as the SR α promoter system, which contains sequences from the SV40 early promoter and the long terminal repeat of human T cell leukemia virus type 1 (Takebe et al., *MCB* 1988;8:466-72).

In addition to the antibody chain genes and regulatory sequences, recombinant expression vectors may carry additional sequences, such as sequences that regulate replication of the vector

in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, *e.g.*, U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies described herein in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. Prokaryotic expression of antibody genes has been reported to be ineffective for production of high yields of active antibody (Boss et al., *Immunology Today* 1985;6:12-13).

Preferred mammalian host cells for expressing the recombinant antibodies described herein include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, *PNAS* 1980;77:4216-220, used with a DHFR selectable marker, *e.g.*, as described in Kaufman et al., *Mol Biol* 1982;159:601-21, NSO myeloma cells, COS cells, and SP2 cells. In particular, for use with NSO myeloma cells, another preferred expression system is the GS gene expression system disclosed in WO 87/04462, WO 89/01036 and EP 338,841. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

The chimeric anti-GITR antibodies described herein can be prepared based on the sequence of a human monoclonal antibody prepared as described above. For instance, in one embodiment, DNA encoding the human heavy and light variable regions of the 6G10 antibody can be operatively linked to a non-human constant region (e.g., a murine constant region) using routine recombinant technology.

Methods of detection of human GITR on cells

Provided herein are methods for detecting GITR protein expression in a biological sample, e.g., a human tumor tissue sample, by contacting the sample with an anti-GITR antibody, e.g., described herein, such as an anti-GITR antibody comprising the heavy and light chain variable region CDR sequences set forth in SEQ ID NOs: 5-7 and 8-10, respectively, heavy and light chain variable region sequences set forth in SEQ ID NOs: 11 and 12, respectively, or full length heavy and light chain sequences of antibody 6G10 (a fully human anti-GITR antibody) set forth in SEQ ID NOs: 13 and 14, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR, and detecting the binding of the antibody to GITR in the sample, e.g., by immunohistochemistry. In certain embodiments, the heavy chain lacks the C-terminal lysine. In some embodiments, the anti-GITR antibody is a chimeric antibody comprising the variable regions of antibody 6G10, but non-human constant regions (e.g., mouse constant regions), e.g., a chimeric 6G10 antibody comprising the heavy and light chain sequences set forth in SEQ ID NOs: 15 and 16, respectively. In certain embodiments, the heavy chain of the chimeric antibody lacks the C-terminal lysine.

In certain embodiments, exemplary biological samples include blood samples, serum samples, cells, surgically-resected tissue, and biopsied tissue (e.g. cancer tissue) obtained from the cancer patient. Biological samples for use in the methods described herein can be fresh, frozen, or fixed. Biological samples, e.g., tumor samples, can be obtained from the patient using routine methods, such as, but not limited to, biopsy, surgical resection, or aspiration.

The methods used to detect GITR expression in human tissue, e.g., tumor tissue, can be qualitative, semi-quantitative, or quantitative. Art-recognized antibody-based methods for detecting GITR protein levels in biological samples include, but are not limited to, enzyme-linked immunosorbent assay (ELISA), radioimmunoassays, electrochemiluminescence (ECL) assays, surface plasmon resonance, western blot, immunoprecipitation, fluorescence-activated

cell sorting (FACS), immunofluorescence, immunohistochemistry, and the like. Methods for detecting GITR expression in a biological sample may include control samples (negative and positive controls). For example, a negative control sample may be a sample containing no GITR protein, and a positive controls sample is a sample containing GITR protein. Comparing the results with the negative and positive controls can confirm the presence or absence of GITR in the biological sample.

In one embodiment, GITR expression in a tumor tissue sample is assessed by immunohistochemistry. This technique allows detection of GITR on specific cell types, e.g., Treg cells, e.g., Treg cells within a tumor, and tumor cells.

General immunohistochemical methods are well-known in the art. Specific conditions, considerations, and guidance for performing immunohistochemistry are described in, e.g., Immunohistochemical Staining Methods 6th Edition 2013, Taylor CR, Rudbeck L, eds., Dako North America, available as an Education Guide at www.dako.com; "Immunohistochemical Staining Method guide"). Immunohistochemical staining may be conducted on fixed tissue sections or on frozen tissue sections. When using fixed tissue sections, e.g., formalin fixed paraffin embedded (FFPE) tissue sections, the procedure generally involves the following steps: obtaining a tumor tissue sample (e.g., by biopsy), fixation of the tumor sample; embedding (e.g., in paraffin); sectioning and mounting; antigen retrieval; incubation with a primary antibody (e.g., chimeric 6G10 antibody described herein), detection (e.g., after amplification of the antigen/antibody complex signal), and interpretation by a pathologist (e.g., using an art-recognized scoring system).

Suitable, non-limiting fixatives include, for example, paraformaldehyde, glutaraldehyde, formaldehyde, acetic acid, acetone, osmium tetroxide, chromic acid, mercuric chloride, picric acid, alcohols (e.g., methanol, ethanol), Gendre's fluid, Rossman's fluid, B5 fixative, Bouin's fluid, Carnoy's fixative, and methacarn. In a preferred embodiment, the tumor sample is fixed in formaldehyde (e.g., 10% buffered formalin, which corresponds to 4% formaldehyde in a buffered solution). Once fixed, the tumor sample is serially dehydrated using alcohol or xylene, embedded in paraffin, and cut with a microtome to generate tumor tissue sections (e.g., with a thickness of about 4-5 μm), which can then be mounted onto microscope slides (e.g., adhesive-coated glass slides).

Exemplary embedding materials include paraffin, celloidin, OCT™ compound, agar, plastics, or acrylics. In a preferred embodiment, the embedding agent is paraffin.

Antigen retrieval can be performed using any method known in the art. Antigen retrieval treatment can involve heat (e.g., heat-induced epitope retrieval (HIER), as described in Example 3), or enzymatic degradation (proteolytic-induced epitope retrieval (PIER)). Exemplary antigen retrieval methods include microwave heating, proteolytic treatment (e.g., with proteinase K), pressure cooker heating, and water bath heating. Detailed general methods for antigen retrieval are described in Chapter 3 of the Immunohistochemical Staining Method guide. In certain embodiments, the antigen retrieval is performed by HIER in combination with commercially available antigen retrieval buffers (as described in Example 3).

The methods described herein employ anti-GITR antibodies to detect the expression levels of human GITR protein. In certain embodiments, the diagnostic anti-GITR antibody comprises the heavy and light chain variable region CDR sequences set forth in SEQ ID NOs: 5-7 and 8-10, respectively, heavy and light chain variable region sequences set forth in SEQ ID NOs: 11 and 12, respectively, or full length heavy and light chain sequences of antibody 6G10 (a fully human anti-GITR antibody) set forth in SEQ ID NOs: 13 and 14, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR, as described *supra*. In certain embodiments, the heavy chain lacks the C-terminal lysine. In some embodiments, the anti-GITR antibody is a chimeric antibody comprising the variable regions of antibody 6G10, but non-human constant regions (e.g., mouse constant regions), e.g., a chimeric 6G10 antibody comprising the heavy and light chain sequences set forth in SEQ ID NOs: 15 and 16, respectively. In certain embodiments, the heavy chain of the chimeric antibody lacks the C-terminal lysine. Such antibodies are particularly useful for detecting GITR expression in tumor tissue sections (see, e.g., Example 3).

GITR protein expression in tissue, e.g., tumor, samples can be detected using direct or indirect methods. For example, the primary antibody may comprise a detectable moiety, such as the enzyme horseradish peroxidase (HRP) or a fluorescent label (e.g., FITC, TRITC), as described herein. In certain embodiments, the primary antibody does not itself comprise a detectable moiety, but is detected by binding of a secondary antibody to it, for indirect immunohistochemistry. Accordingly, in certain embodiments, the secondary antibody comprises a detectable label, e.g., an enzymatic, chromogenic, or fluorescent label. In certain embodiments,

the primary antibody is a chimeric antibody comprising human variable region sequences and a non-human Fc region. A secondary antibody can be used to recognize the non-human Fc region of the primary antibody in order to reduce background staining.

In some embodiments, GTR protein expression can be detected using the avidin-biotin complex method (ABC method). In these embodiments, the secondary antibody is biotinylated and can serve as a bridge between the primary antibody and biotin-avidin-peroxidase complex. Other suitable non-limiting methods for immunohistochemistry include those described in Chapter 6 of the Immunohistochemical Staining Method guide, e.g., methods described in Chilosi et al., *Biotech Histochem* 1994;69:235; Sabattini et al., *J Clin Pathol* 1998;51:506-11; and Gross et al., *JBC* 1959;234:1622.

General methods for preparing and staining frozen tissue sections are well known in the art, and are described in, e.g., the Immunohistochemical Staining Method guide, Chapter 3 of *Immunohistochemistry and Methods* (Buchwalow and Bocker, Springer-Verlag Berlin Heidelberg 2010), and Chapter 21 of *Theory and Practice of Histological Techniques* (Bancroft and Gamble, 6th Edition, Elsevier Ltd., 2008). Frozen tissue section staining can be performed as described in Example 3.

GTR protein can be detected as described in the Examples.

In some embodiments, slides stained with the anti-GTR antibodies described herein can be further counterstained with, e.g., hematoxylin and/or eosin, using methods well known in the art.

In the interpretation step, GTR protein expression detected by immunohistochemistry can be scored using art-recognized scoring methods. Non-limiting examples of scoring methods are described below.

For example, in certain embodiments, GTR expression is evaluated using the H-score (histochemical score) system, which is widely used in the art and is useful given its dynamic range and use of weighted percentiles. The H-score is a semiquantitative scoring system based on the formula: 3 x percentage of strongly staining cells (3+ staining) + 2 x percentage of moderately staining (2+ staining) cells + 1 x percentage of weakly staining (1+ staining) cells + 0 x percentage of non-stained (0 staining) cells, giving a score range of 0 to 300. See, e.g., McCarty et al., *Cancer Res* 1986;46:4244-8; Bosman et al., *J Clin Pathol* 1992;45:120-4; Dieset et al., *Analyt Quant Cytol Histol* 1996;18:351-4. Control tissue can include, e.g., matching non-

tumor tissue from the same subject. Accordingly, in certain embodiments, the number of cells staining positive for GITR using the anti-GITR antibodies herein, and the intensity of staining, can be used to determine an H-score.

In other embodiments, GITR expression is evaluated using the Allred scoring system (Allred et al., *Mod Pathol* 1998;11:155-68; Harvey et al., *J Clin Oncol* 1999;17:1474-91). This scoring system involves adding proportion and intensity scores to obtain a total score. Accordingly, in some embodiments, the proportion score is obtained based on the estimated proportion of tumor cells that are positive for GITR (0: none, 1: <1/100, 2: 1/100 to 1/10, 3: 1/10 to 1/3; 4: 1/3 to 2/3; and 5: >2/3), and the intensity score is obtained based on the average intensity of GITR expression in positive tumor cells (0: none, 1: weak, 2: intermediate, 3: strong). Thus, Allred scores range from 0 to 8, with a score ranging from 3 to 8 considered positive (i.e., positive detection). Accordingly, in certain embodiments, tumors with an Allred score of 3 to 8, for example, an Allred score of 3, 4, 5, 6, 7, or 8, for GITR staining are considered GITR-positive tumors.

In other embodiments, the scoring system is automated, e.g., computerized, and quantified by image analysis. Automated methods are well known in the art. For example, an average threshold measure (ATM) score, which obtains an average of 255 staining intensity levels, can be calculated as described in, e.g., Choudhury et al., *J Histochem Cytochem* 2010;58:95-107, Rizzardi et al., *Diagnostic Pathology* 2012; 7:42-52. Another automated scoring system is AQUA[®] (automated quantitative analysis), which is performed using, e.g., tissue microarrays (TMAs), on a continuous scale. AQUA[®] is a hybrid of standard immunohistochemistry and flow cytometry that provides an objective numeric score ranging from 1-255, and involves antigen retrieval, use of primary and secondary antibodies, and multiplexed fluorescent detection. Optimal cutoff points can be determined as described in Camp et al. *Clin Cancer Res* 2004;10:7252-9. The AQUA[®] scoring system is described in detail in Camp et al., *Nat Med* 2002;8:1323-7; Camp et al., *Cancer Res* 2003;63:1445-8; Ghosh et al., *Hum Pathol* 2008;39:1835-43; Bose et al., *BMC Cancer* 2012;12:332; Mascaux et al., *Clin Cancer Res* 2011;17:7796-807. Other suitable automated immunohistochemistry platforms include commercially available platforms, such as the Leica BOND RX staining platform. The platform detects GITR expression in tumor tissue on the basis of staining intensity on the following scale: minimal, <1 cells per 20x objective field; mild, 1~<10 cells per 20x objective

field; moderate, 10~<50 cells per 20x objective field, marked, 50~<200 cells per 20x objective; and intense, >200 cells per 20x objective field.

Accordingly, provided herein are methods for determining whether a tumor of a cancer patient is GITR-positive and/or comprises GITR positive cells, comprising contacting a tumor sample from the patient with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR.

Also provided herein are methods for determining whether a tumor of a cancer patient is GITR-positive and/or comprises GITR positive cells, comprising:

(a) contacting a tumor sample from the patient with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR, and

(b) detecting the binding of the antibody to GITR in the sample.

Also provided herein are methods for determining whether a tumor of a cancer patient is GITR-positive and/or comprises GITR positive cells comprising:

(a) contacting a tumor sample from the patient with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR,

(b) detecting the binding of the antibody to GITR in the sample, and

(c) determining the level of GITR protein expression in the sample, wherein a level of GITR protein above a threshold level, e.g., level of detection using a given detection method, indicates that the tumor is GITR-positive tumor.

Also provided herein are methods for identifying GITR positive cells in a tissue (e.g., tumor) sample comprising:

(a) contacting a tissue (e.g., tumor) sample from the patient with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR; and

(b) detecting the binding of the antibody to GITR on specific or individual cells of the tissue (e.g., tumor) sample; and optionally

(c) staining the tissue (e.g., tumor) sample, e.g., with markers of given cell types (e.g., CD3, CD4, CD8, CD25, or FoxP3 markers) and/or with hematoxylin and eosin, to identify the cells that were identified as GITR positive in step (b).

Step (c) allows identification of GITR positive cells as being, e.g., T cells, such as Treg cells, or tumor cells. Thus, provided herein are the following methods:

A method for detecting the presence of, and/or identifying the number of, T cells expressing GITR in a tissue (e.g., tumor) sample, and/or level of GITR on T cells of a tissue (e.g., tumor) sample comprising:

(a) contacting a tissue (e.g., tumor) sample, e.g., from a subject, with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR; and

(b) detecting the binding of the antibody to GITR on T cells of the tissue (e.g., tumor) sample; and optionally

(c) staining the tissue (e.g., tumor) sample, e.g., with markers of T cells (e.g., CD3, CD4, CD8, CD25, or FoxP3 markers) and/or with hematoxylin and eosin, to identify the T cells that were identified as GITR positive in step (b).

A method for detecting the presence of, and/or identifying the number of, Treg cells expressing GITR in a tissue (e.g., tumor) sample, and/or the level of GITR on Treg cells of a tissue (e.g., tumor) sample comprising:

(a) contacting a tissue (e.g., tumor) sample, e.g., from a subject, with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR; and

(b) detecting the binding of the antibody to GITR on Treg cells of the tissue (e.g., tumor) sample; and optionally

(c) staining the tissue (e.g., tumor) sample, e.g., with markers of Treg cells (e.g., a FoxP3 marker) and/or with hematoxylin and eosin, to identify the Treg cells that were identified as GITR positive in step (b).

A method for detecting the presence of, and/or identifying the number of, Teff cells expressing GITR in a tissue (e.g., tumor) sample, and/or the level of GITR on Teff cells of a tissue (e.g., tumor) sample comprising:

(a) contacting a tissue (e.g., tumor) sample, e.g., from a subject, with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR; and

(b) detecting the binding of the antibody to GITR on Teff cells of the tissue (e.g., tumor) sample; and optionally

(c) staining the tissue (e.g., tumor) sample, e.g., with markers of Teff cells (e.g., a FoxP3 marker) and/or with hematoxylin and eosin, to identify the Teff cells that were identified as GITR positive in step (b).

A method for detecting the presence of, and/or identifying the number of Treg and Teff cells expressing GITR in a tissue (e.g., tumor) sample, and/or the level of GITR on Treg and Teff cells of a tissue (e.g., tumor) sample comprising:

(a) contacting a tissue (e.g., tumor) sample, e.g., from a subject, with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR; and

(b) detecting the binding of the antibody to GITR on Treg and Teff cells of the tissue (e.g., tumor) sample; and optionally

(c) staining the tissue (e.g., tumor) sample, e.g., with markers of Treg and Teff cells (e.g., CD3, CD4, CD8, CD25, and/or FoxP3 markers) and/or with hematoxylin and eosin, to identify the Treg and Teff cells that were identified as GITR positive in step (b).

A method for detecting the presence of, or identifying the number of, tumor cells expressing GITR in a tumor sample, and/or level of GITR on tumor cells of a tumor sample comprising:

(a) contacting a tumor sample, e.g., from a subject, with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR; and

(b) detecting the binding of the antibody to GITR on tumor cells of the tumor sample; and optionally

(c) staining the tumor sample, e.g., with markers of tumor cells or other cell type and/or with hematoxylin and eosin, to identify the tumor cells that were identified as GITR positive in step (b).

A method for detecting the presence of, and/or identifying the number of, tumor cells and Treg cells expressing GITR in a tissue (e.g., tumor) sample, and/or the level of GITR on tumor cells and Treg cells of a tissue (e.g., tumor) sample comprising:

(a) contacting a tissue (e.g., tumor) sample, e.g., from a subject, with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR; and

(b) detecting the binding of the antibody to GITR on tumor cells and Treg cells of the tissue (e.g., tumor) sample; and optionally

(c) staining the tissue (e.g., tumor) sample, e.g., with markers of tumor and/or Treg cells (e.g., a FoxP3 marker) and/or with hematoxylin and eosin, to identify the tumor and Treg cells that were identified as GITR positive in step (b).

A method for detecting the presence of, and/or identifying the number of, tumor cells, Treg cells and Teff cells expressing GITR in a tissue (e.g., tumor) sample, and/or the level of GITR on tumor cells, Treg cells and Teff cells of a tissue (e.g., tumor) sample comprising:

(a) contacting a tissue (e.g., tumor) sample, e.g., from a subject, with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR; and

(b) detecting the binding of the antibody to GITR on tumor cells, Treg cells and Teff cells of the tissue (e.g., tumor) sample; and optionally

(c) staining the tissue (e.g., tumor) sample, e.g., with markers of tumor cells, Treg cells and/or Teff cells (e.g., CD3, CD4, CD8, CD25, and/or FoxP3 markers) and/or with hematoxylin and eosin, to identify the tumor cells, Treg cells and Teff cells that were identified as GITR positive in step (b).

Exemplary tumor markers include, for example, ErbB receptors, Melan A [MART1], gp100, tyrosinase, TRP-1/gp 75, TRP-2, MAGE-1, MAGE-3, HPV E6 and E7 proteins, Mucin [MUC-1], prostate-specific antigen [PSA], carcinoembryonic antigen [CEA] PIA tumor antigen, MAGE-2, MAGE-4, MAGE-6, MAGE-10, MAGE-12, BAGE-1, CAGE-1,2,8, CAGE-3 to 7, LAGE-1, NY-ESO-1/LAGE-2, NA-88, GnTV, and TRP2-INT2.

T reg cell surface markers include CD4, CD25 and FOXP3.

In certain embodiments, a combination of detection methods is used to increase the accuracy of designating a tumor as being GITR-positive or GITR-negative. For example, in one embodiment, a combination of immunohistochemistry and fluorescence *in situ* hybridization (FISH) is used to type a tumor as being GITR positive or negative. Such approaches have been used to, e.g., optimize Her-2/neu testing. See, e.g., Ridolfi et al., *Mod Pathol* 2000;13:866-73. Any of the antibody-based methods of detection described herein can be used in the combination, or in combination with RNA-based detection methods, such as RNAscope and quantitative PCR.

The methods of detection described above are typically performed in conjunction with a reference or control sample. In some embodiments, the control sample is a section of matched normal tissue. In some embodiments, the control or reference sample is derived from a subject that is not afflicted with a GITR-expressing tumor or cancer (e.g., a healthy subject). Such samples may provide a standardized reference for the amount of GITR-expressing cells in a biological sample.

