

region having the amino acid sequence of SEQ ID NO: 34, and a variable light chain region having the amino acid sequence of SEQ ID NO: 36; a variable heavy chain region having the amino acid sequence of SEQ ID NO: 38, and a variable light chain region having the amino acid sequence of SEQ ID NO: 40; a variable heavy chain region having the amino acid sequence of SEQ ID NO: 42, and a variable light chain region having the amino acid sequence of SEQ ID NO: 44; a variable heavy chain region having the amino acid sequence of SEQ ID NO: 46, and a variable light chain region having the amino acid sequence of SEQ ID NO: 48; a variable heavy chain region having the amino acid sequence of SEQ ID NO: 50, and a variable light chain region having the amino acid sequence of SEQ ID NO: 52.

[0009a] In a first aspect, the present disclosure refers to an isolated humanized monoclonal antibody or antigen-binding fragment thereof that binds to the human-glucocorticoid-induced tumor necrosis factor receptor (GITR) comprising:

- a. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 2, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 4;
- b. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 6, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 8;
- c. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 10, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 12;
- d. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 14, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 16;
- e. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 18, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 20;
- f. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 22, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 24;
- g. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 26, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 28;
- h. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 30, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 32;
- i. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 34, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 36;
- j. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 38, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 40; or
- k. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 42, and

determining region 1, 2 or 3 (VL-CDR) having the amino acid sequences of SEQ ID NO. 90, 91, or 92; a variable heavy chain complementarity determining region 1, 2, or 3 (VH-CDR) having the amino acid sequences of SEQ ID NO. 93, 94, or 95, respectively; and, a variable light chain complementarity determining region 1, 2 or 3 (VL-CDR) having the amino acid sequences of SEQ ID NO. 96, 97, or 98; a variable heavy chain complementarity determining region 1, 2, or 3 (VH-CDR) having the amino acid sequences of SEQ ID NO. 99, 100, or 101, respectively; and, a variable light chain complementarity determining region 1, 2 or 3 (VL-CDR) having the amino acid sequences of SEQ ID NO. 102, 103, or 104; a variable heavy chain complementarity determining region 1, 2, or 3 (VH-CDR) having the amino acid sequences of SEQ ID NO. 65, 72, or 105, respectively; and, a variable light chain complementarity determining region 1, 2 or 3 (VL-CDR) having the amino acid sequences of SEQ ID NO. 106, 107, or 108; a variable heavy chain complementarity determining region 1, 2, or 3 (VH-CDR) having the amino acid sequences of SEQ ID NO. 109, 72, or 110, respectively; and, a variable light chain complementarity determining region 1, 2 or 3 (VL-CDR) having the amino acid sequences of SEQ ID NO. 111, 112, or 113; a variable heavy chain complementarity determining region 1, 2, or 3 (VH-CDR) having the amino acid sequences of SEQ ID NO. 65, 72, or 114, respectively; and, a variable light chain complementarity determining region 1, 2 or 3 (VL-CDR) having the amino acid sequences of SEQ ID NO. 115, 116, or 117; and a variable heavy chain complementarity determining region 1, 2, or 3 (VH-CDR) having the amino acid sequences of SEQ ID NO. 101, 118, or 119, respectively; and, a variable light chain complementarity determining region 1, 2 or 3 (VL-CDR) having the amino acid sequences of SEQ ID NO. 120, 121, or 122.

[0010a] In another aspect, the present disclosure refers to an isolated humanized monoclonal antibody or antigen-binding fragment thereof wherein the antibody or antigen-binding fragment comprises:

- (a) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 53, 54 and 55, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 56, 57, and 58, respectively;
- (b) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 59, 60, and 61, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the

amino acid sequences of SEQ ID NO. 62, 63, and 64, respectively;

(c) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 65, 66, and 67, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 68, 69, and 70, respectively;

(d) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 71, 72, and 73, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 74, 75, and 76, respectively;

(e) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 77, 78, and 79, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 80, 81, and 82, respectively;

(f) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 83, 84, and 85, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 86, 87, and 88, respectively;

(g) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 65, 72, and 89, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 90, 91, and 92, respectively;

(h) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 93, 94, and 95, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 96, 97, and 98, respectively;

(i) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 99, 100, and 101, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 102, 103, and 104, respectively;

(j) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 65, 72, and 105, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 106, 107, and 108, respectively;

(k) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 109, 72, and 110, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 111, 112, and 113, respectively;

(l) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 65, 72, and 114, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 115, 116, and 117, respectively; or

(m) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 101, 118, and 119, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 120, 121, and 122, respectively;

wherein said antibody or antibody binding fragment binds human-glucocorticoid-induced tumor necrosis factor receptor (GITR).