Exemplary Prognostic Methods

Provided herein are methods for identifying a cancer patient that is likely to respond to an anti-GITR immunotherapy or predicting whether a cancer patient will respond to anti-GITR immunotherapy comprising:

(a) contacting a tumor sample from the patient with an anti-GITR antibody, or antigen-binding portion thereof,

(b) detecting the binding of the antibody to GITR in the tumor sample,

(c) determining the level of GITR protein expression in the sample, wherein a level of GITR protein above a threshold level indicates that the tumor is a GITR-positive tumor and that the patient is likely to or predicted to respond to anti-GITR immunotherapy. In certain

embodiments, the level of expression of GITR on specific immune cells, e.g., tumor infiltration lymphocytes (TILs), such as T cells, e.g., Treg cells, e.g., in a tumor, is detected or monitored, and, e.g., determines likelihood of response of a subject to an anti-GITR therapy. In certain embodiments, the levels of expression of GITR on tumor cells is is detected or monitored, and, e.g., determines likelihood of response of a subject to an anti-GITR therapy.

In certain embodiments, the anti-GITR antibody is an antibody comprising the heavy and light chain variable region CDR sequences set forth in SEQ ID NOs: 5-7 and 8-10, respectively, heavy and light chain variable region sequences set forth in SEQ ID NOs: 11 and 12, respectively, or full length heavy and light chain sequences of antibody 6G10 (a fully human anti-GITR antibody) set forth in SEQ ID NOs: 13 and 14, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR. In certain embodiments, the heavy chain lacks the C-terminal lysine. In some embodiments, the anti-GITR antibody is a chimeric antibody comprising the variable regions of antibody 6G10, but non-human constant regions (e.g., mouse constant regions), e.g., a chimeric 6G10 antibody comprising the heavy and light chain sequences set forth in SEQ ID NOs: 15 and 16, respectively. In certain embodiments, the heavy chain of the chimeric antibody lacks the C-terminal lysine. In certain embodiments, the detection of GITR expression is performed by immunohistochemistry, as described *supra*. In one embodiment, immunohistochemistry is combined with another detection method, e.g., FISH.

In certain embodiments, the tumor sample is prepared as FFPE sections. In other embodiments, the tumor sample is prepared as frozen tissue sections.

In some embodiments, a tumor is considered GITR-positive and likely to benefit from anti-GITR immunotherapy if a sample of the tumor or immune cells therein, e.g., tumor infiltration lymphocytes (TILs), e.g., Treg cells, has more GITR-positive cells than a matching normal tissue, or immune cell, e.g., T reg cell, from the same cancer patient.

In certain embodiments, the cancer patient is likely to or predicted to benefit from anti-GITR immunotherapy if the number of GITR-positive cells, e.g., immune cells, e.g., tumor infiltration lymphocytes (TILs), such as T reg cells, and/or tumor cells, in the tumor sample exceeds a threshold level. A threshold level may be the lowest level of GITR as determined with a given detection system, e.g., immunohistochemistry, as further described herein, such as in the Examples. In certain embodiments, the cancer patient is likely to or predicted to benefit from anti-GITR immunotherapy if the number of GITR-positive cells, e.g., immune cells, e.g., tumor

infiltration lymphocytes (TILs), such as T reg cells, and/or tumor cells, in the tumor sample exceeds a threshold number or proportion using an art-recognized scoring system described *supra*. Thus, in some embodiments, the number or proportion of GITR-positive cells exceeds a certain threshold number or proportion using a scoring system described herein and is defined as being “GITR-positive.”

In certain embodiments, a tumor sample having at least 1%, for example, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 90%, or at least 95% of cells in the tumor, e.g., tumor cells and/or immune cells, e.g., tumor infiltration lymphocytes (TILs), such as T reg cells, expressing GITR protein is designated as being “GITR-positive” and indicates that the cancer patient is likely to or predicted to respond to anti-GITR immunotherapy.

In some embodiments, an H score is calculated for the tumor sample. Accordingly, in certain embodiments, a tumor sample with an H score of at least 5, such as at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, at least 250, at least 275, or at least 290 is designated as being “GITR-positive” and indicates that the cancer patient is likely to or predicted to respond to anti-GITR immunotherapy. The score may be calculated based on all cells in a tumor, or a specific type of cells, e.g., immune cells, e.g., tumor infiltration lymphocytes (TILs), such as Treg cells, and/or tumor cells.

In some embodiments, an Allred score is calculated for the tumor sample. Accordingly, in certain embodiments, a tumor sample with an Allred score of at least 3, such as at least 4, at least 5, at least 6, at least 7, or 8 is designated as being “GITR-positive” and indicates that the cancer patient is likely to or predicted to respond to anti-GITR immunotherapy. The score may be calculated based on all cells in a tumor, or a specific type of cells, e.g., immune cells, e.g., tumor infiltration lymphocytes (TILs), such as Treg cells, and/or tumor cells.

In some embodiments, an AQUA score is calculated. Accordingly, in certain embodiments, a tumor sample with an AQUA score of at least 5, such as at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, or at least 250 is designated as being “GITR-positive” and indicates that the cancer patient is likely to or

predicted to respond to anti-GITR immunotherapy. The score may be calculated based on all cells in a tumor, or a specific type of cells, e.g., immune cells, e.g., tumor infiltration lymphocytes (TILs), such as Treg cells, and/or tumor cells.

In certain embodiments, a combination of one of the scoring systems above with another detection modality, such as FISH, is used to increase the accuracy of determining whether a tumor is GITR-positive or GITR-negative.

In certain embodiments, the patient has a cancer selected from the group consisting of squamous cell carcinoma, small-cell lung cancer, non-small cell lung cancer, squamous non-small cell lung cancer (NSCLC), non squamous NSCLC, glioma, gastrointestinal cancer, renal cancer (e.g. clear cell carcinoma), ovarian cancer, liver cancer, colorectal cancer, endometrial cancer, kidney cancer (e.g., renal cell carcinoma (RCC)), prostate cancer (e.g. hormone refractory prostate adenocarcinoma), thyroid cancer, neuroblastoma, pancreatic cancer, glioblastoma (glioblastoma multiforme), cervical cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, and head and neck cancer (or carcinoma), gastric cancer, germ cell tumor, pediatric sarcoma, sinonasal natural killer, melanoma (e.g., metastatic malignant melanoma, such as cutaneous or intraocular malignant melanoma), bone cancer, skin cancer, uterine cancer, cancer of the anal region, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, cancer of the ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain cancer, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally-induced cancers including those induced by asbestos, virus-related cancers or cancers of viral origin (e.g., human papilloma virus (HPV-related or -originating tumors)), and hematologic malignancies derived from either of the two major blood cell lineages, i.e., the myeloid cell line (which produces granulocytes, erythrocytes, thrombocytes, macrophages and mast cells) or lymphoid cell line (which produces B, T, NK and plasma cells), such as all types of leukemias, lymphomas, and myelomas, e.g., acute, chronic, lymphocytic and/or myelogenous leukemias, such as acute leukemia (ALL), acute myelogenous leukemia (AML), chronic

lymphocytic leukemia (CLL), and chronic myelogenous leukemia (CML), undifferentiated AML (M0), myeloblastic leukemia (M1), myeloblastic leukemia (M2; with cell maturation), promyelocytic leukemia (M3 or M3 variant [M3V]), myelomonocytic leukemia (M4 or M4 variant with eosinophilia [M4E]), monocytic leukemia (M5), erythroleukemia (M6), megakaryoblastic leukemia (M7), isolated granulocytic sarcoma, and chloroma; lymphomas, such as Hodgkin's lymphoma (HL), non-Hodgkin's lymphoma (NHL), B cell hematologic malignancy, e.g., B-cell lymphomas, T-cell lymphomas, lymphoplasmacytoid lymphoma, monocytoid B-cell lymphoma, mucosa-associated lymphoid tissue (MALT) lymphoma, anaplastic (e.g., Ki 1+) large-cell lymphoma, adult T-cell lymphoma/leukemia, mantle cell lymphoma, angio immunoblastic T-cell lymphoma, angiocentric lymphoma, intestinal T-cell lymphoma, primary mediastinal B-cell lymphoma, precursor T-lymphoblastic lymphoma, T-lymphoblastic; and lymphoma/leukaemia (T-Lbly/T-ALL), peripheral T- cell lymphoma, lymphoblastic lymphoma, post-transplantation lymphoproliferative disorder, true histiocytic lymphoma, primary central nervous system lymphoma, primary effusion lymphoma, B cell lymphoma, lymphoblastic lymphoma (LBL), hematopoietic tumors of lymphoid lineage, acute lymphoblastic leukemia, diffuse large B-cell lymphoma, Burkitt's lymphoma, follicular lymphoma, diffuse histiocytic lymphoma (DHL), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, cutaneous T-cell lymphoma (CTLC) (also called mycosis fungoides or Sezary syndrome), and lymphoplasmacytoid lymphoma (LPL) with Waldenstrom's macroglobulinemia; myelomas, such as IgG myeloma, light chain myeloma, nonsecretory myeloma, smoldering myeloma (also called indolent myeloma), solitary plasmacytoma, and multiple myelomas, chronic lymphocytic leukemia (CLL), hairy cell lymphoma; hematopoietic tumors of myeloid lineage, tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma; seminoma, teratocarcinoma, tumors of the central and peripheral nervous, including astrocytoma, schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer and teratocarcinoma, hematopoietic tumors of lymphoid lineage, for example T-cell and B-cell tumors, including but not limited to T-cell disorders such as T-prolymphocytic leukemia (T-PLL), including of the small cell and cerebriform cell type; large granular lymphocyte leukemia (LGL) preferably of the T-cell type; a/d T-NHL hepatosplenic lymphoma; peripheral/post-thymic T cell lymphoma

(pleomorphic and immunoblastic subtypes); angiocentric (nasal) T-cell lymphoma; cancer of the head or neck, renal cancer, rectal cancer, cancer of the thyroid gland; acute myeloid lymphoma, as well as any combinations of said cancers. In certain embodiments, the cancer is a metastatic cancer, refractory cancer, or recurrent cancer.

Once a cancer patient has been identified as being likely to benefit from anti-GITR immunotherapy (i.e., has a GITR-positive tumor), the patient can be treated with a therapeutic anti-GITR antibody (i.e., anti-GITR immunotherapy).

Accordingly, provided herein are method of treating a cancer patient with a GITR-positive tumor, as determined by the level of GITR protein expression using the anti-GITR antibodies and methods provided herein, comprising administering to the patient a therapeutically-effective amount of an anti-GITR antibody, or antigen-binding portion thereof.

In certain embodiments, the anti-GITR antibody used to determine the level of GITR protein expression is an antibody comprising the heavy and light chain variable region CDR sequences set forth in SEQ ID NOs: 5-7 and 8-10, respectively, heavy and light chain variable region sequences set forth in SEQ ID NOs: 11 and 12, respectively, or full length heavy and light chain sequences of antibody 6G10 (a fully human anti-GITR antibody) set forth in SEQ ID NOs: 13 and 14, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR. In certain embodiments, the heavy chain lacks the C-terminal lysine. In some embodiments, the antibody is a chimeric antibody comprising the variable regions of antibody 6G10, but non-human constant regions (e.g., mouse constant regions), e.g., a chimeric 6G10 antibody comprising the heavy and light chain sequences set forth in SEQ ID NOs: 15 and 16, respectively. In certain embodiments, the heavy chain of the chimeric antibody lacks the C-terminal lysine. In certain embodiments, the detection of GITR expression is performed by immunohistochemistry, as described *supra*.

In certain embodiments, a cancer patient determined as having a GITR-positive tumor (ie., GITR-positive tumor cells and/or immune cells, e.g., tumor infiltration lymphocytes (TILs), such as T reg cells) using the methods described herein is treated with any agent that modulates GITR signaling (e.g., an agonist anti-GITR antibody) and results in, e.g., the induction and/or enhancement of T cell activation (e.g., increase in IL-2 and/or IFN- γ production by T cells and/or increase in proliferation of T cells), depletion of regulatory T cells, etc. Accordingly, provided herein are methods of treating a cancer patient comprising:

- (a) determining whether a tumor (tumor cells and/or immune cells, e.g., tumor infiltration lymphocytes (TILs), such as Treg cells) of a cancer patient is GITR-positive using the methods described herein, e.g., contacting a tumor sample from the patient with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR, detecting the binding of the antibody to GITR in the sample, and determining the level of GITR protein expression in the sample, wherein a level of GITR protein above a threshold level in tumor cells and/or immune cells therein, e.g., tumor infiltration lymphocytes (TILs), such as Treg cells, indicates that the tumor is GITR-positive, and
- (b) if the tumor is determined to be GITR-positive, then administering a therapeutically effective amount of an agent that modulates GITR signaling, e.g., an agent that increases IL-2, increases IFN- γ production by T cells, and/or increases the proliferation of T cells and/or depletion of regulatory T cells.

For example, in one embodiment, the patient is treated with a therapeutically-effective amount of an agonist anti-GITR antibody disclosed in WO15187835, the contents of which are herein incorporated by reference, e.g., antibodies having the heavy and light chain variable region CDRs, heavy and light chain variable regions, or heavy and light chains of antibodies 28F3, 19D3, 18E10, 3C3-1, 3C3-2, 2G6, 9G7-1, 9G7-2, 14E3, 19H8-1, 19H8-2, and/or 6G10, and variants thereof. The sequences of the antibodies disclosed in PCT Patent Application No. WO15187835 are provided in Table 2 (see SEQ ID NOs: 5-14 and 27-228). The patient may also be treated with any other anti-GITR antibodies, e.g., TRX518 (Leap Therapeutics), MK-4166 (Merck), LKZ-145 (Novartis), GWN-323 (Novartis Pharmaceuticals Corp.), Medi 1873 (MedImmune), INBRX-110 (Inhibrx), GITR-Fc protein (OncoMed) and antibodies described in WO2006105021, WO2009009116, WO2011028683, US2014/0072565, US20140072566, US20140065152, WO2015031667, WO15184099, WO2015184099, or WO2016054638.

Exemplary methods of monitoring GITR-positive tumors/cancer

Provided herein are methods for monitoring a GITR-expressing tumor(s) in a cancer patient. Methods for monitoring may be performed in a similar manner to methods for

determining whether a cancer patient is likely to respond to anti-GITR immunotherapy described above.

The presence of GITR may be monitored in whole tissues, e.g., tumors, or in specific cells thereof. For example, the presence of GITR may be monitored on tumor cells and/or tumor infiltrating lymphocytes (TILs), e.g., T reg cells and Teff cells.

Monitoring GITR presence (or expression) may comprise determining GITR presence at two or more time points. For example, a first and a second tissue sample may be obtained (and the presence and/or level of GITR determined) from a subject, wherein, e.g., the first and the second tissue sample are obtained within 3-7 days, 1 week to 3 weeks, or 1 month to 3 months from each other. A tissue sample may also be obtained for determining the presence and/or level of GITR every week or every month.

Accordingly, in certain embodiments, a method for monitoring a GITR-expressing tumor in a cancer patient comprises:

- (a) detecting GITR protein expression in a tissue (e.g., tumor) sample at a first time point by using an anti-GITR antibody, or antigen-binding portion thereof,
- (b) determining the level of GITR protein expression at the first time point,
- (c) detecting GITR protein expression in a tissue (e.g., tumor, which may be the same tumor as the one that was biopsied at the first time point) sample at a second time point using the same antibody used in step (a), and
- (d) determining the level of GITR protein expression in the tumor from the second time point.

Also provided is a method for monitoring a GITR-expressing tumor in a cancer patient comprising:

- (a) detecting GITR protein expression in Treg cells in a tissue (e.g., tumor) sample at a first time point by using an anti-GITR antibody, or antigen-binding portion thereof,
- (b) determining the level of GITR protein expression at the first time point,
- (c) detecting GITR protein expression in Treg cells in a tissue (e.g., tumor, which may be the same tumor as the one that was biopsied at the first time point) sample at a second time point using the same antibody used in step (a),
- (d) determining the level of GITR protein expression in the Treg cells from the second time.

Also provided is a method for monitoring a GITR-expressing tumor in a cancer patient comprising:

- (a) detecting GITR protein expression in Teff cells in a tissue (e.g., tumor) sample at a first time point by using an anti-GITR antibody, or antigen-binding portion thereof,
- (b) determining the level of GITR protein expression at the first time point,
- (c) detecting GITR protein expression in Teff cells in a tissue (e.g., tumor, which may be the same tumor as the one that was biopsied at the first time point) sample at a second time point using the same antibody used in step (a),
- (d) determining the level of GITR protein expression in the Teff cells from the second time.

Also provided is a method for monitoring a GITR-expressing tumor in a cancer patient comprising:

- (a) detecting GITR protein expression in tumor cells in a tumor sample at a first time point by using an anti-GITR antibody, or antigen-binding portion thereof,
- (b) determining the level of GITR protein expression at the first time point,
- (c) detecting GITR protein expression in tumor cells in a tumor sample (which may be from the same tumor as the one that was biopsied at the first time point) at a second time point using the same antibody used in step (a),
- (d) determining the level of GITR protein expression in the tumor cells from the second time.

In certain methods, the presence and/or level of GITR are determined in Treg cells and/or Teff cells and/or tumor cells.

Accordingly, in certain embodiments, a method for monitoring a GITR-expressing tumor in a cancer patient comprises:

- (a) detecting GITR protein expression in the GITR-positive tumor at a first time point by using an anti-GITR antibody, or antigen-binding portion thereof,
- (b) determining the level of GITR protein expression at the first time point,
- (c) detecting GITR protein expression in the tumor at a second time point using the same antibody used in step (a),
- (d) determining the level of GITR protein expression in the tumor from the second time point; and

(e) comparing the levels of GITR protein expression determined at the first and second time points.

GITR expression may be determined in the tumor cells and/or in immune cells, e.g., tumor infiltration lymphocytes (TILs), such as T reg cells and/or Teff cells within the tumor. A higher level at the first time point relative to the second time point may be indicative of tumor regression, a lower score at the first time point relative to the second time point may be indicative of tumor progression, and a relatively unchanged level at the first time point relative to the second time point may be indicative of a stable tumor.

In certain embodiments, the anti-GITR antibody used to determine the level of GITR protein expression is an antibody comprising the heavy and light chain variable region CDR sequences set forth in SEQ ID NOs: 5-7 and 8-10, respectively, heavy and light chain variable region sequences set forth in SEQ ID NOs: 11 and 12, respectively, or full length heavy and light chain sequences of antibody 6G10 (a fully human anti-GITR antibody) set forth in SEQ ID NOs: 13 and 14, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR. In certain embodiments, the heavy chain lacks the C-terminal lysine. In some embodiments, the antibody is a chimeric antibody comprising the variable regions of antibody 6G10, but non-human constant regions (e.g., mouse constant regions), e.g., a chimeric 6G10 antibody comprising the heavy and light chain sequences set forth in SEQ ID NOs: 15 and 16, respectively. In certain embodiments, the heavy chain of the chimeric antibody lacks the C-terminal lysine. In a preferred embodiment, the detection of GITR expression is performed by immunohistochemistry, as described herein. In some embodiments, the level of GITR protein expression is determined using a scoring system selected from the group consisting of: the H score system, the Allred score system, an AQUA[®] system, or an automated scoring system, as described herein.

Such methods of monitoring are also useful for determining whether or not anti-GITR immunotherapy may be effective.

Accordingly, provided herein are methods for monitoring the efficacy of anti-GITR immunotherapy in a patient having a GITR-positive tumor comprising:

(a) detecting GITR protein expression in the GITR-positive tumor at a first time point before or after initiating anti-GITR immunotherapy by using an anti-GITR antibody,

(b) determining the level of GITR protein expression in the tumor from the first time point,

(c) detecting GITR protein expression in the GITR-positive tumor at a second time point after initiating anti-GITR immunotherapy using the same antibody from step (a),

(d) determining the level of GITR protein expression in the tumor from the second time point;

(e) comparing the levels of GITR protein expression determined at the first and second time points, wherein a higher level at the first time point relative to the second time point may be indicative of effective anti-GITR immunotherapy, a lower score at the first time point relative to the second time point is indicative of ineffective anti-GITR immunotherapy, and an unchanged score at the first time point relative to the second time point may be indicative of anti-GITR immunotherapy being stabilizing. GITR expression may be determined in the tumor cells and/or in immune cells, e.g., tumor infiltration lymphocytes (TILs), such as T reg cells, within the tumor.

In certain embodiments, the anti-GITR antibody used to determine the level of GITR protein expression is an antibody comprising the heavy and light chain variable region CDR sequences set forth in SEQ ID NOs: 5-7 and 8-10, respectively, heavy and light chain variable region sequences set forth in SEQ ID NOs: 11 and 12, respectively, or full length heavy and light chain sequences of antibody 6G10 (a fully human anti-GITR antibody) set forth in SEQ ID NOs: 13 and 14, respectively, wherein the antibody or antigen-binding fragment thereof specifically binds to human GITR. In certain embodiments, the heavy chain lacks the C-terminal lysine. In some embodiments, the antibody is a chimeric antibody comprising the variable regions of antibody 6G10, but non-human constant regions (e.g., mouse constant regions), e.g., a chimeric 6G10 antibody comprising the heavy and light chain sequences set forth in SEQ ID NOs: 15 and 16, respectively. In certain embodiments, the heavy chain of the chimeric antibody lacks the C-terminal lysine. In a preferred embodiment, the detection of GITR expression is performed by immunohistochemistry, as described herein. In some embodiments, the level of GITR protein expression is determined using a scoring system selected from the group consisting of: the H score system, the Allred score system, an AQUA[®] system, or an automated scoring system, as described herein.

In certain embodiments, the anti-GITR immunotherapy comprises administering to the cancer patient a therapeutically-effective amount of an anti-GITR antibody disclosed in WO15187835, the contents of which are herein incorporated by reference, e.g., antibodies having the heavy and light chain variable region CDRs, heavy and light chain variable regions, or heavy and light chains of antibodies 28F3, 19D3, 18E10, 3C3-1, 3C3-2, 2G6, 9G7-1, 9G7-2, 14E3, 19H8-1, 19H8-2, and/or 6G10, and variants thereof. The sequences of the antibodies disclosed in PCT Patent Application No. WO15187835 are provided in Table 2 (see SEQ ID NOs: 5-14 and 27-228). The patient may also be treated with any other anti-GITR antibodies, e.g., TRX518 (Leap Therapeutics), MK-4166 (Merck), LKZ-145 (Novartis), GWN-323 (Novartis Pharmaceuticals Corp.), Medi 1873 (MedImmune), INBRX-110 (Inhibrx), GITR-Fc protein (OncoMed) and antibodies described in WO2006105021, WO2009009116, WO2011028683, US2014/0072565, US20140072566, US20140065152, WO2015031667, WO15184099, WO2015184099, or WO2016054638.

In certain embodiments, the status of a GITR-expressing tumor can be monitored repeatedly after initial diagnosis (i.e., after making a determination that the tumor is GITR-positive), such as one month after initial diagnosis, two months after initial diagnosis, three months after initial diagnosis, four months after initial diagnosis, five months after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc. In other embodiments, the efficacy of anti-GITR immunotherapy against a GITR-expressing tumor can be monitored repeatedly after initiating anti-GITR immunotherapy, such as one month after initiation of therapy, two months after initiation of therapy, three months after initiation of therapy, four months after initiation of therapy, five months after initiation of therapy, six months after initiation of therapy, one year after initiation of therapy, etc.

Diagnostic Kits

Provided herein are kits which include the diagnostic anti-GITR antibodies described herein and instructions for use. Accordingly, in some embodiments, the kit comprises an antibody comprising the heavy and light chain variable region CDR sequences set forth in SEQ ID NOs: 5-7 and 8-10, respectively, heavy and light chain variable region sequences set forth in SEQ ID NOs: 11 and 12, respectively, wherein the antibody or antigen-binding fragment thereof

specifically binds to human GITR, or full length heavy and light chain sequences of antibody 6G10 set forth in SEQ ID NOs: 13 and 14, respectively. In certain embodiments, the heavy chain lacks the C-terminal lysine. In some embodiments, the anti-GITR antibody is a chimeric antibody comprising the variable regions of antibody 6G10, but non-human constant regions (e.g., mouse constant regions), e.g., a chimeric antibody comprising the heavy and light chain sequences set forth in SEQ ID NOs: 15 and 16, respectively. In certain embodiments, the heavy chain of the chimeric antibody lacks the C-terminal lysine. In certain embodiments, the antibody comprises a detectable label, e.g., an antibody-label conjugate. Such kits may comprise at least one additional reagent. For example, in certain embodiments, the kits comprise buffers, stabilizers, substrates, immunodetection reagent (e.g., secondary antibodies for use in immunohistochemistry), and/or cofactors required for the assay. In certain embodiments, the antibody and, optionally the reagents, are suitably aliquoted. In certain embodiments, the kit comprises a means for obtaining the biological sample from a cancer patient. Such means can comprise, for example, reagents that can be used to obtain fluid or tissue sample from the cancer patient.

The present disclosure is further illustrated by the following examples, which should not be construed as further limiting. The contents of all figures and all references, Genbank sequences, patents and published patent applications cited throughout this application are expressly incorporated herein by reference. In particular, the disclosure of WO15187835 is expressly incorporated herein by reference.

EXAMPLES

Example 1: Generation of the 6G10 antibody

The human anti-GITR antibody 6G10 was generated during the course of immunizing HuMAb® transgenic mice (“HuMAb” is a Trade Mark of Medarex, Inc., Princeton, New Jersey) and KM mice (the KM Mouse® strain contains the SC20 transchromosome as described in PCT Publication WO 02/43478) and screening fusions for antibodies that bound to GITR (see concurrently filed PCT Application No. PCT/US15/33991). cDNA sequencing identified one

heavy and one light chain for the 6G10 antibody. The variable region amino acid sequences and the isotype of the 6G10 antibody is set forth in Figures 1-4 and Table 2.

Example 2: Binding properties of the 6G10 antibody

Binding of the 6G10 antibody to soluble GITR was determined by Biacore. The 6G10 antibody was captured on human kappa coated chips (~5KRUs; Southernbiotech), and recombinant human GITR (rHGITR/Fc; R&D systems) was flowed across the chip at concentrations of 500 nM, 250 nM, 125 nM, 62 nM, and 31 nM. The capture concentration of the antibody/volume was 2-40 µg/mL (5 µL at 10 µL/min). The antigen association time was 5 minutes at 15 µL/min, the antigen dissociation time was 6 minutes, and regeneration was performed with 50 mM HCl/50 mM NaOH (12 µL each at 100 µL/min). The 6G10 exhibited a K_D of 1.87×10^{-9} M for binding to soluble human GITR, with a k_a of $3.83 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ and a k_d of $7.15 \times 10^{-4} \text{ s}^{-1}$.

Example 3: Immunohistochemistry of cancer tissue with a chimeric 6G10 antibody

Two immunohistochemistry (IHC) techniques were used to detect GITR in tissue sections: one IHC method was conducted on formalin fixed paraffin embedded (FFPE) tissue sections, and the other one was conducted on frozen tissue sections.

Several anti-GITR antibodies have been tested in these IHC methods and 6G10 was consistently better.

To detect GITR expression in human tissues, a fluoresceinated form of the 6G10 antibody was prepared and designated 6G10-FITC. To further develop a user and clinical friendly method, the 6G10 antibody was engineered to a mouse chimeric IgG2a format and optimized for automated immunohistochemistry using the Leica BondRX platform. The heavy and light chain sequences of the chimeric 6G10 antibody is set forth in Figures 5 and 6, respectively, and Table 2. The heavy chain may or may not contain a C-terminal lysine.

Briefly, slides were deparaffinized and rehydrated following routine histology procedures, and subjected to HIER (heat-induced antigen retrieval) manually with pH6 target retrieval buffer (Dako) for 10 min at 110°C using Decloaking Chamber (BioCare Medical). Slides were then loaded onto the Leica BondRX automated immunohistochemistry stainer. Tissue sections were

incubated with the chimeric 6G10 antibody at 0.5 µg/ml for 60 minutes, followed by detection with the Leica Bond Polymer Refine Detection System (DS9800) using the manufacturer's instructions. Briefly, the tissue sections were incubated with rabbit anti-mouse linker (Leica) for 30 minutes and Novolink Max polymer (Leica) for 30 minutes. Finally, slides were reacted with a DAB substrate-chromogen solution for 6 minutes. Slides were then counterstained with Mayer's hematoxylin, dehydrated, cleared, and coverslipped with Permount following routine histological procedure. Dako protein block supplemented with 0.5% Hu-gamma-globulins (Sigma) was used to block non-specific binding and PBS supplemented with 0.5% human gamma globulins and 0.5% BSA was used as diluent for both primary and secondary antibodies.

To profile tumor infiltrating lymphocytes (TILs), commercial monoclonal antibodies to CD3 (T cell marker) and FoxP3 (regulatory T cell marker) were used to stain adjacent sections. In-house engineered mouse chimeric human anti-KLH IgG2a and commercial mouse IgG1 were used as negative control antibodies. HEK-293T cells stably expressing human GITR and hyperplastic human tonsil tissue sections were used as positive controls. FFPE tissue samples from 5 tumor types were purchased from commercial tissue vendors, with 13~26 cases for each tumor type.

After immunostaining, the slides were examined under a light microscope. For scoring of GITR and TILs, an automated and quantitative analysis using Aperio® ScanScope and HALO™ image analysis software was performed on the whole slide. Representative images were taken either with Aperio ScanScope or Olympus DP71 digital microscope camera.

Table 1 summarizes the distribution of GITR positive TILs in cervical carcinoma (CC), melanoma (Mel), hepatocellular carcinoma (HCC), colorectal carcinoma (CRC), and head and neck squamous cell carcinoma (HNSCC). A manual score of 13 to 26 cases by estimation of number of positive cells under 20x objective of microscope. Minimum, <1 cells per 20x objective field; Mild, 1~<10 cells per 20x objective field; Moderate, 10~<50 cells per 20x objective field; Marked, 50~<200 cells per 20x objective; Intense, >200 cells per 20x objective field. At least 1/3 of the whole tumor section met the above criteria.

Table 1.

Staining intensity	Cervical carcinoma (n=24)	Melanoma (n=17)	Hepatocellular carcinoma (n=25)	Colorectal carcinoma (n=26)	Head and neck squamous cell carcinoma (n=13)
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Minimum	0%	6%	8%	12%	15%
Mild	0%	24%	16%	15%	23%
Moderate	58%	29%	36%	46%	38%
Marked	33%	29%	40%	27%	24%
Intense	8%	12%	0%	0%	0%

In hyperplastic tonsil, strong scattered positive cells were distributed in the interfollicular region and germinal center, as well as scattered epithelium-infiltrating lymphocytes. There was a tendency for more positive cells to be distributed in focal clusters of MNC beneath the epithelium. CD3+ TILs were present in all cancer samples examined, although the quantity varied across cancers and the distribution within the same tissue was heterogeneous. CD8+ TILs were present in the vast majority of tissues. In multiple cancers examined, GITR-positive staining was present in a small fraction of TILs. In general, the abundance of GITR+ cells was in proportion to that of TILs. GITR-positive TILs were abundant in cervical carcinoma (CC), melanoma (Mel), and hepatocellular carcinoma (HCC), and some staining was also observed in colorectal carcinoma (CRC) and head and neck squamous cell carcinoma (HNSCC), among which cervical squamous cell carcinoma showed the most abundant staining.

GITR-positive staining was also observed in tumor cells in NSCLC (non-small cell lung carcinoma), CC, HNSCC, and HCC (Figure 7). In NSCLC and CC, the specificity of the staining was confirmed by complete displacement of staining upon incubation with GITR fusion protein.

Immunohistochemistry was also performed on frozen tissue samples. Cryostat sections at 5 μ m were fixed with acetone for 10 minutes and immunostained using an indirect immunoperoxidase method at room temperature. For staining with CD3 (T cell marker), CD8 (cytotoxic T cell marker), and FoxP3 (regulatory T cell marker) monoclonal antibodies, slides were further fixed with 10% NBF for 2 minutes. For staining with 6G10-FITC and HuIgG2-FITC, slides were immersed in 10% NBF for 1 minute. Peroxidase block supplied with the EnVision+System and Dako protein block supplemented with 0.5% human gamma globulins were used for endogenous peroxidase activity and nonspecific binding blocks, respectively. Primary antibodies (6G10-FITC at 5 μ g/mL, CD3 at 1:100, CD8 at 1:100 or FoxP3 at 5 or 10 μ g/mL) or an isotype control antibody (HuIgG2-FITC at 5 μ g/mL) were applied to sections and incubated for 1 hour. The EnVison+system for either mouse or rabbit IgG was used as the detection system. For 6G10-FITC and its isotype control HuIgG2-FITC, rabbit anti-FITC

antibody (5 µg/mL) was used as bridging antibody. Finally, slides were reacted with the DAB substrate-chromogen solution for 6 minutes, and then counterstained with Mayer's hematoxylin, dehydrated, cleared, and coverslipped with Permount using routine histological procedures. For CD3, CD8, and FoxP3 monoclonal antibodies, Dako protein block was used as the diluent, whereas for 6G10-FITC and HuIgG2-FITC, PBS supplemented with 0.5% human gamma globulins and 0.5% BSA was used as the diluent for both primary and secondary antibodies. Frozen tissue samples from 3 tumor types were purchased from commercial tissue vendors.

Different amounts of GITR+ TILs were observed in the 3 tumor types. FoxP3 was present in a small subset of TILs. Staining of a subset of tumor cells with the anti-GITR antibody was also observed, and the specificity of the staining was confirmed by complete displacement of staining upon incubation with GITR fusion protein. The presence of GITR mRNA in GITR tumor cells was also observed by RNAscope on the frozen sections, which further confirms the IHC results.

These results indicate that anti-GITR antibody 6G10 can efficiently detect human GITR protein in fixed and frozen tissue samples.

Table 2: Summary table of sequences

SEQ ID	DESCRIPTION	SEQUENCE
1	human GITR isoform 1	MAQHGMGAFRALCGLALLCALSLGQRPTGGPGCGPGRLLLG TGTDARCCRVHTTRCCRDYPGEECCSEWDCMCVQPEFHCGDP CCTTCRHHPCPPGQGVQSQGKFSFGFQCIDCASGTFSGGHEG HCKPWT DCTQFGFLT VFPGNKTHNAV CVPGSPPAEPLGWLT V VLLAVAACVLLLTSAQLGLHIWQLRSQCMWPRETQLLLEVPP STEDARSCQFP EEERGERSAEEKGRLGDLWV
2	human GITR isoform 2	MAQHGMGAFRALCGLALLCALSLGQRPTGGPGCGPGRLLLG TGTDARCCRVHTTRCCRDYPGEECCSEWDCMCVQPEFHCGDP CCTTCRHHPCPPGQGVQSQGKFSFGFQCIDCASGTFSGGHEG HCKPWT DCCWRCRRRPKTPEAASSPRKSGASDRQRRRGWET CGCEPGRPPGPPTAASPSPGAPQAAGALRSALGRALLPWQQK WVQEGGSDQRPGPCSSAAAAGPCRRERETQSWPPSSLAGPDG VGS
3	human GITR isoform 3	MAQHGMGAFRALCGLALLCALSLGQRPTGGPGCGPGRLLLG TGTDARCCRVHTTRCCRDYPGEECCSEWDCMCVQPEFHCGDP CCTTCRHHPCPPGQGVQSQGKFSFGFQCIDCASGTFSGGHEG HCKPWT DCTQFGFLT VFPGNKTHNAV CVPGSPPAEPLGWLT V VLLAVAACVLLLTSAQLGLHIWQLRKTQLLLEVPPSTEDARS CQFP EEERGERSAEEKGRLGDLWV
4	extracellular portion of mature human GITR	QRPTGGPGCGPGRLLLG TGTDARCCRVHTTRCCRDYPGEECC SEWDCMCVQPEFHCGDP CCTTCRHHPCPPGQGVQSQGKFSFG FQCIDCASGTFSGGHEGHCKPWT DCTQFGFLT VFPGNKTHNA VCVPGSPPAEP
5	6G10 VH CDR1	TYGMH
6	6G10 VH CDR2	VTWYAGSNKFYADSVKG
7	6G10 VH CDR3	GGSMVRGLYYYGMDV
8	6G10 VL CDR1	RASQGISSALA
9	6G10 VL CDR2	DASSLES
10	6G10 VL CDR3	QQFNSYPYT
11	6G10 VH	QVQLVESGGDVVQPGRSRLRLSCAASGFTFSTYGMHWVRQAPG KGLEWVAVTWYAGSNKFYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARGGSMVRGLYYYGMDVWGQGTTVTVSS
12	6G10 VL	AIQLTQSPSSLSASVGDRVITTCRASQGISSALAWYQQKPGK APKLLIYDASSLESQVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQFNSYPYTFGGGTKLEIK
13	6G10 full length heavy chain	QVQLVESGGDVVQPGRSRLRLSCAASGFTFSTYGMHWVRQAPG KGLEWVAVTWYAGSNKFYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARGGSMVRGLYYYGMDVWGQGTTVTVSSAS <u>TKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG</u> <u>ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVD</u> <u>HKPSNTKVDKTKVERKCCVECPPCPAPPVAGPSVFLFPPKPKD</u> <u>TLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKP</u> <u>REEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIE</u>

		<u>KTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS</u> <u>DIAVEWESNGQPENNYKTPPMLDSDGSFFLYSKLTVDKSRW</u> <u>QQGNVFSCSVMHEALHNHYTQKSLSLSPGK</u>
14	6G10 full length light chain	AIQLTQSPSSLSASVGDRVITTCRASQGIISSALAWYQQKPGK APKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQFNSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLK <u>SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS</u> <u>KDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN</u> <u>RGEC</u>
15	chimeric 6G10 antibody—full length heavy chain The constant region is underlined	QVQLVESGGDVVQPGRSRLRLSCAASGFTFSTYGMHWVRQAPG KGLEWVAVTWYAGSNKFYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARGGSMVRGLYYYGMDVWGQGTITVTVSSAK <u>TTAPSVYPLAPVCGDITGSSVTLGCLVKGYFPEPVTLTWSNG</u> <u>SLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAH</u> <u>PASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPP</u> <u>KIKDVLMI SLSPIVTCVVVDVSEDDPDVQISW FVNNVEVHTA</u> <u>QTQTHREDYNSTLRVVSALPIQH QDWM S GKEFKCKVNNKDLP</u> <u>APIERTISKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTD</u> <u>FMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVE</u> <u>KKNWVERN SYSCSVVHEGLHNHHTTKSF SRTPGK</u>
16	chimeric 6G10 antibody—full length light chain The constant region is underlined	AIQLTQSPSSLSASVGDRVITTCRASQGIISSALAWYQQKPGK APKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQFNSYPYTFGQGTKLEIKRADAAPTVSI FPPSSEQLT <u>SGGASVVCFLNNFYPKDINVWKIDGSERQNGVLNSWTDQDS</u> <u>KDSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPIVKSFN</u> <u>RNEC</u>
17	6G10 (VH) nucleotide sequence	CAGGTGCAGCTGGTGGAGTCTGGGGGAGACGTGGT CCAGCCTGGGAGGTCCCTGAGACTCTCCTGTGCAG CGTCTGGATTACCTTCAGTACCTATGGCATGCAC TGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTG GGTGGCAGTTACATGGTATGCTGGAAGTAATAAAT TTTATGCAGACTCCGTGAAGGGCCGATTCACCATC TCCAGAGACAATTCCAAGAACACGCTGTATCTGCA AATGAACAGCCTGAGAGCCGAGGACACGGCTGTGT ATTACTGTGCGAGAGGAGGTAGTATGGTTCGGGGA CTTTATTATTACGGTATGGACGTCTGGGGCCAAGG GACCACGGTCACCGTCTCCTCA
18	6G10 (VL) nucleotide sequence	GCCATCCAGTTGACCCAGTCTCCATCCTCCCTGTC TGCATCTGTAGGAGACAGAGTCACCATCACTTGCC GGGCAAGTCAGGGCATTAGCAGTGCTTTAGCCTGG TATCAGCAGAAACCAGGGAAAGCTCCTAAGCTCCT GATCTATGATGCCTCCAGTTTGGAAAGTGGGGTCC CATCAAGGTT CAGCGGCAGTGGATCTGGGACAGAT TTC ACTCTC ACCATCAGCAGCCTGCAGCCTGAAGA TTTTGCAACTTATTACTGTCAACAGTTTAATAGTT ACCCGTACACTTTTGGCCAGGGGACCAAGCTGGAG ATCAAA