[0011] The antibody is monovalent or bivalent. For example the antibody is a single chain antibody. The antibody has a binding affinity within the range of 10^{-5} M to 10^{-12} M. In some examples the antibody has a IgG4 heavy chain constant region. In other examples the antibody has an Fc region that contains mutations at amino acid positions 234 and 235. The mutations are for example, L234A and L235A.

[0012] In other examples the disclosure includes a bi-specific antibody containing the human GITR antibody of the disclosure and an antibody that also binds to a tumor-associated antigen, a cytokine or a cell surface receptor.

[0013] Optionally the antibodies of the disclosure are s linked to a therapeutic agent, such as a toxin, a radiolabel, a siRNA, a small molecule, or a cytokine.

[0014] Also disclosed are cells producing the antibody according to the disclosure.

[0014a] In another aspect, the present disclosure refers to a cell producing the antibody as described herein.

[0015] Disclosed are methods for depleting regulatory T-cells in a subject, by administering to a subject in need thereof a composition comprising an antibody according to the disclosure.

[0015a] In another aspect, the present disclosure refers to use of a composition comprising an antibody as described herein in the manufacture of a medicament for depleting regulatory T-cells in a subject.

[0016] Other methods of the disclosure include augmenting an immune response to an antigen by administering to a subject in need thereof a composition comprising an antibody according to the disclosure. The antigen is a viral antigen, a bacterial antigen or a tumor associated antigen.

[0016a] In another aspect, the present disclosure refers to use of a composition comprising an antibody as described herein in the manufacture of a medicament for augmenting an immune response to an antigen, wherein the composition is to be administered to a subject in need thereof.

[0017] In various examples administering an antibody according to the disclosure result in an increase in antigen specific T cell activity and/or an increase NK cell cytotoxicity.

[0018] In some examples the methods of the disclosure further includes administering to the subject IL-15.

[0019] Disclosed are methods of treating or alleviating a symptom of cancer by administering to a subject in need thereof a composition comprising an antibody according to the disclosure. The cancer is a cancer in which GITR or its ligand, GITR-L, is overexpressed. Optionally the subject is further administered a cytokine, such as IL-15 or a chemotherapeutic agent.

[0019a] In another aspect, the present disclosure refers to use of a composition comprising an antibody as described herein in the manufacture of a medicament for treating or alleviating a symptom of cancer, wherein the composition is to be administered to a subject in need thereof.

[0020] The disclosure further provides a nucleic acid having the nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, and 51.

[0020a] In another aspect, the present disclosure refers to a nucleic acid comprising the nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, or 51.

[0021] Disclosed is a nucleic acid encoding the polypeptide of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52 or polypeptide having the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52. Vectors containing the nucleic acids according to the disclosure are also provided. Also included in the disclosure are cells containing the vectors according to the disclosure.

[0021a] In another aspect, the present disclosure refers to a nucleic acid encoding the polypeptide of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, or 52. In another aspect, the present disclosure refers to a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, or 52. In another aspect, the present disclosure refers to a vector comprising the nucleic acid as described herein. In another aspect, the present disclosure refers to a cell comprising the vector as described herein.