19	6G10 (full length wild-type heavy chain) nucleotide sequence The sequence encoding the constant region is underlined	CAGGTGCAGCTGGTGGAGTCTGGGGGAGACGTGGTCCAGCCT GGGAGGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACC TTCAGTACCTATGGCATGCACTGGGTCCGCCAGGCTCCAGGC AAGGGGCTGGAGTGGGTGGCAGTTACATGGTATGCTGGAAGT AATAAATTTTATGCAGACTCCGTGAAGGGCCGATTCACCATC TCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAAC AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGA GGAGGTAGTATGGTTCGGGGACTTTATTATTACGGTATGGAC GTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGCCTCC <u>ACCAAGGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGG</u> <u>AGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAG</u> <u>GACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGC</u> <u>GCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGTCCTACAG</u> <u>TCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC</u> <u>TCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGAT</u> <u>CACAAGCCCAGCAACACCAAGGTGGACAAGACAGTTGAGCGC</u> <u>AAATGTTGTGTCGAGTGCCACCGTGCCAGCACCACCTGTG</u> <u>GCAGGACCGTCAGTCTTCTCTTCCCCC AAAACCCAAGGAC</u> <u>ACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTG</u> <u>GTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGG</u> <u>TACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCA</u> <u>CGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTC</u> <u>CTCACCGTTGTGCACCAGGACTGGCTGAACGGCAAGGAGTAC</u> <u>AAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCCATCGAG</u> <u>AAAACCATCTCCAAAACCAAGGGCAGCCCCGAGAACCACAG</u> <u>GTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAAC</u> <u>CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGC</u> <u>GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC</u> <u>AACTACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCC</u> <u>TTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG</u> <u>CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT</u> <u>CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCG</u> <u>GGTAAA</u>
20	6G10 (full length wild-type light chain) nucleotide sequence The sequence encoding the constant region is underlined	GCCATCCAGTTGACCCAGTCTCCATCCTCCCTGTCTGCATCT GTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGGGC ATTAGCAGTGCTTTAGCCTGGTATCAGCAGAAACCAGGGAAA GCTCCTAAGCTCCTGATCTATGATGCCTCCAGTTTGGAAAGT GGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAT TTCACTCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCA ACTTATTACTGTCAACAGTTTAAATAGTTACCCGTACACTTTT GGCCAGGGGACCAAGCTGGAGATCAAACGAACTGTGGCTGCA CCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAA <u>TCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTAT</u> <u>CCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTC</u> <u>CAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGC</u> <u>AAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGC</u> <u>AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTC</u> <u>ACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAAC</u> <u>AGGGGAGAGTGT</u>
21	chimeric 6G10 heavy chain The sequence encoding the	CAGGTGCAGCTGGTGGAGTCTGGGGGAGACGTGGTCCAGCCT GGGAGGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACC TTCAGTACCTATGGCATGCACTGGGTCCGCCAGGCTCCAGGC AAGGGGCTGGAGTGGGTGGCAGTTACATGGTATGCTGGAAGT

	constant region is underlined	<u>AATAAATTTTATGCAGACTCCGTGAAGGGCCGATTACCATC</u> <u>TCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAAC</u> <u>AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGA</u> <u>GGAGGTAGTATGGTTCGGGGACTTTATTATTACGGTATGGAC</u> <u>GTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGCCAAA</u> <u>ACAACAGCCCCATCGGTCTATCCGCTAGCCCCTGTGTGTGGA</u> <u>GATACAACTGGCTCCTCGGTGACTCTAGGATGCCTGGTCAAG</u> <u>GGTTATTTCCCTGAGCCAGTGACCTTGACCTGGAACCTCTGGA</u> <u>TCCCTGTCCAGTGGTGTGCACACCTTCCCAGCTGTCCTGCAG</u> <u>TCTGACCTCTACACCCCTCAGCAGCTCAGTGACTGTAACCTCG</u> <u>AGCACCTGGCCCAGCCAGTCCATCACCTGCAATGTGGCCCAC</u> <u>CCGGCAAGCAGCACCAAGGTGGACAAGAAAATTGAGCCCAGA</u> <u>GGGCCCACAATCAAGCCCTGTCCTCCATGCAAATGCCCAGCA</u> <u>CCTAACCTCTTGGGTGGACCATCCGTCTTCATCTTCCCTCCA</u> <u>AAGATCAAGGATGTACTCATGATCTCCCTGAGCCCCATAGTC</u> <u>ACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTC</u> <u>CAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCT</u> <u>CAGACACAAACCCATAGAGAGGATTACAACAGTACTCTCCGG</u> <u>GTGGTCAGTGCCCTCCCCATCCAGCACCCAGGACTGGATGAGT</u> <u>GGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGACCTCCCA</u> <u>GCGCCCATCGAGAGAACCATCTCAAAACCCAAAGGGTCAGTA</u> <u>AGAGCTCCACAGGTATATGTCTTGCCTCCACCAGAAGAAGAG</u> <u>ATGACTAAGAAACAGGTCACTCTGACCTGCATGGTCACAGAC</u> <u>TTCATGCCTGAAGACATTTACGTGGAGTGGACCAACAACGGG</u> <u>AAACAGAGCTAAACTACAAGAACACTGAACCAGTCCTGGAC</u> <u>TCTGATGGTTCTTACTTCATGTACAGCAAGCTGAGAGTGGAA</u> <u>AAGAAGAACTGGGTGGAAAGAAATAGCTACTCCTGTTCAGTG</u> <u>GTCCACGAGGGTCTGCACAATCACCACACGACTAAGAGCTTC</u> <u>TCCCGGACTCCGGGTAAATGA</u>
22	chimeric 6G10 light chain The sequence encoding the constant region is underlined	<u>GCCATCCAGTTGACCCAGTCTCCATCCTCCCTGTCTGCATCT</u> <u>GTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGGGC</u> <u>ATTAGCAGTGCTTTAGCCTGGTATCAGCAGAAACCAGGGAAA</u> <u>GCTCCTAAGCTCCTGATCTATGATGCCTCCAGTTTGGAAGT</u> <u>GGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACAGAT</u> <u>TTCACTCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCA</u> <u>ACTTATTACTGTCAACAGTTTAAATAGTTACCCGTACACTTTT</u> <u>GGCCAGGGGACCAAGCTGGAGATCAAACGGGGCTGATGCTGCA</u> <u>CCAAGTGTATCCATCTTCCCACCATCCAGTGAGCAATTGACA</u> <u>TCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACCTTCTAC</u> <u>CCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAA</u> <u>CGACAAAATGGCGTCCTGAACAGTTGGACCGATCAGGACAGC</u> <u>AAAGACAGCACCTACAGCATGAGCAGCACCCCTCACGTTGACC</u> <u>AAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCC</u> <u>ACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAAC</u> <u>AGGAATGAGTGTTAG</u>
23	human IgG2 heavy chain constant region	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWN SGALTSQVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCN VDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKT KPREEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAP IEKTIISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPMLDSGDGSFFLYSKLTVDKS RWQQGNVFSCSVMEALHNHYTQKSLSLSPGK
24	human kappa light chain	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV

	constant region	YACEVTHQGLSSPVTKSFNRGEC
25	mouse IgG2a heavy chain constant region	AKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWN SGSLSSGVHTFPAYLQSDLYTLSSSVTVTSSTWPSQSITCNV AHPASSTKVDDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIF PPKIKDVLMISSLPIVTCVVVDVSEDDPDVQISWFEVNNVEVH TAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKD LPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMV TDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLR VEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK
26	mouse kappa light chain constant region	RADAAPTVSIFFPSSEQLTSGGASVVCFLNIFYPKDINVKWK IDGSEKQNGVLNSWTDQDSKDYMSSTLTTLTKDEYERHNS YTCEATHKTSTSPIVKSFNRNEC
27	28F3 (VH)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW VAVIWEYSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARGGSMVRGDYYYGMDVWGQGTTVTVSS
28	28F3 (VL)	AIQLTQSPSSLSASVGDRVITITCRASQGIISSALAWYQQKPGKAPKLL IYDASSLESGVPSRFSGSGSGTDFTLTIISSLPEDFATYYCQQFNSY PYTFGQGTKLEIK
29	28F3 (full length wild-type heavy chain) The constant region is underlined	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW VAVIWEYSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARGGSMVRGDYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPCSRS <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCA</u> <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV</u> <u>DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNK</u> <u>GLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMEALHNHYTQKSLSLSPGK</u>
30	28F3 (full length wild-type light chain) The constant region is underlined	AIQLTQSPSSLSASVGDRVITITCRASQGIISSALAWYQQKPGKAPKLL IYDASSLESGVPSRFSGSGSGTDFTLTIISSLPEDFATYYCQQFNSY PYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYF <u>REAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEK</u> <u>HKVYACEVTHQGLSSPVTKSFNREGC</u>
31	28F3.IgG1 (VH + IgG1)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW VAVIWEYSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARGGSMVRGDYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMEALHNHYTQKSLSLSPG
32	28F3.IgG1.1 (VH + IgG1.1)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW VAVIWEYSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARGGSMVRGDYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK

		VSNKALPSSIEKTIISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG
33	28F3.IgG1 (VL + CL)	AIQLTQSPSSLSASVGDRVTTITCRASQGIISSALAWYQQKPGKAPKLL IYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFNSY PYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
34	28F3 VH CDR1	SYGMH
35	28F3 VH CDR2	VIWYEGSNKYYADSVKG
36	28F3 VH CDR3	GGSMVRGDYYYGMDV
37	28F3 VL CDR1	RASQGIISSALA
38	28F3 VL CDR2	DASSLES
39	28F3 VL CDR3	QQFNSYPYT
40	28F3 (VH + G2(C219S)) or 28F3-IgG2-C219S	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW</u> <u>VAVIWIWYEGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGSMVRGDYYYGMDVWGQGTTTVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKSCVECPPCPA</u> <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV</u> <u>DGVEVHNAKTKPREEQFNSTFRVVS VLTVVHQDWLNGKEYKCKVSNK</u> <u>GLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTT PMLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
41	28F3 (VH + G2.g1) or 28F3-IgG2-IgG1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW</u> <u>VAVIWIWYEGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGSMVRGDYYYGMDVWGQGTTTVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCPA</u> <u>PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY</u> <u>VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN</u> <u>KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF</u> <u>YPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQG</u> <u>GNVFSCSVMHEALHNHYTQKSLSLSPG</u>
42	28F3 (VH + G2.g1.1) or 28F3-IgG2-IgG1.1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW</u> <u>VAVIWIWYEGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGSMVRGDYYYGMDVWGQGTTTVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECPPCPA</u> <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV</u> <u>DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK</u> <u>ALPSSIEKTIISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
43	28F3 (VH + G2(C219S).g1) or 28F3-IgG2-C219S-IgG1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW</u> <u>VAVIWIWYEGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGSMVRGDYYYGMDVWGQGTTTVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKSCVECPPCPA</u>

		<u>PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY</u> <u>VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN</u> <u>KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF</u> <u>YPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQ</u> <u>GNVFSCSVMHEALHNHYTQKSLSLSPG</u>
44	28F3 (VH + G2(C219S).g1.1) or 28F3-IgG2-C219S-IgG1.1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFS</u> <u>SYGMHWVRQAPGKGLEW</u> <u>VAVIWYEGSNKYYADSVKGRFTISRDN</u> <u>SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGSMVRGDYYYGMDVWGQGT</u> <u>TVTVSS</u> <u>ASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVT</u> <u>SVWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNV</u> <u>DHKPSNTKVDKTV</u> <u>VERKSCVECP</u> <u>PCPA</u> <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV</u> <u>DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK</u> <u>ALPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
45	28F3 (VH) (SEQ ID NO: 13) with signal peptide The signal peptide is underlined	<u>MRAWIFFLLCLAGRALA</u> <u>QVQLVESGGGVVQPGRSLRLSCAASGFTFS</u> <u>SYGMHWVRQAPGKGLEW</u> <u>VAVIWYEGSNKYYADSVKGRFTISRDN</u> <u>SKN</u> <u>TLYLQMNSLRAEDTAVYYCARGGSMVRGDYYYGMDVWGQGT</u> <u>TVTVSS</u>
46	28F3 (VL) (SEQ ID NO: 14) with signal peptide The signal peptide is underlined	<u>MRAWIFFLLCLAGRALAAIQLTQSPSSLSASVGDRVTITCRASQGIS</u> <u>SALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLT</u> <u>ISS</u> <u>LQPEDFATYYCQQFN</u> <u>SYPTFGQGTKLEIK</u>
47	28F3.IgG1 (VH + IgG1) with signal peptide The signal peptide and constant region are underlined	<u>MRAWIFFLLCLAGRALA</u> <u>QVQLVESGGGVVQPGRSLRLSCAASGFTFS</u> <u>SYGMHWVRQAPGKGLEW</u> <u>VAVIWYEGSNKYYADSVKGRFTISRDN</u> <u>SKN</u> <u>TLYLQMNSLRAEDTAVYYCARGGSMVRGDYYYGMDVWGQGT</u> <u>TVTVSS</u> <u>ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT</u> <u>SVWNSGALT</u> <u>SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV</u> <u>NHKPSNTKV</u> <u>DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE</u> <u>VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV</u> <u>LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL</u> <u>P</u> <u>PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLD</u> <u>SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG</u>
48	28F3.IgG1.1 (VH + IgG1.1) with signal peptide The signal peptide and constant region are underlined	<u>MRAWIFFLLCLAGRALA</u> <u>QVQLVESGGGVVQPGRSLRLSCAASGFTFS</u> <u>SYGMHWVRQAPGKGLEW</u> <u>VAVIWYEGSNKYYADSVKGRFTISRDN</u> <u>SKN</u> <u>TLYLQMNSLRAEDTAVYYCARGGSMVRGDYYYGMDVWGQGT</u> <u>TVTVSS</u> <u>ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT</u> <u>SVWNSGALT</u> <u>SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV</u> <u>NHKPSNTKV</u> <u>DKRVEPKSCDKTHTCPPCPA</u> <u>EAEGAPSVFLFPPKPKDTLMISRTPE</u> <u>VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV</u> <u>LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVYTL</u> <u>P</u> <u>PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLD</u> <u>SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG</u>
49	28F3.IgG1 (VL+CL) with signal peptide The signal peptide and constant region are underlined	<u>MRAWIFFLLCLAGRALAAIQLTQSPSSLSASVGDRVTITCRASQGIS</u> <u>SALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLT</u> <u>ISS</u> <u>LQPEDFATYYCQQFN</u> <u>SYPTFGQGTKLEIKRTVAAPSVFIFPPSDEQ</u> <u>LKSGTASVVCLLNNFY</u> <u>P</u> <u>REAKVQWKVDNALQSGNSQESVTEQDSKDS</u> <u>TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT</u> <u>KSFNRGEC</u>
50	19D3 (VH)	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFS</u> <u>SYGFHWVRQAPGKGLEW</u> <u>VAVIWYAGSNKF</u> <u>YADSVKGRFTISRDN</u> <u>SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGQLDYYYYYVMDVWGQGT</u> <u>TVTVSS</u>

51	19D3 (VL)	DIQMTQSPSSLSASVGDRVTITCRASQGIISSWLAWYQQKPEKAPKSL IYAASSLQSGVP SRFSGSGSGTDFTLTIISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK
52	19D3 (full length wild-type heavy chain) The constant region is underlined	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGFHWVRQAPGKGLEW VAVIWIYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARGGQLDYIIIIYVMDVWGQGTITVTVSSASTKGPSVFPLAPCSRST <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYTCNV DHKPSNTKVDKTV ERKCCVECP PCPAP</u> <u>PVAGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVQFNWYVD</u> <u>GVEVHNAKTKPREEQFNSTFRVVS VLT TVVHQDWLNGKEYKCKVSNKGL</u> <u>LPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP</u> <u>SDIAVEWESNGQPENNYKTT PPM L DSDGSFFLYSKLTVDKSRWQQGN</u> <u>VFSCSVMHEALHNHYTQKSLSLSPGK</u>
53	19D3 (full length wild-type light chain) The constant region is underlined	DIQMTQSPSSLSASVGDRVTITCRASQGIISSWLAWYQQKPEKAPKSL IYAASSLQSGVPSRFSGSGSGTDFTLTIISSLQPEDFATYYCQQYNSY PYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYF <u>REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK</u> <u>HKVYACEVTHQGLSSPVTKSFNRGEC</u>
54	19D3.IgG1 (VH + IgG1)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGFHWVRQAPGKGLEW VAVIWIYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARGGQLDYIIIIYVMDVWGQGTITVTVSSASTKGPSVFPLAPSSKST SGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYP SDIAVEWESNGQPENNYKTT P PVL DSDGSFFLYSKLTVDKSRW QQGNVVFSCSVMHEALHNHYTQKSLSLSPG
55	19D3.IgG1.1 (VH + IgG1.1)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGFHWVRQAPGKGLEW VAVIWIYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARGGQLDYIIIIYVMDVWGQGTITVTVSSASTKGPSVFPLAPSSKST SGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAEGAPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYP SDIAVEWESNGQPENNYKTT P PVL DSDGSFFLYSKLTVDKSRW QQGNVVFSCSVMHEALHNHYTQKSLSLSPG
56	19D3.IgG1 (VL + CL)	DIQMTQSPSSLSASVGDRVTITCRASQGIISSWLAWYQQKPEKAPKSL IYAASSLQSGVPSRFSGSGSGTDFTLTIISSLQPEDFATYYCQQYNSY PYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYF REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
57	19D3 VH CDR1	SYGFH
58	19D3 VH CDR2	VIWIYAGSNKFYADSVK G
59	19D3 VH CDR3	GGQLDYIIIIYVMDV
60	19D3 VL CDR1	RASQGISSWLA

61	19D3 VL CDR2	AASSLQS
62	19D3 VL CDR3	QQYNSYPYT
63	19D3 (VH + G2(C219S)) or 19D3-IgG2-C219S	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGFHWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGQLDYYYYYVMDVWGQGTTVTVSSASTKGPSVFPLAPCSRST</u> <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYTCNV DHKPSNTKVDK TVERKSCVECPPCPAP</u> <u>PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVD</u> <u>GVEVHNAKTKPREEQFNSTFRVVS VLT TVVHQDWLNGKEYKCKVSNKG</u> <u>LPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP</u> <u>SDIAVEWESNGQPENNYKTT P PMLDSDGSFFLYSKLTVDKSRWQQGN</u> <u>VFSCSVMHEALHNHYTQKSLSLSPG</u>
64	19D3 (VH + G2.g1) or 19D3-IgG2-IgG1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGFHWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGQLDYYYYYVMDVWGQGTTVTVSSASTKGPSVFPLAPCSRST</u> <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYTCNV DHKPSNTKVDK TVERKCCVECPPCPAP</u> <u>ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV</u> <u>DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK</u> <u>ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTT P PVLDS DGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
65	19D3 (VH + G2.g1.1) or 19D3-IgG2-IgG1.1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGFHWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGQLDYYYYYVMDVWGQGTTVTVSSASTKGPSVFPLAPCSRST</u> <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYTCNV DHKPSNTKVDK TVERKCCVECPPCPAP</u> <u>PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD</u> <u>GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA</u> <u>LPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP</u> <u>SDIAVEWESNGQPENNYKTT P PVLDS DGSFFLYSKLTVDKSRWQQGN</u> <u>VFSCSVMHEALHNHYTQKSLSLSPG</u>
66	19D3 (VH + G2(C219S).g1) or 19D3-IgG2-C219S-IgG1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGFHWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGQLDYYYYYVMDVWGQGTTVTVSSASTKGPSVFPLAPCSRST</u> <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYTCNV DHKPSNTKVDK TVERKSCVECPPCPAP</u> <u>ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV</u> <u>DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK</u> <u>ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTT P PVLDS DGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
67	19D3 (VH + G2(C219S).g1.1) or 19D3-IgG2-C219S-IgG1.1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGFHWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGQLDYYYYYVMDVWGQGTTVTVSSASTKGPSVFPLAPCSRST</u> <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYTCNV DHKPSNTKVDK TVERKSCVECPPCPAP</u> <u>PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD</u> <u>GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA</u> <u>LPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP</u> <u>SDIAVEWESNGQPENNYKTT P PVLDS DGSFFLYSKLTVDKSRWQQGN</u> <u>VFSCSVMHEALHNHYTQKSLSLSPG</u>

68	19D3 (VH + G2(C219S)) or 19D3-IgG2-C219S	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGFWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGQLDYIIIIYVMDVWGQGTTVTVSSASTKGPSVFPLAPCSRST</u> <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYYTCNV DHKPSNTKVDKTV ERKSCVECPPCPAP</u> <u>PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVD</u> <u>GVEVHNAKTKPREEQFNSTFRVVS VLT TVVHQDWLNGKEYKCKVSNKG</u> <u>LPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP</u> <u>SDIAVEWESNGQPENNYKTT PPM L DSDGSFFLYSKLTVDKSRWQQGN</u> <u>VFSCSVMHEALHNHYTQKSLSLSPG</u>
69	19D3 (VH + G2.g1) or 19D3-IgG2-IgG1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGFWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGQLDYIIIIYVMDVWGQGTTVTVSSASTKGPSVFPLAPCSRST</u> <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYYTCNV DHKPSNTKVDKTV ERKCCVECPPCPAP</u> <u>ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV</u> <u>DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK</u> <u>ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
70	19D3 (VH + G2.g1.1) or 19D3-IgG2-IgG1.1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGFWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGQLDYIIIIYVMDVWGQGTTVTVSSASTKGPSVFPLAPCSRST</u> <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYYTCNV DHKPSNTKVDKTV ERKCCVECPPCPAP</u> <u>PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD</u> <u>GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA</u> <u>LPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP</u> <u>SDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGN</u> <u>VFSCSVMHEALHNHYTQKSLSLSPG</u>
71	19D3 (VH + G2(C219S).g1) or 19D3-IgG2-C219S-IgG1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGFWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGQLDYIIIIYVMDVWGQGTTVTVSSASTKGPSVFPLAPCSRST</u> <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYYTCNV DHKPSNTKVDKTV ERKSCVECPPCPAP</u> <u>ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV</u> <u>DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK</u> <u>ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
72	19D3 (VH + G2(C219S).g1.1) or 19D3-IgG2-C219S-IgG1.1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGFWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGQLDYIIIIYVMDVWGQGTTVTVSSASTKGPSVFPLAPCSRST</u> <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYYTCNV DHKPSNTKVDKTV ERKSCVECPPCPAP</u> <u>PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD</u> <u>GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA</u> <u>LPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP</u> <u>SDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGN</u> <u>VFSCSVMHEALHNHYTQKSLSLSPG</u>
73	18E10 (VH)	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGRIAVAFYY SMDVWGQGTTVTVSS</u>

74	18E10 (VL)	DIQMTQSPSSLSASVGDRVTITCRASQGIISSWLAWYQQKPEKAPKSL IYAASSLQSGVPSRFSGSGSGTDFTLTIISSSLQPEDFATYYCQQYNSY PYTFGQGGTKLEIK
75	18E10 (full length wild-type heavy chain) The constant region is underlined	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW VAVIWIYAGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARGGRIAVAFYYSM DVWGQGT TTVTVSSASTKGPSVFPLAPCSRST SESTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL SSVTVTPSSNFGTQT YTCNV DHKPSNTKVDK TVERKCCVECP PCPAP PVAGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVQFNWYVD GVEVHNAKTKPREEQFNSTFRVVS VLT VVHQDWLNGKEYKCKVSNKG LPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
76	18E10 (full length wild-type light chain) The constant region is underlined	DIQMTQSPSSLSASVGDRVTITCRASQGIISSWLAWYQQKPEKAPKSL IYAASSLQSGVPSRFSGSGSGTDFTLTIISSSLQPEDFATYYCQQYNSY PYTFGQGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYЕК HKVYACEVTHQGLSSPVTKSFNRGEC
77	18E10.IgG1 (VH + IgG1)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW VAVIWIYAGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARGGRIAVAFYYSM DVWGQGT TTVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL SSVTVTPSSSLGTQTYICNV NHKPSNTKVDKRVEPKSCDKTHTCPPC PAPELLGGPSVFLF PPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLT VLVHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVVFSCSVMHEALHNHYTQKSLSLSPG
78	18E10.IgG1.1 (VH + IgG1.1)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW VAVIWIYAGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARGGRIAVAFYYSM DVWGQGT TTVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL SSVTVTPSSSLGTQTYICNV NHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAEGAPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLVHQDWLNGKEYKCKV SNKALPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVVFSCSVMHEALHNHYTQKSLSLSPG
79	18E10.IgG1 (VL + CL)	DIQMTQSPSSLSASVGDRVTITCRASQGIISSWLAWYQQKPEKAPKSL IYAASSLQSGVPSRFSGSGSGTDFTLTIISSSLQPEDFATYYCQQYNSY PYTFGQGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYЕК HKVYACEVTHQGLSSPVTKSFNRGEC
80	18E10 VH CDR1	SYGMH
81	18E10 VH CDR2	VIWIYAGSNKYYADSVKG
82	18E10 VH CDR3	GGRIAVAFYYSM DV
83	18E10 VL CDR1	RASQGIISSWLA

84	18E10 VL CDR2	AASSLQS
85	18E10 VL CDR3	QQYNSYPYT
86	18E10 (VH + G2(C219S)) or 18E10-IgG2-C219S	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGRIAVAFYYSDMVWGQGTTVTVSSASTKGPSVFPLAPCSRST</u> <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYTCNV DHKPSNTKVDK TVERKSCVECPPCPAP</u> <u>PVAGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVQFNWYVD</u> <u>GVEVHNAKTKPREEQFNSTFRVVS VLT TVVHQDWLNGKEYKCKVSNKG</u> <u>LPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP</u> <u>SDIAVEWESNGQPENNYKTT P PMLDSDGSFFLYSKLTVDKSRWQQGN</u> <u>VFSCSVMHEALHNHYTQKSLSLSPG</u>
87	18E10 (VH + G2.g1) or 18E10-IgG2-IgG1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGRIAVAFYYSDMVWGQGTTVTVSSASTKGPSVFPLAPCSRST</u> <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYTCNV DHKPSNTKVDK TVERKCCVECPPCPAP</u> <u>ELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYV</u> <u>DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK</u> <u>ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTT P PVLDS DGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
88	18E10 (VH + G2.g1.1) or 18E10-IgG2-IgG1.1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGRIAVAFYYSDMVWGQGTTVTVSSASTKGPSVFPLAPCSRST</u> <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYTCNV DHKPSNTKVDK TVERKCCVECPPCPAP</u> <u>PVAGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVD</u> <u>GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA</u> <u>LPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP</u> <u>SDIAVEWESNGQPENNYKTT P PVLDS DGSFFLYSKLTVDKSRWQQGN</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
89	18E10 (VH + G2(C219S).g1) or 18E10-IgG2-C219S-IgG1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGRIAVAFYYSDMVWGQGTTVTVSSASTKGPSVFPLAPCSRST</u> <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYTCNV DHKPSNTKVDK TVERKSCVECPPCPAP</u> <u>ELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYV</u> <u>DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK</u> <u>ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTT P PVLDS DGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
90	18E10 (VH + G2(C219S).g1.1) or 18E10-IgG2-C219S-IgG1.1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW</u> <u>VAVIWIYAGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGRIAVAFYYSDMVWGQGTTVTVSSASTKGPSVFPLAPCSRST</u> <u>SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL</u> <u>SSVTVTPSSNFGTQTYTCNV DHKPSNTKVDK TVERKSCVECPPCPAP</u> <u>PVAGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVD</u> <u>GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA</u> <u>LPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP</u> <u>SDIAVEWESNGQPENNYKTT P PVLDS DGSFFLYSKLTVDKSRWQQGN</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>

91	3C3 (VH)	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWTWIRQPPGKGLEW IGKINHSGNTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYY CARLGAFDAFDIWGQGTMVTVSS
92	3C3 (VL1)	DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSL IYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSY PYTFGQGGTKLEIK
93	3C3 (VL2)	EIVLTQSPATLSLSPGERATLSCRASQGVSSYLAWYQQKPGQAPRLL IYDASN RATGIPARFSGSGPGTDFTLTISSLEPEDFAVYYCQQRSNW HTFGQGGTKLEIK
94	3C3 (full length wild-type heavy chain) The constant region is underlined	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWTWIRQPPGKGLEW IGKINHSGNTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYY CARLGAFDAFDIWGQGTMVTVSS <u>ASTKGPSVFPLAPSSKSTSGGTAA</u> <u>LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV</u> <u>PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELL</u> <u>GGPSVFLFPPKPKDTLMI</u> <u>SRTPEVTCVVVDVSHEDPEVKFNWYVDGV</u> <u>EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP</u> <u>APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD</u> <u>IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF</u> <u>SCSVMHEALHNHYTQKSLSLSPGK</u>
95	3C3 L1 (full length wild-type light chain 1) The constant region is underlined	DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSL IYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSY PYTFGQGGTKLEIK <u>KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP</u> <u>REAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEK</u> <u>HKVYACEVTHQGLSSPVTKSFNRGEC</u>
96	3C3 L2 (full length wild-type light chain 2) The constant region is underlined	EIVLTQSPATLSLSPGERATLSCRASQGVSSYLAWYQQKPGQAPRLL IYDASN RATGIPARFSGSGPGTDFTLTISSLEPEDFAVYYCQQRSNW HTFGQGGTKLEIK <u>KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR</u> <u>EAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKH</u> <u>KVYACEVTHQGLSSPVTKSFNRGEC</u>
97	3C3.IgG1 (VH + IgG1)	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWTWIRQPPGKGLEW IGKINHSGNTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYY CARLGAFDAFDIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELL GGPSVFLF PPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG
98	3C3.IgG1.1 (VH + IgG1.1)	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWTWIRQPPGKGLEW IGKINHSGNTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYY CARLGAFDAFDIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEA <u>E</u> <u>G</u> APSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP <u>SSI</u> IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPG

99	3C3.IgG1 (VL1 + CL)	DIQMTQSPSSLSASVGDRVTITCRASQGIISSWLAWYQQKPEKAPKSL IYAASSLQSGVPSRFSGSGSGTDFTLTIISSLQPEDFATYYCQQYNSY PYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
100	3C3IgG1.2 (VL2 + CL)	EIVLTQSPATLSLSPGERATLSCRASQGVSSYLAWYQQKPGQAPRLL IYDASNRATGIPARFSGSGPGTDFTLTIISSLEPEDFAVYYCQQRSNW HTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC
101	3C3 VH CDR1	GYWT
102	3C3 VH CDR2	KINHSGNTNYPNPSLKS
103	3C3 VH CDR3	LGAFDAFDI
104	3C3 VL1 CDR1	RASQGISSWLA
105	3C3 VL1 CDR2	AASSLQS
106	3C3 VL1 CDR3	QQYNSYPYT
107	3C3 VL2 CDR1	RASQGVSSYLA
108	3C3 VL2 CDR2	DASNRAT
109	3C3 VL2 CDR3	QQRSNWHT
110	3C3 (VH + G2) or 3C3-IgG2	<u>QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWTWIRQPPGKGLEW</u> <u>IGKINHSGNTNYPNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYY</u> <u>CARLGAFDAFDIWGQGTMTVTVSSASTKGPSVFPLAPCSRSTSESTAA</u> <u>LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV</u> <u>PSSNFGTQTYTCNVDPKPSNTKVDKTVRKCCVECPPCPAPPVAGPS</u> <u>VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHN</u> <u>AKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIE</u> <u>KTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE</u> <u>WESNGQPENNYKTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV</u> <u>MHEALHNHYTQKSLSLSPG</u>
111	3C3 (VH + G2(C219S)) or 3C3-IgG2-C219S	<u>QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWTWIRQPPGKGLEW</u> <u>IGKINHSGNTNYPNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYY</u> <u>CARLGAFDAFDIWGQGTMTVTVSSASTKGPSVFPLAPCSRSTSESTAA</u> <u>LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV</u> <u>PSSNFGTQTYTCNVDPKPSNTKVDKTVRKSCVECPPCPAPPVAGPS</u> <u>VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHN</u> <u>AKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIE</u> <u>KTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE</u> <u>WESNGQPENNYKTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV</u> <u>MHEALHNHYTQKSLSLSPG</u>
112	3C3 (VH + G2.g1) or 3C3-IgG2-IgG1	<u>QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWTWIRQPPGKGLEW</u> <u>IGKINHSGNTNYPNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYY</u> <u>CARLGAFDAFDIWGQGTMTVTVSSASTKGPSVFPLAPCSRSTSESTAA</u> <u>LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV</u> <u>PSSNFGTQTYTCNVDPKPSNTKVDKTVRKCCVECPPCPAPELLGGP</u> <u>SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH</u> <u>NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI</u>

		<u>EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV</u> <u>EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS</u> <u>VMHEALHNHYTQKSLSLSPG</u>
113	3C3 (VH + G2.g1.1) or 3C3-IgG2-IgG1.1	<u>QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWTWIRQPPGKGLEW</u> <u>IGKINHSGNTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYY</u> <u>CARLGAFDAFDIWGQGTMTVTVSSASTKGPSVFPLAPCSRSTSESTAA</u> <u>LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV</u> <u>PSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS</u> <u>VFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN</u> <u>AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPSSIE</u> <u>KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE</u> <u>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV</u> <u>MHEALHNHYTQKSLSLSPG</u>
114	3C3 (VH + G2(C219S).g1) or 3C3-IgG2-C219S-IgG1	<u>QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWTWIRQPPGKGLEW</u> <u>IGKINHSGNTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYY</u> <u>CARLGAFDAFDIWGQGTMTVTVSSASTKGPSVFPLAPCSRSTSESTAA</u> <u>LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV</u> <u>PSSNFGTQTYTCNVDHKPSNTKVDKTVERKSCVECPPCPAPELLGGP</u> <u>SVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH</u> <u>NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI</u> <u>EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV</u> <u>EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS</u> <u>VMHEALHNHYTQKSLSLSPG</u>
115	3C3 (VH + G2(C219S).g1.1) or 3C3-IgG2-C219S-IgG1.1	<u>QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWTWIRQPPGKGLEW</u> <u>IGKINHSGNTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYY</u> <u>CARLGAFDAFDIWGQGTMTVTVSSASTKGPSVFPLAPCSRSTSESTAA</u> <u>LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV</u> <u>PSSNFGTQTYTCNVDHKPSNTKVDKTVERKSCVECPPCPAPPVAGPS</u> <u>VFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN</u> <u>AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPSSIE</u> <u>KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE</u> <u>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV</u> <u>MHEALHNHYTQKSLSLSPG</u>
116	2G6 (VH)	<u>QVQLVESGGGVVQPGGSLRLS</u> <u>CAASGFILSDYGMHWVRQAPGKGLEW</u> <u>VTVIWYDGSNKFYVDSVKGRFTISRDN</u> <u>SKNTLYLQMNSLRVEDTAVY</u> <u>YCARGGRLATGHFYVMDVWGQGT</u> <u>TVTVSS</u>
117	2G6 (VL)	<u>DIQMTQSPSSLSASVGDRVTITCRASQGISSW</u> <u>LAWYQQKPEKAPKSL</u> <u>IYAASSLQSGVPSR</u> <u>FGSGSGTDFTLT</u> <u>ISSLPEDFATYYCQQYNSY</u> <u>PYTFGQGTKLEIK</u>
118	2G6 (full length wild-type heavy chain) The constant region is underlined	<u>QVQLVESGGGVVQPGGSLRLS</u> <u>CAASGFILSDYGMHWVRQAPGKGLEW</u> <u>VTVIWYDGSNKFYVDSVKGRFTISRDN</u> <u>SKNTLYLQMNSLRVEDTAVY</u> <u>YCARGGRLATGHFYVMDVWGQGT</u> <u>TVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECP</u> <u>PCPA</u> <u>PPVAGPSVFLFPPKPKDTLMI</u> <u>SRTPEVTCVVVDVSHEDPEVQFNWYV</u> <u>DGVEVHNAKTKPREEQFNSTFRV</u> <u>SVLTVVHQDWLNGKEYKCKVSNK</u> <u>GLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTTP</u> <u>PMLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSV</u> <u>MHEALHNHYTQKSLSLSPGK</u>
119	2G6 (full length wild-type light chain)	<u>DIQMTQSPSSLSASVGDRVTITCRASQGISSW</u> <u>LAWYQQKPEKAPKSL</u> <u>IYAASSLQSGVPSR</u> <u>FGSGSGTDFTLT</u> <u>ISSLPEDFATYYCQQYNSY</u> <u>PYTFGQGTKLEIK</u> <u>RTVAAPSVFIFPPSDEQLKSGTASV</u> <u>VCLLNNFYP</u> <u>REAKVQWKVDNALQSGNSQESVTEQDSK</u> <u>DSTYLSSTLTLSKADYEK</u>

	The constant region is underlined	<u>HKVYACEVTHQGLSSPVTKSFNRGEC</u>
120	2G6.IgG1 (VH + IgG1)	QVQLVESGGGVVQPGGSLRLSCAASGFI ¹ LSDYGMHWVRQAPGKGLEW VTVIWYDGSNKFYVDSVKGRFTISRDN ² SKNTLYLQMN ³ SLRVEDTAVY YCARGGRLATGHFY ⁴ YVMDVWGQGT ⁵ TVTVSSASTKGPSVFPLAPSSK ⁶ S TSGGTAALGCLVKDYFPEPVT ⁷ SVWNSGALTSGVHTFPAVLQSSGLY ⁸ S LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK ⁹ RVEPKSCDKTHTCPP CPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF ¹⁰ NWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQD ¹¹ WLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYK ¹² TPPVLDSDGSFFLYSKLTVDKSRW QQGNV ¹³ FSCSVMHEALHNHYTQKSLSLSPG
121	2G6.IgG1.1 (VH + IgG1.1)	QVQLVESGGGVVQPGGSLRLSCAASGFI ¹ LSDYGMHWVRQAPGKGLEW VTVIWYDGSNKFYVDSVKGRFTISRDN ² SKNTLYLQMN ³ SLRVEDTAVY YCARGGRLATGHFY ⁴ YVMDVWGQGT ⁵ TVTVSSASTKGPSVFPLAPSSK ⁶ S TSGGTAALGCLVKDYFPEPVT ⁷ SVWNSGALTSGVHTFPAVLQSSGLY ⁸ S LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK ⁹ RVEPKSCDKTHTCPP CPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF ¹⁰ NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD ¹¹ WLNGKEYKCK VSNKALPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYK ¹² TPPVLDSDGSFFLYSKLTVDKSR WQQGNV ¹³ FSCSVMHEALHNHYTQKSLSLSPG
122	2G6.IgG1 (VL + CL)	DIQMTQSPSSLSASVGDRVTITCRASQGIISSWLAWYQQKPEKAPKSL IYAASSLQSGVPSRFSGSGSGTDFTLT ¹ TISSLQPEDFATYYCQQYNSY PYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY ² P REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSLTTLTKADY ³ EK HKVYACEVTHQGLSSPVTKSFNRGEC
123	2G6 VH CDR1	DYGMH
124	2G6 VH CDR2	VIWYDGSNKFYVDSVKG
125	2G6 VH CDR3	GGRLATGHFY ¹ YVMDV
126	2G6 VL CDR1	RASQGISSWLA
127	2G6 VL CDR2	AASSLQS
128	2G6 VL CDR3	QQYNSYPYT
129	2G6 (VH + G2) or 2G6-IgG2	QVQLVESGGGVVQPGGSLRLSCAASGFI ¹ LSDYGMHWVRQAPGKGLEW VTVIWYDGSNKFYVDSVKGRFTISRDN ² SKNTLYLQMN ³ SLRVEDTAVY YCARGGRLATGHFY ⁴ YVMDVWGQGT ⁵ TVTVSSASTKGPSVFPLAPCSRS <u>TSESTAALGCLVKDYFPEPVT</u> ⁶ <u>SVWNSGALTSGVHTFPAVLQSSGLY</u> ⁷ <u>S</u> <u>LSSVVTVPSSNFGTQTYTCNV</u> ⁸ <u>DHKPSNTKVDKTVERKCCVECP</u> ⁹ <u>PCPA</u> <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV</u> ¹⁰ <u>DGVEVHNAKTKPREEQFNSTFRVSVLTVVHQD</u> ¹¹ <u>WLNGKEYKCKVSNK</u> <u>GLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> ¹² <u>PSDIAVEWESNGQPENNYK</u> ¹³ <u>TPPMLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
130	2G6 (VH + G2(C219S)) or 2G6-IgG2-C219S	QVQLVESGGGVVQPGGSLRLSCAASGFI ¹ LSDYGMHWVRQAPGKGLEW VTVIWYDGSNKFYVDSVKGRFTISRDN ² SKNTLYLQMN ³ SLRVEDTAVY YCARGGRLATGHFY ⁴ YVMDVWGQGT ⁵ TVTVSSASTKGPSVFPLAPCSRS <u>TSESTAALGCLVKDYFPEPVT</u> ⁶ <u>SVWNSGALTSGVHTFPAVLQSSGLY</u> ⁷ <u>S</u>