[0022] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety. In cases of

What is claimed is:

1. An isolated humanized monoclonal antibody or antigen-binding fragment thereof that binds to the human-glucocorticoid-induced tumor necrosis factor receptor (GITR) comprising:
 - a. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 2, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 4;
 - b. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 6, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 8;
 - c. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 10, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 12;
 - d. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 14, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 16;
 - e. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 18, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 20;
 - f. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 22, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 24;
 - g. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 26, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 28;
 - h. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 30, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 32;

- i. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 34, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 36;
 - j. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 38, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 40; or
 - k. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 42, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 44.
 - l. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 46, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 48
 - m. a variable heavy chain region comprising the amino acid sequence of SEQ ID NO: 50, and a variable light chain region comprising the amino acid sequence of SEQ ID NO: 52.
2. An isolated humanized monoclonal antibody or antigen-binding fragment thereof wherein the antibody or antigen-binding fragment comprises:
- (a) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 53, 54 and 55, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 56, 57, and 58, respectively;
 - (b) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 59, 60, and 61, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 62, 63, and 64, respectively;
 - (c) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 65, 66, and 67, respectively; and, a variable light chain complementarity

- determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 68, 69, and 70, respectively;
- (d) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 71, 72, and 73, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 74, 75, and 76, respectively;
- (e) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 77, 78, and 79, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 80, 81, and 82, respectively;
- (f) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 83, 84, and 85, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 86, 87, and 88, respectively;
- (g) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 65, 72, and 89, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 90, 91, and 92, respectively;
- (h) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 93, 94, and 95, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 96, 97, and 98, respectively;
- (i) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 99, 100, and 101, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 102, 103, and 104, respectively;

- (j) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 65, 72, and 105, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 106, 107, and 108, respectively;
- (k) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 109, 72, and 110, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 111, 112, and 113, respectively;
- (l) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 65, 72, and 114, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 115, 116, and 117, respectively; or
- (m) a variable heavy chain complementarity determining region 1, 2, and 3 (VH-CDR) comprising the amino acid sequences of SEQ ID NO. 101, 118, and 119, respectively; and, a variable light chain complementarity determining region 1, 2 and 3 (VL-CDR) comprising the amino acid sequences of SEQ ID NO. 120, 121, and 122, respectively;

wherein said antibody or antibody binding fragment binds human-glucocorticoid-induced tumor necrosis factor receptor (GITR).

- 3. The antibody of claim 1, wherein said antibody is monovalent or bivalent.
- 4. The antibody of claim 1, wherein said antibody is a single chain antibody.
- 5. The antibody of claim 1, wherein said antibody has a binding affinity within the range of 10^{-5} M to 10^{-12} M.
- 6. The antibody of claim 1, wherein said antibody has a IgG4 heavy chain constant region.
- 7. The antibody of claim 1, wherein the Fc region contains mutations at amino acid positions 234 and 235.

8. The antibody of claim 7, wherein the mutations are L234A and L235A.
9. The antibody according to claim 1 wherein said antibody is a bi-specific antibody that also binds to a tumor-associated antigen, a cytokine or a cell surface receptor.
10. The antibody according to any one of preceding claims linked to a therapeutic agent.
11. The antibody of claim 10, wherein said therapeutic agent is a toxin, a radiolabel, a siRNA, a small molecule, or a cytokine.
12. A cell producing the antibody of any one of claims 1-11.
13. Use of a composition comprising an antibody according to any one of claims 1-11 in the manufacture of a medicament for depleting regulatory T-cells in a subject.
14. Use of a composition comprising an antibody of any one of claims 1-11 in the manufacture of a medicament for augmenting an immune response to an antigen, wherein the composition is to be administered to a subject in need thereof.
15. The use of claim 14, wherein said antigen is a viral antigen, a bacterial antigen or a tumor associated antigen.
16. The use of claim 14, wherein said antibody causes an increase in antigen specific T cell activity.
17. The use of claim 14, wherein said antibody causes an increase NK cell cytotoxicity.
18. The use of claim 14, wherein IL-15 is to be further administered to said subject.
19. Use of a composition comprising an antibody according to any one of claims 1-11 in the manufacture of a medicament for treating or alleviating a symptom of cancer, wherein the composition is to be administered to a subject in need thereof.
20. The use of claim 19, wherein said cancer is a cancer in which GITR or its ligand, GITR-L, is overexpressed.
21. The use of claim 20, wherein a cytokine or a chemotherapeutic agent is to be further administered to said subject.

- 22. The use of claim 21, wherein the cytokine is IL-15.
- 23. A nucleic acid comprising the nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, or 51.
- 24. A nucleic acid encoding the polypeptide of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, or 52.
- 25. A polypeptide comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, or 52.
- 26. A vector comprising the nucleic acid of claim 23 or 24.
- 27. A cell comprising the vector of claim 26.