		<u>LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKSCVECPPCPA</u> <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV</u> <u>DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNK</u> <u>GLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
131	2G6 (VH + G2.g1) or 2G6-IgG2-IgG1	<u>QVQLVESGGGVVQPGGSLRLSCAASGFI</u> <u>LSDYGMHWVRQAPGKGLEW</u> <u>VTVIWYDGSNKFYVDSVKGRFTISRDN</u> <u>SKNTLYLQMNSLRVEDTAVY</u> <u>YCARGGRLATGHFYVMDVWGQGT</u> <u>TVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVT</u> <u>SWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPA</u> <u>PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY</u> <u>VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN</u> <u>KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF</u> <u>YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ</u> <u>GNVFSCSVMHEALHNHYTQKSLSLSPG</u>
132	2G6 (VH + G2.g1.1) or 2G6-IgG2-IgG1.1	<u>QVQLVESGGGVVQPGGSLRLSCAASGFI</u> <u>LSDYGMHWVRQAPGKGLEW</u> <u>VTVIWYDGSNKFYVDSVKGRFTISRDN</u> <u>SKNTLYLQMNSLRVEDTAVY</u> <u>YCARGGRLATGHFYVMDVWGQGT</u> <u>TVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVT</u> <u>SWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPA</u> <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV</u> <u>DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK</u> <u>ALPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
133	2G6 (VH + G2(C219S).g1) or 2G6-IgG2-C219S-IgG1	<u>QVQLVESGGGVVQPGGSLRLSCAASGFI</u> <u>LSDYGMHWVRQAPGKGLEW</u> <u>VTVIWYDGSNKFYVDSVKGRFTISRDN</u> <u>SKNTLYLQMNSLRVEDTAVY</u> <u>YCARGGRLATGHFYVMDVWGQGT</u> <u>TVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVT</u> <u>SWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKSCVECPPCPA</u> <u>PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY</u> <u>VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN</u> <u>KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF</u> <u>YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ</u> <u>GNVFSCSVMHEALHNHYTQKSLSLSPG</u>
134	2G6 (VH + G2(C219S).g1.1) or 2G6-IgG2-C219S-IgG1.1	<u>QVQLVESGGGVVQPGGSLRLSCAASGFI</u> <u>LSDYGMHWVRQAPGKGLEW</u> <u>VTVIWYDGSNKFYVDSVKGRFTISRDN</u> <u>SKNTLYLQMNSLRVEDTAVY</u> <u>YCARGGRLATGHFYVMDVWGQGT</u> <u>TVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVT</u> <u>SWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKSCVECPPCPA</u> <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV</u> <u>DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK</u> <u>ALPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
135	8A6 (VH)	<u>QVQLVESGGGVVQPG</u> <u>RSRLSCTASGFTFSSYGMQWVRQAPGKGLEW</u> <u>VAVIWE</u> <u>GSNKYYADSVKGRFTISRENSKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGLMVRGLFY</u> <u>YGMDVWGQGT</u> <u>TVTVSS</u>
136	8A6 (VL)	<u>AIQLTQSPSSLSASV</u> <u>GDRVTITCRASQGISSALAWYQQKPGKAPKFL</u> <u>IYDASSLESGVPSR</u> <u>FSGSGSGTDFTLT</u> <u>ISSLPEDFATYYCQQFN</u> <u>SY</u> <u>PYTFGQG</u> <u>TKLEIK</u>

137	8A6 (full length wild-type heavy chain) The constant region is underlined	<u>QVQLVESGGGVVQPGRSLRLSCTASGFTFSSYGMQWVRQAPGKGLEW</u> <u>VAVIWYEGSNKYYADSVKGRFTISRENSKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGLMVRGLFYYGMDVWGQGTITVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPA</u> <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV</u> <u>DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNK</u> <u>GLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPGK</u>
138	8A6 (full length wild-type light chain) The constant region is underlined	<u>AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKFL</u> <u>IYDASSLESGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQFN</u> <u>SYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP</u> <u>REAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADY</u> <u>EYEHKVYACEVTHQGLSSPVTKSFNRGEC</u>
139	8A6.IgG1 (VH + IgG1)	QVQLVESGGGVVQPGRSLRLSCTASGFTFSSYGMQWVRQAPGKGLEW VAVIWYEGSNKYYADSVKGRFTISRENSKNTLYLQMNSLRAEDTAVY YCARGGLMVRGLFYYGMDVWGQGTITVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG
140	8A6.IgG1.1 (VH + IgG1.1)	QVQLVESGGGVVQPGRSLRLSCTASGFTFSSYGMQWVRQAPGKGLEW VAVIWYEGSNKYYADSVKGRFTISRENSKNTLYLQMNSLRAEDTAVY YCARGGLMVRGLFYYGMDVWGQGTITVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG
141	8A6.IgG1 (VL + CL)	AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKFL IYDASSLESGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQFN SYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADY EYEHKVYACEVTHQGLSSPVTKSFNRGEC
142	8A6 VH CDR1	SYGMQ
143	8A6 VH CDR2	VIWYEGSNKYYADSVKG
144	8A6 VH CDR3	GGLMVRGLFYYGMDV
145	8A6 VL CDR1	RASQGISSALA
146	8A6 VL CDR2	DASSLES
147	8A6 VL CDR3	QQFN SYPYT

148	8A6 (VH + G2(C219S)) or 8A6-IgG2-C219S	<u>QVQLVESGGGVVQPGRSLRLSCTASGFTFSSYGMQWVRQAPGKGLEW</u> <u>VAVIWYEGSNKYYADSVKGRFTISRENSKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGLMVRGLFYYGMDVWGQGTITVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKSCVECPPCPA</u> <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV</u> <u>DGVEVHNAKTKPREEQFNSTFRVVSVLTIVVHQDWLNGKEYKCKVSNK</u> <u>GLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
149	8A6 (VH + G2.g1) or 8A6 -IgG2-IgG1	<u>QVQLVESGGGVVQPGRSLRLSCTASGFTFSSYGMQWVRQAPGKGLEW</u> <u>VAVIWYEGSNKYYADSVKGRFTISRENSKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGLMVRGLFYYGMDVWGQGTITVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPA</u> <u>PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY</u> <u>VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN</u> <u>KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF</u> <u>YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ</u> <u>GNVFSCSVMHEALHNHYTQKSLSLSPG</u>
150	8A6 (VH + G2.g1.1) or 8A6 -IgG2-IgG1.1	<u>QVQLVESGGGVVQPGRSLRLSCTASGFTFSSYGMQWVRQAPGKGLEW</u> <u>VAVIWYEGSNKYYADSVKGRFTISRENSKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGLMVRGLFYYGMDVWGQGTITVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPA</u> <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV</u> <u>DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK</u> <u>ALPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
151	8A6 (VH + G2(C219S).g1) or 8A6-IgG2-C219S-IgG1	<u>QVQLVESGGGVVQPGRSLRLSCTASGFTFSSYGMQWVRQAPGKGLEW</u> <u>VAVIWYEGSNKYYADSVKGRFTISRENSKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGLMVRGLFYYGMDVWGQGTITVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKSCVECPPCPA</u> <u>PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY</u> <u>VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN</u> <u>KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF</u> <u>YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ</u> <u>GNVFSCSVMHEALHNHYTQKSLSLSPG</u>
152	8A6 (VH + G2(C219S).g1.1) or 8A6-IgG2-C219S-IgG1.1	<u>QVQLVESGGGVVQPGRSLRLSCTASGFTFSSYGMQWVRQAPGKGLEW</u> <u>VAVIWYEGSNKYYADSVKGRFTISRENSKNTLYLQMNSLRAEDTAVY</u> <u>YCARGGLMVRGLFYYGMDVWGQGTITVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKSCVECPPCPA</u> <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV</u> <u>DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK</u> <u>ALPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSVMHEALHNHYTQKSLSLSPG</u>
153	9G7 (VH)	<u>EVQLVESGGGLVKPGGSLRLSCAASGFTFSTVWMSWVRQAPGKGLEW</u> <u>VGRIKSKTDGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLHTEDTA</u> <u>VYYCTTGQLIPYSYYYGMDVWGQGTISVTVSS</u>

154	9G7 (VL1)	EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRL LIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGS SPWTFGQGTKVEIK
155	9G7 (VL2)	EIVLTQSPGTLSSLSPGERATLSCRASQSVTSSYLAWYQQKPGQAPRL LIYGASSRATGIPERFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGS SPITFGQGTRLEIK
156	9G7 (full length wild-type heavy chain) The constant region is underlined	EVQLVESGGGLVKPGGSLRLSCAASGFTFSTVWMSWVRQAPGKGLEW VGRIKSKTDGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLHTEDTA VYYCTTGQLIPYSYYYGMDVWGQGTSTVTVSSASTKGPSVFPLAPCSR <u>STSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY</u> <u>SLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVKDKRVESKYGP</u> <u>PCPCAPAEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSD</u> <u>QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD</u> <u>WNLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS</u> <u>REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK</u> <u>TTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV</u> <u>FSCSVMHEALHNHYTQKSLSLSPG</u>
157	9G7 L2 (full length wild-type light chain 2) The constant region is underlined	EIVLTQSPGTLSSLSPGERATLSCRASQSVTSSYLAWYQQKPGQAPRL LIYGASSRATGIPERFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGS SPITFGQGTRLEIK <u>R</u> TVAAPSVFIFPPSDEQLKSGTASVVCLLNMFY <u>P</u> REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE <u>KHKVYACEVTHQGLSSPVTKSFNRGEC</u>
158	9G7.IgG1 (VH + IgG1)	EVQLVESGGGLVKPGGSLRLSCAASGFTFSTVWMSWVRQAPGKGLEW VGRIKSKTDGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLHTEDTA VYYCTTGQLIPYSYYYGMDVWGQGTSTVTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVKDKRVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDNLNGKEYKC KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
159	9G7.IgG1.1 (VH + IgG1.1)	EVQLVESGGGLVKPGGSLRLSCAASGFTFSTVWMSWVRQAPGKGLEW VGRIKSKTDGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLHTEDTA VYYCTTGQLIPYSYYYGMDVWGQGTSTVTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVKDKRVEPKSCDKTHTCP PCPAPEA <u>E</u> GAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDNLNGKEYKC KVSNAKALP <u>S</u> SIIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
160	9G7.IgG1 (VL1 + CL)	EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRL LIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGS SPWTFGQGTKVEIK <u>R</u> TVAAPSVFIFPPSDEQLKSGTASVVCLLNMFY <u>P</u> REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE <u>KHKVYACEVTHQGLSSPVTKSFNRGEC</u>
161	9G7.IgG1.2 (VL2 + CL)	EIVLTQSPGTLSSLSPGERATLSCRASQSVTSSYLAWYQQKPGQAPRL LIYGASSRATGIPERFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGS SPITFGQGTRLEIK <u>R</u> TVAAPSVFIFPPSDEQLKSGTASVVCLLNMFY <u>P</u> REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE <u>KHKVYACEVTHQGLSSPVTKSFNRGEC</u>

162	9G7 VH CDR1	TVWMS
163	9G7 VH CDR2	RIKSKTDGGTTDYAAPVKG
164	9G7 VH CDR3	GQLIPYSYYYGMDV
165	9G7 VL1 CDR1	RASQSVSSSYLA
166	9G7 VL1 CDR2	GASSRAT
167	9G7 VL1 CDR3	QQYGSSPWT
168	9G7 VL2 CDR1	RASQSVTSSYLA
169	9G7 VL2 CDR2	GASSRAT
170	9G7 VL2 CDR3	QQYGSSPIT
171	9G7 (VH + G2) or 9G7-IgG2	<u>EVQLVESGGGLVKPGGSLRLS</u> <u>CAASGFTFSTVWMSWVRQAPGKGLEW</u> <u>VGRIKSKTDGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLH</u> <u>TEDTA</u> <u>VYYCTTGQLIPYSYYYGMDVWGQGT</u> <u>SVTVSSASTKGPSVFPLAPCSR</u> <u>STSESTAALGCLVKDYFPEPVT</u> <u>SVWNSGALTSGVHTFPAVLQSSGLY</u> <u>SLSSVVTVPSSNFGTQTYTCNV</u> <u>DHKPSNTKVDKTVERKCCVECP</u> <u>PCP</u> <u>APPVAGPSVFLFPPKPKDTLMISRTPEV</u> <u>TCVVVDVSHEDPEVQFNWY</u> <u>VDGVEVHNAKTKPREEQFNSTFRVVS</u> <u>VLTVVHQDWLNGKEYKCKVSN</u> <u>KGLPAPIEKTISKTKGQPREPQVY</u> <u>TLPPSREEMTKNQVSLTCLVKGF</u> <u>YPSDIAVEWESNGQPENNYKTTPPMLD</u> <u>SDGSFFLYSKLTVDKSRWQQ</u> <u>GNVFSCSVMHEALHNHYTQKSLSLSPG</u>
172	9G7 (VH + G2(C219S)) or 9G7-IgG2-C219S	<u>EVQLVESGGGLVKPGGSLRLS</u> <u>CAASGFTFSTVWMSWVRQAPGKGLEW</u> <u>VGRIKSKTDGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLH</u> <u>TEDTA</u> <u>VYYCTTGQLIPYSYYYGMDVWGQGT</u> <u>SVTVSSASTKGPSVFPLAPCSR</u> <u>STSESTAALGCLVKDYFPEPVT</u> <u>SVWNSGALTSGVHTFPAVLQSSGLY</u> <u>SLSSVVTVPSSNFGTQTYTCNV</u> <u>DHKPSNTKVDKTVERKSCVECP</u> <u>PCP</u> <u>APPVAGPSVFLFPPKPKDTLMISRTPEV</u> <u>TCVVVDVSHEDPEVQFNWY</u> <u>VDGVEVHNAKTKPREEQFNSTFRVVS</u> <u>VLTVVHQDWLNGKEYKCKVSN</u> <u>KGLPAPIEKTISKTKGQPREPQVY</u> <u>TLPPSREEMTKNQVSLTCLVKGF</u> <u>YPSDIAVEWESNGQPENNYKTTPPMLD</u> <u>SDGSFFLYSKLTVDKSRWQQ</u> <u>GNVFSCSVMHEALHNHYTQKSLSLSPG</u>
173	9G7 (VH + G2.g1) or 9G7-IgG2-IgG1	<u>EVQLVESGGGLVKPGGSLRLS</u> <u>CAASGFTFSTVWMSWVRQAPGKGLEW</u> <u>VGRIKSKTDGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLH</u> <u>TEDTA</u> <u>VYYCTTGQLIPYSYYYGMDVWGQGT</u> <u>SVTVSSASTKGPSVFPLAPCSR</u> <u>STSESTAALGCLVKDYFPEPVT</u> <u>SVWNSGALTSGVHTFPAVLQSSGLY</u> <u>SLSSVVTVPSSNFGTQTYTCNV</u> <u>DHKPSNTKVDKTVERKCCVECP</u> <u>PCP</u> <u>APELLGGPSVFLFPPKPKDTLMISRTPEV</u> <u>TCVVVDVSHEDPEVKFNW</u> <u>YVDGVEVHNAKTKPREEQYNSTYRVVS</u> <u>VLTVLHQDWLNGKEYKCKVS</u> <u>NKALPAPIEKTISKAKGQPREPQVY</u> <u>TLPPSREEMTKNQVSLTCLVKG</u> <u>FYP</u> <u>SDIAVEWESNGQPENNYKTTPVL</u> <u>SDGSFFLYSKLTVDKSRWQ</u> <u>QGNVFSCSVMHEALHNHYTQKSLSLSPG</u>
174	9G7 (VH + G2.g1.1) or 9G7-IgG2-IgG1.1	<u>EVQLVESGGGLVKPGGSLRLS</u> <u>CAASGFTFSTVWMSWVRQAPGKGLEW</u> <u>VGRIKSKTDGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLH</u> <u>TEDTA</u> <u>VYYCTTGQLIPYSYYYGMDVWGQGT</u> <u>SVTVSSASTKGPSVFPLAPCSR</u> <u>STSESTAALGCLVKDYFPEPVT</u> <u>SVWNSGALTSGVHTFPAVLQSSGLY</u> <u>SLSSVVTVPSSNFGTQTYTCNV</u> <u>DHKPSNTKVDKTVERKCCVECP</u> <u>PCP</u> <u>APPVAGPSVFLFPPKPKDTLMISRTPEV</u> <u>TCVVVDVSHEDPEVKFNWY</u> <u>VDGVEVHNAKTKPREEQYNSTYRVVS</u> <u>VLTVLHQDWLNGKEYKCKVSN</u> <u>KALPSSI</u> <u>EKTISKAKGQPREPQVY</u> <u>TLPPSREEMTKNQVSLTCLVKGF</u>

		<u>YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ</u> <u>GNVFSCSVMHEALHNHYTQKSLSLSPG</u>
175	9G7 (VH + G2(C219S).g1) or 9G7-IgG2-C219S-IgG1	<u>EVQLVESGGGLVQPGGSLRLS</u> <u>CAASGFTFSTVWMSWVRQAPGKGLEW</u> <u>VGRIKSKTDGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLHTE</u> <u>DTA</u> <u>VYYCTTGQLIPYSYYYGMDVWGQGTSTVTVSSASTKGPSVFPLAPCSR</u> <u>STSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY</u> <u>SLSSVVTVPSSNFGTQTYTCNV</u> <u>DKPSNTKVDK</u> <u>TV</u> <u>ERKSCVECP</u> <u>PCP</u> <u>APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW</u> <u>YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS</u> <u>NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG</u> <u>FYP</u> <u>SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ</u> <u>QGNVFSCSVMHEALHNHYTQKSLSLSPG</u>
176	9G7 (VH + G2(C219S).g1.1) or 9G7-IgG2-C219S-IgG1.1	<u>EVQLVESGGGLVQPGGSLRLS</u> <u>CAASGFTFSTVWMSWVRQAPGKGLEW</u> <u>VGRIKSKTDGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLHTE</u> <u>DTA</u> <u>VYYCTTGQLIPYSYYYGMDVWGQGTSTVTVSSASTKGPSVFPLAPCSR</u> <u>STSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY</u> <u>SLSSVVTVPSSNFGTQTYTCNV</u> <u>DKPSNTKVDK</u> <u>TV</u> <u>ERKSCVECP</u> <u>PCP</u> <u>APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY</u> <u>VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN</u> <u>KALPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF</u> <u>YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ</u> <u>GNVFSCSVMHEALHNHYTQKSLSLSPG</u>
177	9G7 L1 (full length wild-type light chain 1) The constant region is underlined	<u>EIVLTQSPGTL</u> <u>SLSPGERATL</u> <u>SCRASQSVSSSYLAWYQQKPGQAPRL</u> <u>LIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGS</u> <u>SPWTFGQGTKVEIKRTVAAPS</u> <u>VFIFPPSDEQLKSGTASV</u> <u>VCLLN</u> <u>NFY</u> <u>PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLS</u> <u>SSTLTLSKADYE</u> <u>KHKVYACEVTHQGLSSPVTKSFNRGEC</u>
178	14E3 (VH)	<u>QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEW</u> <u>IGEINHSGNTYYNPSL</u> <u>KSRVTISVDTSKNQ</u> <u>LSLKLSSVTAADTAVYY</u> <u>CARFGSNDAFDIWGQGM</u> <u>TVSS</u>
179	14E3 (VL)	<u>DIQMTQSPSSLSASV</u> <u>GDRVTITCRASQGISSWLAWYQQKPEKAPKSL</u> <u>IYAASSLQSGVPSR</u> <u>FSGSGSGTDFTLTIS</u> <u>SLQPEDFATYYCQQYNSY</u> <u>PPTFGQGTKVEIK</u>
180	14E3 (full length wild-type heavy chain) The constant region is underlined	<u>QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEW</u> <u>IGEINHSGNTYYNPSL</u> <u>KSRVTISVDTSKNQ</u> <u>LSLKLSSVTAADTAVYY</u> <u>CARFGSNDAFDIWGQGM</u> <u>TVSSASTKGPSVFPLAPSSKSTSGGTAA</u> <u>LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV</u> <u>PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELL</u> <u>GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV</u> <u>EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP</u> <u>APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP</u> <u>SD</u> <u>I</u> <u>AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF</u> <u>SCSVMHEALHNHYTQKSLSLSPGK</u>
181	14E3 (full length wild-type light chain) The constant region is underlined	<u>DIQMTQSPSSLSASV</u> <u>GDRVTITCRASQGISSWLAWYQQKPEKAPKSL</u> <u>IYAASSLQSGVPSR</u> <u>FSGSGSGTDFTLTIS</u> <u>SLQPEDFATYYCQQYNSY</u> <u>PPTFGQGTKVEIKRTVAAPS</u> <u>VFIFPPSDEQLKSGTASV</u> <u>VCLLN</u> <u>NFY</u> <u>P</u> <u>REAKVQWKVDNALQSGNSQESVTEQDSKDSTYLS</u> <u>SSTLTLSKADYEK</u> <u>HKVYACEVTHQGLSSPVTKSFNRGEC</u>
182	14E3.IgG1 (VH + IgG1)	<u>QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEW</u> <u>IGEINHSGNTYYNPSL</u> <u>KSRVTISVDTSKNQ</u> <u>LSLKLSSVTAADTAVYY</u>

		CARFGSNDAFDIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELL GGPSVFLF PPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFCSCVMHEALHNHYTQKSLSLSPG
183	14E3.IgG1.1 (VH + IgG1.1)	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEW IGEINHSGNTYYNPSLKSRTISVDTSKNQLSLKLSSVTAADTAVYY CARFGSNDAFDIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAE GAPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP SSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPG
184	14E3.IgG1 (VL + CL)	DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSL IYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSY PPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYF REAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
185	14E3 VH CDR1	GYYS
186	14E3 VH CDR2	EINHSGNTYYNPSLKS
187	14E3 VH CDR3	FGSNDAFDI
188	14E3 VL CDR1	RASQGISSWLA
189	14E3 VL CDR2	AASSLQS
190	14E3 VL CDR3	QQYNSYPPT
191	14E3 (VH + G2) or 14E3-IgG2	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEW IGEINHSGNTYYNPSLKSRTISVDTSKNQLSLKLSSVTAADTAVYY CARFGSNDAFDIWGQGTMVTVSSASTKGPSVFPLAPCSRSTSESTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSNFGTQTYTCNVDHKPSNTKVDKTKVERKCCVECPPCPAPPVAGPS VFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIE KTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFCSCV MHEALHNHYTQKSLSLSPG
192	14E3 (VH + G2(C219S)) or 14E3-IgG2-C219S	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEW IGEINHSGNTYYNPSLKSRTISVDTSKNQLSLKLSSVTAADTAVYY CARFGSNDAFDIWGQGTMVTVSSASTKGPSVFPLAPCSRSTSESTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSNFGTQTYTCNVDHKPSNTKVDKTKVERKSCVECPPCPAPPVAGPS VFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIE KTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFCSCV

		<u>MHEALHNHYTQKSLSLSPG</u>
193	14E3 (VH + G2.g1) or 14E3-IgG2-IgG1	<u>QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEW</u> <u>IGEINHSGNTYYNPSLKSRVTISVDTSKNQLSLKLSSVTAADTAVYY</u> <u>CARFGSNDAFDIWGQGTMTVTSSASTKGPSVFPLAPCSRSTSESTAA</u> <u>LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV</u> <u>PSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPELLGGP</u> <u>SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH</u> <u>NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI</u> <u>EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV</u> <u>EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCS</u> <u>VMHEALHNHYTQKSLSLSPG</u>
194	14E3 (VH + G2.g1.1) or 14E3-IgG2-IgG1.1	<u>QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEW</u> <u>IGEINHSGNTYYNPSLKSRVTISVDTSKNQLSLKLSSVTAADTAVYY</u> <u>CARFGSNDAFDIWGQGTMTVTSSASTKGPSVFPLAPCSRSTSESTAA</u> <u>LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV</u> <u>PSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS</u> <u>VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN</u> <u>AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPSSIE</u> <u>KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE</u> <u>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSV</u> <u>MHEALHNHYTQKSLSLSPG</u>
195	14E3 (VH + G2(C219S).g1) or 14E3-IgG2-C219S-IgG1	<u>QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEW</u> <u>IGEINHSGNTYYNPSLKSRVTISVDTSKNQLSLKLSSVTAADTAVYY</u> <u>CARFGSNDAFDIWGQGTMTVTSSASTKGPSVFPLAPCSRSTSESTAA</u> <u>LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV</u> <u>PSSNFGTQTYTCNVDHKPSNTKVDKTVERKSCVECPPCPAPELLGGP</u> <u>SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH</u> <u>NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI</u> <u>EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV</u> <u>EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCS</u> <u>VMHEALHNHYTQKSLSLSPG</u>
196	14E3 (VH + G2(C219S).g1.1) or 14E3-IgG2-C219S-IgG1.1	<u>QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEW</u> <u>IGEINHSGNTYYNPSLKSRVTISVDTSKNQLSLKLSSVTAADTAVYY</u> <u>CARFGSNDAFDIWGQGTMTVTSSASTKGPSVFPLAPCSRSTSESTAA</u> <u>LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV</u> <u>PSSNFGTQTYTCNVDHKPSNTKVDKTVERKSCVECPPCPAPPVAGPS</u> <u>VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN</u> <u>AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPSSIE</u> <u>KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE</u> <u>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSV</u> <u>MHEALHNHYTQKSLSLSPG</u>
197	19H8 (VH)	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMHWVRQAPGKGLEW</u> <u>MAVIWYGGSNKFYADSVKGRFTISRDN SKNSLSLQMN SLRAEDTAVY</u> <u>YCARGGAMVRGVYYYGMDVWGQGTTVTVSS</u>
198	19H8 (VL1)	<u>AIQLTQSPSSLSASVGDRVITITCRASQGISSALAWYQQKPGKAPKFL</u> <u>IYDASSLESGVPSRFSGSGSGTDFTLTITSS LQPEDFATYYCQQFN SY</u> <u>PQTFGGGTKVEIK</u>
199	19H8 (VL2)	<u>EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRL</u> <u>IYDASN RATGIPARFSGSGSGTDFTLTITSSLEPEDFAVYYCQQRSNW</u> <u>PLTFGGGTKVEIK</u>
200	19H8 (full length wild-type heavy chain)	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMHWVRQAPGKGLEW</u> <u>MAVIWYGGSNKFYADSVKGRFTISRDN SKNSLSLQMN SLRAEDTAVY</u> <u>YCARGGAMVRGVYYYGMDVWGQGTTVTVSS</u> <u>ASTKGPSVFPLAPCSRS</u>

	The constant region is underlined	<u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECP</u> <u>PCPA</u> <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV</u> <u>DGVEVHNAKTKPREEQFNSTFRVVS</u> <u>VLTVVHQDWLNGKEYKCKVSNK</u> <u>GLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTTTPMLDS</u> <u>DG</u> <u>SFFLYSKLTVDKSRWQQG</u> <u>NVFS</u> <u>CSVMHEALHNHYTQKSLSLSPGK</u>
201	19H8 L1 (full length wild-type light chain 1) The constant region is underlined	<u>AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKFL</u> <u>IYDASSLESGVPSRFSGSGSGTDFTLT</u> <u>TISSLPEDFATYYCQQFNSY</u> <u>PQTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY</u> <u>P</u> <u>REAKVQWKVDNALQSGNSQESVTEQDSKDSTYLS</u> <u>SSTLTLSKADYEK</u> <u>HKVYACEVTHQGLSSPVTKSFNRGEC</u>
202	19H8 L2 (full length wild-type light chain 2)	<u>EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRL</u> <u>L</u> <u>IYDASN</u> <u>RATGIPARFSGSGSGTDFTLT</u> <u>TISSLEPEDFAVYYCQQRSNW</u> <u>PLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY</u> <u>P</u> <u>REAKVQWKVDNALQSGNSQESVTEQDSKDSTYLS</u> <u>SSTLTLSKADYEK</u> <u>HKVYACEVTHQGLSSPVTKSFNRGEC</u>
203	19H8.IgG1 (VH + IgG1)	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMHWVRQAPGKGLEW</u> <u>MAVIWYGGSNKFYADSVKGRFTISRDN</u> <u>SKNSLSLQMN</u> <u>SLRAEDTAVY</u> <u>YCARGGAMVRGVYYYGMDVWGQGT</u> <u>TVTVSSASTKGPSVFPLAPSSKS</u> <u>TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP</u> <u>CPAPELLGGPSVFLF</u> <u>PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE</u> <u>VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV</u> <u>SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK</u> <u>GFYPSDIAVEWESNGQPENNYKTT</u> <u>PPVLDSDGSFFLYSKLTVDKSRW</u> <u>QQGNVFS</u> <u>CSVMHEALHNHYTQKSLSLSPG</u>
204	19H8.IgG1.1 (VH + IgG1.1)	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMHWVRQAPGKGLEW</u> <u>MAVIWYGGSNKFYADSVKGRFTISRDN</u> <u>SKNSLSLQMN</u> <u>SLRAEDTAVY</u> <u>YCARGGAMVRGVYYYGMDVWGQGT</u> <u>TVTVSSASTKGPSVFPLAPSSKS</u> <u>TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP</u> <u>CPAPEA</u> <u>EGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF</u> <u>NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK</u> <u>VSNKALP</u> <u>SSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV</u> <u>KGFYPSDIAVEWESNGQPENNYKTT</u> <u>PPVLDSDGSFFLYSKLTVDKSR</u> <u>WQQGNVFS</u> <u>CSVMHEALHNHYTQKSLSLSPG</u>
205	19H8.IgG1 (VL1 + CL)	<u>AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKFL</u> <u>IYDASSLESGVPSRFSGSGSGTDFTLT</u> <u>TISSLPEDFATYYCQQFNSY</u> <u>PQTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY</u> <u>P</u> <u>REAKVQWKVDNALQSGNSQESVTEQDSKDSTYLS</u> <u>SSTLTLSKADYEK</u> <u>HKVYACEVTHQGLSSPVTKSFNRGEC</u>
206	19H8.IgG1.2 (VL2 + CL)	<u>EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRL</u> <u>L</u> <u>IYDASN</u> <u>RATGIPARFSGSGSGTDFTLT</u> <u>TISSLEPEDFAVYYCQQRSNW</u> <u>PLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY</u> <u>P</u> <u>REAKVQWKVDNALQSGNSQESVTEQDSKDSTYLS</u> <u>SSTLTLSKADYEK</u> <u>HKVYACEVTHQGLSSPVTKSFNRGEC</u>
207	19H8 VH CDR1	NYGMH
208	19H8 VH CDR2	VIWYGGSNKFYADSVKG

209	19H8 VH CDR3	GGAMVRGVYYYGMDV
210	19H8 VL1 CDR1	RASQGISSALA
211	19H8 VL1 CDR2	DASSLES
212	19H8 VL1 CDR3	QQFNSYPQT
213	19H8 VL2 CDR1	RASQSVSSYLA
214	19H8 VL2 CDR2	DASNRAT
215	19H8 VL2 CDR3	QQRSNWPLT
216	19H8 (VH + G2(C219S)) or 19H8-IgG2-C219S	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMHWVRQAPGKGLEW</u> <u>MAVIWYGGSNKFYADSVKGRFTISRDN SKNSLSLQMN SLRAEDTAVY</u> <u>YCARGGAMVRGVYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKSCVECP PCA</u> <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV</u> <u>DGVEVHNAKTKPREEQFNSTFRVVS VLT TVVHQDWLNGKEYKCKVSNK</u> <u>GLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTT P PMLDSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSV MHEALHNHYTQKSLSLSPG</u>
217	19H8 (VH + G2.g1) or 19H8-IgG2-IgG1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMHWVRQAPGKGLEW</u> <u>MAVIWYGGSNKFYADSVKGRFTISRDN SKNSLSLQMN SLRAEDTAVY</u> <u>YCARGGAMVRGVYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECP PCA</u> <u>PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY</u> <u>VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN</u> <u>KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF</u> <u>YPSDIAVEWESNGQPENNYKTT P PVL DSDGSFFLYSKLTVDKSRWQQG</u> <u>GNVFSCSV MHEALHNHYTQKSLSLSPG</u>
218	19H8 (VH + G2.g1.1) or 19H8-IgG2-IgG1.1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMHWVRQAPGKGLEW</u> <u>MAVIWYGGSNKFYADSVKGRFTISRDN SKNSLSLQMN SLRAEDTAVY</u> <u>YCARGGAMVRGVYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKCCVECP PCA</u> <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV</u> <u>DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK</u> <u>ALPSSI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY</u> <u>PSDIAVEWESNGQPENNYKTT P PVL DSDGSFFLYSKLTVDKSRWQQG</u> <u>NVFSCSV MHEALHNHYTQKSLSLSPG</u>
219	19H8 (VH + G2(C219S).g1) or 19H8-IgG2-C219S-IgG1	<u>QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMHWVRQAPGKGLEW</u> <u>MAVIWYGGSNKFYADSVKGRFTISRDN SKNSLSLQMN SLRAEDTAVY</u> <u>YCARGGAMVRGVYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPCSRS</u> <u>TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS</u> <u>LSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKTVERKSCVECP PCA</u> <u>PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY</u> <u>VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN</u> <u>KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF</u> <u>YPSDIAVEWESNGQPENNYKTT P PVL DSDGSFFLYSKLTVDKSRWQQG</u> <u>GNVFSCSV MHEALHNHYTQKSLSLSPG</u>

220	19H8 (VH + G2(C219S).g1.1) or 19H8-IgG2-C219S-IgG1.1	QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMHWVRQAPGKGLEW MAVIWYGGSNKFYADSVKGRFTISRDN SKNSLSLQMN SLRAEDTAVY YCARGGAMVRGVYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPCSRS TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSNFGTQTYTCNV DHKPSNTKV DKTVERKSCVECP PCA PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPSSIEKTIISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSV MHEALHNHYTQKSLSLSPG
221	6G10.IgG1 (VH + IgG1)	QVQLVESGGDVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPGKGLEW VAVTWYAGSNKFYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVY YCARGGSMVRGLYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSV MHEALHNHYTQKSLSLSPG
222	6G10.IgG1.1 (VH + IgG1.1)	QVQLVESGGDVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPGKGLEW VAVTWYAGSNKFYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVY YCARGGSMVRGLYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPP CPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPSSIEKTIISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSV MHEALHNHYTQKSLSLSPG
223	6G10.IgG1 (VL + CL)	AIQLTQSPSSLSASVGD RVTITCRASQGISSALAWYQQKPGKAPKLL IYDASSLESGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQQFN SY PYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY P REAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
224	6G10 (VH + G2(C219S)) or 6G10-IgG2-C219S	QVQLVESGGDVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPGKGLEW VAVTWYAGSNKFYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVY YCARGGSMVRGLYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPCSRS TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSNFGTQTYTCNV DHKPSNTKV DKTVERKSCVECP PCA PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTFRV VSVLTVVHQDWLNGKEYKCKVSNK GLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTT PMLDSDGSFFLYSKLTVDKSRWQQG NVFSCSV MHEALHNHYTQKSLSLSPG
225	6G10 (VH + G2.g1) or 6G10-IgG2-IgG1	QVQLVESGGDVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPGKGLEW VAVTWYAGSNKFYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVY YCARGGSMVRGLYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPCSRS TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSNFGTQTYTCNV DHKPSNTKV DKTVERKCCVECP PCA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTT PVLDS DGSFFLYSKLTVDKSRWQQ

		GNVFSCSVMHEALHNHYTQKSLSLSPG
226	6G10 (VH + G2.g1.1) or 6G10-IgG2-IgG1.1	QVQLVESGGDVVQPGRSRLRLSCAASGFTFSTYGMHWVRQAPGKGLEW VAVTWYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARGGSMVRGLYYYGMDVWGQGT TVTVSSASTKGPSVFPLAPCSRS TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKT <u>VERKCCVECP</u> PCPA <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV</u> DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPSSIEKTIISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPG
227	6G10 (VH + G2(C219S).g1) or 6G10-IgG2-C219S-IgG1	QVQLVESGGDVVQPGRSRLRLSCAASGFTFSTYGMHWVRQAPGKGLEW VAVTWYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARGGSMVRGLYYYGMDVWGQGT TVTVSSASTKGPSVFPLAPCSRS TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKT <u>VERKSCVECP</u> PCPA <u>PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY</u> VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPG
228	6G10 (VH + G2(C219S).g1.1) or 6G10-IgG2-C219S-IgG1.1	QVQLVESGGDVVQPGRSRLRLSCAASGFTFSTYGMHWVRQAPGKGLEW VAVTWYAGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARGGSMVRGLYYYGMDVWGQGT TVTVSSASTKGPSVFPLAPCSRS TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSNFGTQTYTCNV DHKPSNTKVDKT <u>VERKSCVECP</u> PCPA <u>PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV</u> DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPSSIEKTIISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPG

Table 2 provides the sequences of the mature variable regions and heavy and light chains and where indicated, sequences with signal peptides.

Equivalents:

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments disclosed herein. Such equivalents are intended to be encompassed by the following claims

We claim

1. A method for determining the level of expression of GITR on tumor infiltrating lymphocytes (TILs), e.g., Treg cells, in a tissue (e.g., tumor) sample, comprising
 - (a) contacting a tissue sample from a patient with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen binding portion thereof binds to human GITR,
 - (b) detecting the binding of the antibody to GITR on TILs, e.g., Treg cells, of the tissue sample; and optionally
 - (c) staining the tissue sample, e.g., with markers of TILs, e.g., Treg cells (e.g., CD3, CD4, CD8, CD25, and/or FoxP3 markers), and/or with hematoxylin and eosin, to identify the TILs, e.g., Treg cells, that were identified as GITR positive in step (b).
2. A method for determining the level of expression of GITR on tumor cells and tumor infiltrating lymphocytes (TILs) and/or tumor cells of a tumor, comprising
 - (a) contacting a tumor sample from the patient with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen binding portion thereof binds to human GITR,
 - (b) detecting the binding of the antibody to GITR on TILs and/or tumor cells of the tumor sample; and optionally
 - (c) staining the tumor sample, e.g., with markers of TILs (e.g., CD3, CD4, CD8, CD25, and/or FoxP3 markers) and/or tumor cells, and/or with hematoxylin and eosin, to identify the TILs and tumor cells that were identified as GITR positive in step (b).
3. A method for separately determining the level of expression of GITR tumor infiltrating lymphocytes (TILs) and tumor cells of a tumor, comprising

(a) contacting a tumor sample from a patient with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen binding portion thereof binds to human GITR,

(b) detecting the binding of the antibody to GITR on TILs and/or tumor cells of the tumor sample; and optionally

(c) staining the tumor sample, e.g., with markers of TILs (e.g., CD3, CD4, CD8, CD25, and/or FoxP3 markers) and/or tumor cells, and/or with hematoxylin and eosin, to identify the TILs and tumor cells that were identified as GITR positive in step (b).

4. A method of detecting human glucocorticoid-induced tumor necrosis factor receptor (GITR) in a biological sample comprising contacting the sample with an antibody, or antigen-binding fragment thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, wherein the antibody or antigen-binding fragment thereof, binds to human GITR, and detecting the binding of the antibody to GITR in the sample.

5. The method of any one of claims 1-4, wherein the sample is a human tissue sample.

6. The method of claim 5, wherein the human tissue sample is a tumor tissue.

7. A method of determining whether a tumor of a cancer patient is GITR-positive comprising:

(a) contacting a tumor sample from the patient with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively,

- (b) detecting the binding of the antibody to GITR in the sample, and
 - (c) determining the level of GITR protein expression in the sample.
8. The method of claim 7, wherein the tumor sample is a formalin-fixed paraffin-embedded tumor sample or frozen tumor tissue.
9. The method of claim 7 or 8, wherein step (b) is performed by immunohistochemistry.
10. The method of any one of claims 7-9, wherein the level of GITR protein expression is determined using a scoring system selected from the group consisting of: the H score system, the Allred score system, the AQUA[®] system, or an automated scoring system.
11. A method of identifying a cancer patient likely to respond to an anti-GITR immunotherapy comprising:
- (a) contacting a tumor sample from the patient with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively,
 - (b) detecting the binding of the antibody to GITR in the tumor sample,
 - (c) determining the level of GITR protein expression in the sample, wherein a level of GITR protein above a threshold level indicates that the tumor is GITR-positive tumor and that the patient is likely to or predicted to respond to anti-GITR immunotherapy.
12. The method of claim 11, wherein the tumor sample is a formalin-fixed paraffin-embedded tumor sample or frozen tumor tissue.

13. The method of claim 11 or 12, wherein step (b) is performed by immunohistochemistry.
14. The method of any one of claims 11-13, wherein the level of GITR protein expression is determined using a scoring system selected from the group consisting of: the H score system, the Allred score system, an AQUA[®] system, or an automated scoring system.
15. A method of treating a cancer patient comprising:
- (a) determining whether a tumor of a cancer patient is GITR-positive by (i) contacting a tumor sample from the patient with an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, (ii) detecting the binding of the antibody to GITR in the tumor sample, and (iii) determining the level of GITR protein expression in the sample, wherein a level of GITR protein above a threshold level indicates that the tumor is GITR-positive, and
- (b) if the tumor is determined to be GITR-positive, administering to the patient an agent that modulates GITR signaling.
16. The method of claim 15, wherein step (b) is performed by immunohistochemistry.
17. The method of claim 15 or 16, wherein the agent is an agonistic anti-GITR antibody.
18. A method of monitoring a GITR-positive tumor in a cancer patient comprising:
- (a) detecting GITR protein expression in the GITR-positive tumor at a first time point by using an antibody comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable

region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively,

- (b) determining the level of GITR protein expression at the first time point,
- (c) detecting GITR protein expression in the tumor at a second time point using the same antibody used in step (a),
- (d) determining the level of GITR protein expression in the tumor from the second time point;
- (e) comparing the levels of GITR protein expression determined at the first and second time points, wherein a higher level at the first time point relative to the second time point is indicative of tumor regression, a lower score at the first time point relative to the second time point is indicative of tumor progression, and a relatively unchanged level at the first time point relative to the second time point is indicative of a stable tumor.

19. The method of claim 18, wherein the level of GITR protein expression is determined by immunohistochemistry.

20. The method of claim 18 or 19, wherein the level of GITR protein expression is determined using a scoring system selected from the group consisting of: the H score system, the Allred score system, an AQUA[®] system, or an automated scoring system.

21. A method of monitoring the efficacy of anti-GITR immunotherapy in a patient having a GITR-positive tumor comprising:

- (a) detecting GITR protein expression in the GITR-positive tumor at a first time point before or after initiating anti-GITR immunotherapy by using an antibody comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively,
- (b) determining the level of GITR protein expression in the tumor from the first time point,

(c) detecting GITR protein expression in the GITR-positive tumor at a second time point after initiating anti-GITR immunotherapy using the same antibody from step (a),

(d) determining the level of GITR protein expression in the tumor from the second time point;

(e) comparing the levels of GITR protein expression determined at the first and second time points, wherein a higher level at the first time point relative to the second time point is indicative of effective anti-GITR immunotherapy, a lower score at the first time point relative to the second time point is indicative of ineffective anti-GITR immunotherapy, and an unchanged score at the first time point relative to the second time point is indicative of anti-GITR immunotherapy being stabilizing.

22. The method of claim 21, wherein the level of GITR protein expression is determined by immunohistochemistry.

23. The method of claim 21 or 22, wherein the level of GITR protein expression is determined using a scoring system selected from the group consisting of: the H score system, the Allred score system, an AQUA[®] system, or an automated scoring system.

24. The method of any one of claims 21-23, wherein the anti-GITR immunotherapy comprises administering to the patient a therapeutically effective amount of an anti-GITR antibody, or antigen binding portion thereof.

25. The method of any one of the preceding claims, wherein the antibody comprises heavy and light chain variable regions comprising the amino acid sequences set forth in SEQ ID NOs: 11 and 12, respectively.

26. The method of any one of the preceding claims, wherein the antibody is an IgG1, IgG2, IgG3, or IgG4 antibody.

27. The method of any one of the preceding claims, wherein the antibody binds to soluble human GTR with a K_D of 10 nM or less, as assessed by Biacore.
28. The method of any one of the preceding claims, wherein the antibody is a humanized, human, or chimeric antibody.
29. The method of any one of the preceding claims, wherein the antibody is a chimeric antibody.
30. The method of claim 29, wherein the chimeric antibody has human variable region sequences and non-human constant region sequences.
31. The method of claim 30, wherein the non-human constant region comprises a mouse Fc region.
32. The method of claim 31, wherein the mouse Fc region is a mouse IgG2a Fc region.
33. The method of claim 30, wherein the chimeric antibody comprises heavy and light chain sequences set forth in SEQ ID NOs: 15 and 16, respectively.
34. The methods of any one of the preceding claims, wherein the antibody, or antigen-binding portion thereof, comprises a detectable moiety.
35. The method of claim 34, wherein the label is a radiolabel, fluorescent label, an enzymatic label, biotin, chromophore, or an ECL label.

36. A diagnostic kit comprising an antibody, or antigen-binding portion thereof, comprising heavy chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 5-7, respectively, and light chain variable region CDR1, CDR2, and CDR3 comprising the amino acid sequences set forth in SEQ ID NOs: 8-10, respectively, and instructions for use.

37. The diagnostic kit of claim 36, wherein the antibody, or antigen-binding portion thereof, comprises the heavy and light chain variable region sequences set forth in SEQ ID NOs: 11 and 12, respectively.

38. The diagnostic kit of claim 36 or 37, wherein the antibody comprises the heavy and light chain sequences set forth in SEQ ID NOs: 13 and 14, wherein the heavy chain optionally lacks the C-terminal lysine residue.

39. The diagnostic kit of claim 36 or 37, wherein the antibody comprises the heavy and light chain sequences set forth in SEQ ID NOs: 15 and 16, wherein the heavy chain optionally lacks the C-terminal lysine residue.

J segment: JH6b

Figure 1

J segment: JK2

1	A	I	Q	L	T	Q	S	P	S	S	L	S	A	S	V	G	D
	GCC	ATC	CAG	TTG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC
	CDR1																
52	R	V	T	I	T	C	R	A	S	Q	G	I	S	S	A	L	A
	AGA	GTC	ACC	ATC	ACT	TGC	CGG	GCA	AGT	CAG	GGC	ATT	AGC	AGT	GCT	TTA	GCC
	CDR2																
103	W	Y	Q	Q	K	P	G	K	A	P	K	L	L	I	Y	D	A
	TGG	TAT	CAG	CAG	AAA	CCA	GGG	AAA	GCT	CCT	AAG	CTC	CTG	ATC	TAT	GAT	GCC
	CDR3																
154	S	S	L	E	S	G	V	P	S	R	F	S	G	S	G	S	G
	TCC	AGT	TTG	GAA	AGT	GGG	GTC	CCA	TCA	AGG	TTC	AGC	GGC	AGT	GGA	TCT	GGG
	CDR4																
205	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T
	ACA	GAT	TTC	ACT	CTC	ACC	ATC	AGC	AGC	CTG	CAG	CCT	GAA	GAT	TTT	GCA	ACT
	CDR5																
256	Y	Y	C	Q	Q	F	N	S	Y	P	Y	T	F	G	Q	G	T
	TAT	TAC	TGT	CAA	CAG	TTT	AAT	AGT	TAC	CCG	TAC	ACT	TTT	GGC	CAG	GGG	ACC
	CDR6																
307	K	L	E	I	K												
	AAG	CTG	GAG	ATC	AAA												

Figure 2

Anti-GITR 6G10 VH (hIgG2)

3-33	Q	V	Q	L	V	E	S	G	G	G	V	V	Q	P	G	R	S	L	R	L	S	C	A
INPUT	-	-	-	-	-	-	-	-	-	D	-	-	-	-	-	-	-	-	-	-	-	-	-
3-33	A	S	G	F	T	F	S	S	Y	G	M	H	W	V	R	Q	A	P	G	K	G	L	E
INPUT	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3-33	W	V	A	V	I	W	Y	D	G	S	N	K	Y	Y	A	D	S	V	K	G	R	F	T
INPUT	-	-	-	-	T	-	-	A	-	-	-	-	F	-	-	-	-	-	-	-	-	-	-
3-33	I	S	R	D	N	S	K	N	T	L	Y	L	Q	M	N	S	L	R	A	E	D	T	A
INPUT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3-33	V	Y	Y	C	A	R																	
3-10										M	V	R	G										
JH6															Y	Y	Y	G	M	D	V	W	G
INPUT	-	-	-	-	-	-	G	G	S	-	-	-	-	L	-	-	-	-	-	-	-	-	-
JH6	Q	G	T	T	V	T	V	S	S														
INPUT	-	-	-	-	-	-	-	-	-														

Figure 3

Anti-GITR 6G10 VK1 (hKappa)

L18	A	I	Q	L	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C
INPUT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<u>CDR1</u>																						
L18	R	A	S	Q	G	I	S	S	A	L	A	W	Y	Q	Q	K	P	G	K	A	P	K	L
INPUT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<u>CDR2</u>																						
L18	L	I	Y	D	A	S	S	L	E	S	G	V	P	S	R	F	S	G	S	G	S	G	T
INPUT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<u>CDR3</u>																						
L18	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	Q	F	N
INPUT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<u>CDR4</u>																						
L18	N	Y																					
JK2				Y	T	F	G	Q	G	T	K	L	E	I	K								
INPUT	S	-	P	-	-	-	-	-	-	-	-	-	-	-	-								

Figure 4

GITR 6G10 VH
~~~~~  
Q V Q L V E S G G D V V Q P G  
1 CAG GTG CAG CTG GTG GAG TCT GGG GGA GAC GTG GTC CAG CCT GGG  
GITR 6G10 VH  
~~~~~  
R S L R L S C A A S G F T F S
46 AGG TCC CTG AGA CTC TCC TGT GCA GCG TCT GGA TTC ACC TTC AGT
GITR 6G10 VH
~~~~~  
T Y G M H W V R Q A P G K G L  
91 ACC TAT GGC ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG  
GITR 6G10 VH  
~~~~~  
E W V A V T W Y A G S N K F Y
136 GAG TGG GTG GCA GTT ACA TGG TAT GCT GGA AGT AAT AAA TTT TAT
GITR 6G10 VH
~~~~~  
A D S V K G R F T I S R D N S  
181 GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC  
GITR 6G10 VH  
~~~~~  
K N T L Y L Q M N S L R A E D
226 AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC
GITR 6G10 VH
~~~~~  
T A V Y Y C A R G G S M V R G  
271 ACG GCT GTG TAT TAC TGT GCG AGA GGA GGT AGT ATG GTT CGG GGA  
GITR 6G10 VH  
~~~~~  
L Y Y Y G M D V W G Q G T T V
316 CTT TAT TAT TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG GTC
mIgG2a constant
~~~~~  
GITR 6G10 VH  
~~~~~  
T V S S A K T T A P S V Y P L
361 ACC GTC TCC TCA GCC AAA ACA ACA GCC CCA TCG GTC TAT CCG CTA
mIgG2a constant
~~~~~  
A P V C G D T T G S S V T L G  
406 GCC CCT GTG TGT GGA GAT ACA ACT GGC TCC TCG GTG ACT CTA GGA  
mIgG2a constant  
~~~~~  
C L V K G Y F P E P V T L T W
451 TGC CTG GTC AAG GGT TAT TTC CCT GAG CCA GTG ACC TTG ACC TGG
mIgG2a constant
~~~~~  
N S G S L S S G V H T F P A V  
496 AAC TCT GGA TCC CTG TCC AGT GGT GTG CAC ACC TTC CCA GCT GTC  
mIgG2a constant  
~~~~~  
L Q S D L Y T L S S S V T V T
541 CTG CAG TCT GAC CTC TAC ACC CTC AGC AGC TCA GTG ACT GTA ACC
mIgG2a constant
~~~~~  
S S T W P S Q S I T C N V A H  
586 TCG AGC ACC TGG CCC AGC CAG TCC ATC ACC TGC AAT GTG GCC CAC  
mIgG2a constant  
~~~~~  
P A S S T K V D K K I E P R G
631 CCG GCA AGC AGC ACC AAG GTG GAC AAG AAA ATT GAG CCC AGA GGG
mIgG2a constant

```
~~~~~
  P   T   I   K   P   C   P   P   C   K   C   P   A   P   N
676 CCC ACA ATC AAG CCC TGT CCT CCA TGC AAA TGC CCA GCA CCT AAC
      mIgG2a constant
~~~~~
  L   L   G   G   P   S   V   F   I   F   P   P   K   I   K
721 CTC TTG GGT GGA CCA TCC GTC TTC ATC TTC CCT CCA AAG ATC AAG
      mIgG2a constant
~~~~~
  D   V   L   M   I   S   L   S   P   I   V   T   C   V   V
766 GAT GTA CTC ATG ATC TCC CTG AGC CCC ATA GTC ACA TGT GTG GTG
      mIgG2a constant
~~~~~
  V   D   V   S   E   D   D   P   D   V   Q   I   S   W   F
811 GTG GAT GTG AGC GAG GAT GAC CCA GAT GTC CAG ATC AGC TGG TTT
      mIgG2a constant
~~~~~
  V   N   N   V   E   V   H   T   A   Q   T   Q   T   H   R
856 GTG AAC AAC GTG GAA GTA CAC ACA GCT CAG ACA CAA ACC CAT AGA
      mIgG2a constant
~~~~~
  E   D   Y   N   S   T   L   R   V   V   S   A   L   P   I
901 GAG GAT TAC AAC AGT ACT CTC CGG GTG GTC AGT GCC CTC CCC ATC
      mIgG2a constant
~~~~~
  Q   H   Q   D   W   M   S   G   K   E   F   K   C   K   V
946 CAG CAC CAG GAC TGG ATG AGT GGC AAG GAG TTC AAA TGC AAG GTC
      mIgG2a constant
~~~~~
  N   N   K   D   L   P   A   P   I   E   R   T   I   S   K
991 AAC AAC AAA GAC CTC CCA GCG CCC ATC GAG AGA ACC ATC TCA AAA
      mIgG2a constant
~~~~~
  P   K   G   S   V   R   A   P   Q   V   Y   V   L   P   P
1036 CCC AAA GGG TCA GTA AGA GCT CCA CAG GTA TAT GTC TTG CCT CCA
      mIgG2a constant
~~~~~
  P   E   E   E   M   T   K   K   Q   V   T   L   T   C   M
1081 CCA GAA GAA GAG ATG ACT AAG AAA CAG GTC ACT CTG ACC TGC ATG
      mIgG2a constant
~~~~~
  V   T   D   F   M   P   E   D   I   Y   V   E   W   T   N
1126 GTC ACA GAC TTC ATG CCT GAA GAC ATT TAC GTG GAG TGG ACC AAC
      mIgG2a constant
~~~~~
  N   G   K   T   E   L   N   Y   K   N   T   E   P   V   L
1171 AAC GGG AAA ACA GAG CTA AAC TAC AAG AAC ACT GAA CCA GTC CTG
      mIgG2a constant
~~~~~
  D   S   D   G   S   Y   F   M   Y   S   K   L   R   V   E
1216 GAC TCT GAT GGT TCT TAC TTC ATG TAC AGC AAG CTG AGA GTG GAA
      mIgG2a constant
~~~~~
  K   K   N   W   V   E   R   N   S   Y   S   C   S   V   V
1261 AAG AAG AAC TGG GTG GAA AGA AAT AGC TAC TCC TGT TCA GTG GTC
```

```

                                mIgG2a constant
~~~~~
      H   E   G   L   H   N   H   H   T   T   K   S   F   S   R
1306 CAC GAG GGT CTG CAC AAT CAC CAC ACG ACT AAG AGC TTC TCC CGG
      mIgG2a constant
~~~~~
      T   P   G   K   *
1351 ACT CCG GGT AAA TGA

```

Figure 5

GITR 6G10 VK

```
~~~~~
  A   I   Q   L   T   Q   S   P   S   S   L   S   A   S   V
1  GCC ATC CAG TTG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA
    GITR 6G10 VK
~~~~~
  G   D   R   V   T   I   T   C   R   A   S   Q   G   I   S
46 GGA GAC AGA GTC ACC ATC ACT TGC CGG GCA AGT CAG GGC ATT AGC
    GITR 6G10 VK
~~~~~
  S   A   L   A   W   Y   Q   Q   K   P   G   K   A   P   K
91 AGT GCT TTA GCC TGG TAT CAG CAG AAA CCA GGG AAA GCT CCT AAG
    GITR 6G10 VK
~~~~~
  L   L   I   Y   D   A   S   S   L   E   S   G   V   P   S
136 CTC CTG ATC TAT GAT GCC TCC AGT TTG GAA AGT GGG GTC CCA TCA
    GITR 6G10 VK
~~~~~
  R   F   S   G   S   G   S   G   T   D   F   T   L   T   I
181 AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC
    GITR 6G10 VK
~~~~~
  S   S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q
226 AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGT CAA CAG
    GITR 6G10 VK
~~~~~
  F   N   S   Y   P   Y   T   F   G   Q   G   T   K   L   E
271 TTT AAT AGT TAC CCG TAC ACT TTT GGC CAG GGG ACC AAG CTG GAG
    murine kappa C
~~~~~
GITR 6G10 VK
~~~~~
  I   K   R   A   D   A   A   P   T   V   S   I   F   P   P
316 ATC AAA CGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA
    ~~~~~
                                DMTm89
~~~~~
                                DMTm88
~~~~~
    murine kappa C
~~~~~
  S   S   E   Q   L   T   S   G   G   A   S   V   V   C   F
361 TCC AGT GAG CAA TTG ACA TCT GGA GGT GCC TCA GTC GTG TGC TTC
    ~~~~~
    .....
    ~~~~~
    ...
    murine kappa C
~~~~~
  L   N   N   F   Y   P   K   D   I   N   V   K   W   K   I
406 TTG AAC AAC TTC TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT
    murine kappa C
~~~~~
  D   G   S   E   R   Q   N   G   V   L   N   S   W   T   D
451 GAT GGC AGT GAA CGA CAA AAT GGC GTC CTG AAC AGT TGG ACC GAT
    murine kappa C
~~~~~
  Q   D   S   K   D   S   T   Y   S   M   S   S   T   L   T
496 CAG GAC AGC AAA GAC AGC ACC TAC AGC ATG AGC AGC ACC CTC ACG
    murine kappa C
~~~~~
  L   T   K   D   E   Y   E   R   H   N   S   Y   T   C   E
```

```
541 TTG ACC AAG GAC GAG TAT GAA CGA CAT AAC AGC TAT ACC TGT GAG
      murine kappa C
      ~~~~~
      A   T   H   K   T   S   T   S   P   I   V   K   S   F   N
586 GCC ACT CAC AAG ACA TCA ACT TCA CCC ATT GTC AAG AGC TTC AAC
      murine kappa C
      ~~~~~
      R   N   E   C   *
631 AGG AAT GAG TGT TAG
```

Figure 6

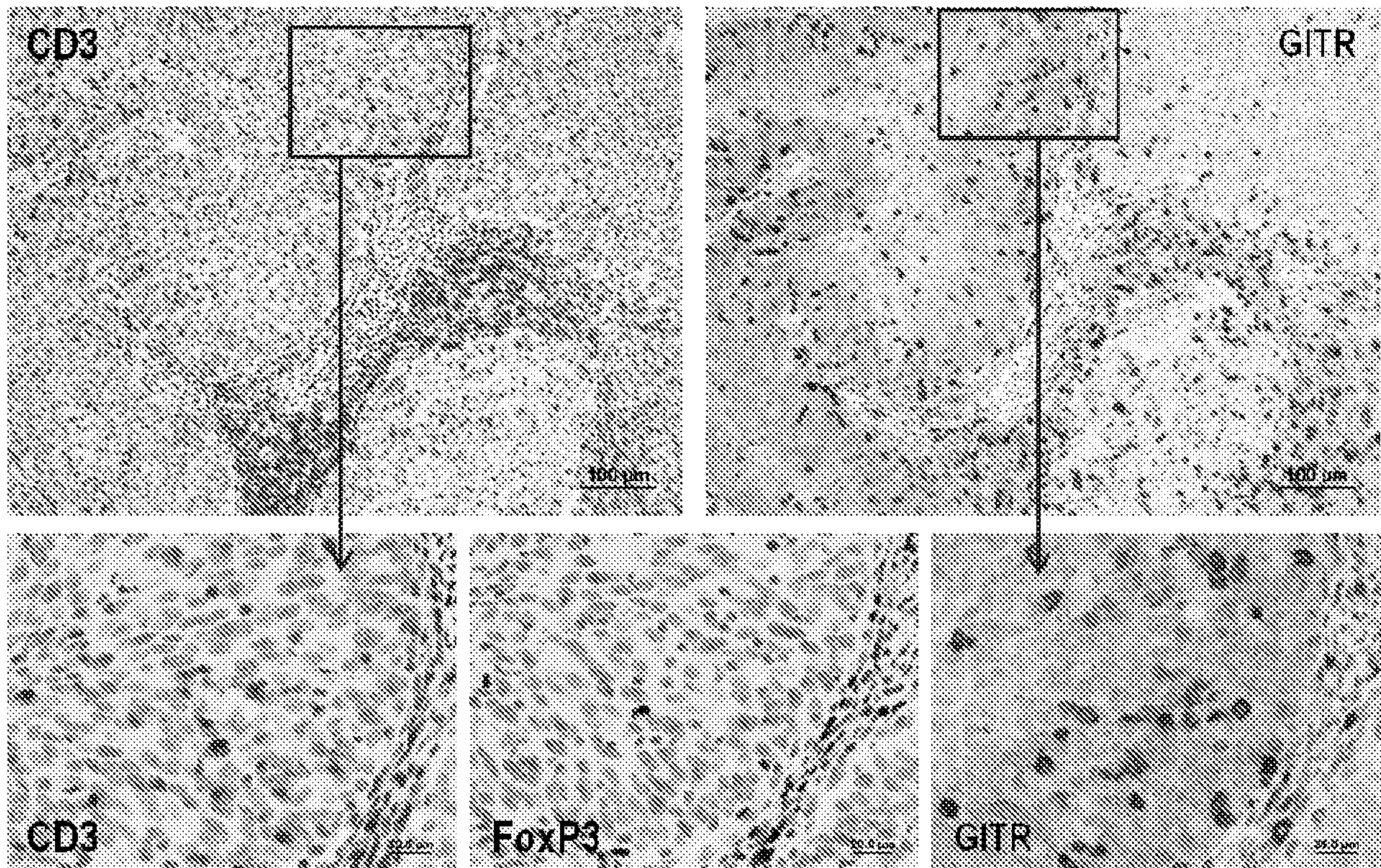


Figure 7