

What is claimed is:

1. A polypeptide that binds glucocorticoid-induced TNFR-related protein (GITR), comprising two or more GITR-binding domain (GITR-BD) that specifically binds GITR.
2. The polypeptide of claim 1, wherein the polypeptide comprises at least two copies of the same GITR-BD.
3. The polypeptide of claim 1, wherein the polypeptide comprises at least three copies of the same GITR-BD.
4. The polypeptide of any one of claims 1-3, wherein two or more GITR-binding domains are operably linked via a linker polypeptide.
5. The polypeptide of any one of claims 1-4, wherein each GITR-BD comprise a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 106, 109, 112, 117, 120, 125, 131, 138, 143, 148, and 149; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 107, 110, 113, 115, 118, 121, 123, 128, 130, 132, 134, 136, 137, 139, 141, 144, and 147; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 108, 111, 114, 116, 119, 122, 124, 126, 127, 129, 133, 135, 140, 142, 145, 146, and 150.
6. A polypeptide that binds glucocorticoid-induced TNFR-related protein (GITR), comprising at least one GITR-binding domain (GITR-BD) comprising a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 106, 109, 112, 117, 120, 125, 131, 138, 143, 148, and 149; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 107, 110, 113, 115, 118, 121, 123, 128, 130, 132, 134, 136, 137, 139, 141, 144, and 147; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group

consisting of SEQ ID NO: 108, 111, 114, 116, 119, 122, 124, 126, 127, 129, 133, 135, 140, 142, 145, 146, and 150.

7. A polypeptide that binds at least glucocorticoid-induced TNFR-related protein (GITR), comprising at least one GITR-binding domain (GITR-BD) that specifically binds GITR and one or more additional binding domain.

8. The polypeptide of claim 7, wherein the at least one GITR-BD comprise a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 106, 109, 112, 117, 120, 125, 131, 138, 143, 148, and 149; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 107, 110, 113, 115, 118, 121, 123, 128, 130, 132, 134, 136, 137, 139, 141, 144, and 147; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 108, 111, 114, 116, 119, 122, 124, 126, 127, 129, 133, 135, 140, 142, 145, 146, and 150.

9. The polypeptide of any one of claims 5, 6 and 8, wherein the GITR-BD comprises a CDR1, CDR2, and CDR3 comprising:

the amino acid sequences set forth in SEQ ID NOS: 106, 107 and 108, respectively;
the amino acid sequences set forth in SEQ ID NOS: 109, 110 and 111, respectively;
the amino acid sequences set forth in SEQ ID NOS: 112, 113 and 114, respectively;
the amino acid sequences set forth in SEQ ID NOS: 106, 115 and 116, respectively;
the amino acid sequences set forth in SEQ ID NOS: 117, 118 and 119, respectively;
the amino acid sequences set forth in SEQ ID NOS: 120, 121 and 122, respectively;
the amino acid sequences set forth in SEQ ID NOS: 149, 123 and 150, respectively;
the amino acid sequences set forth in SEQ ID NOS: 120, 123 and 124, respectively;
the amino acid sequences set forth in SEQ ID NOS: 125, 121 and 124, respectively;
the amino acid sequences set forth in SEQ ID NOS: 120, 121 and 126, respectively;
the amino acid sequences set forth in SEQ ID NOS: 112, 113 and 127, respectively;
the amino acid sequences set forth in SEQ ID NOS: 148, 128 and 129, respectively;
the amino acid sequences set forth in SEQ ID NOS: 120, 130 and 124, respectively;
the amino acid sequences set forth in SEQ ID NOS: 131, 132 and 133, respectively;

the amino acid sequences set forth in SEQ ID NOS: 120, 134 and 135, respectively;
the amino acid sequences set forth in SEQ ID NOS: 120, 121 and 122, respectively;
the amino acid sequences set forth in SEQ ID NOS: 106, 136 and 122, respectively;
the amino acid sequences set forth in SEQ ID NOS: 106, 137 and 122, respectively;
the amino acid sequences set forth in SEQ ID NOS: 120, 137 and 122, respectively;
the amino acid sequences set forth in SEQ ID NOS: 138, 137 and 122, respectively;
the amino acid sequences set forth in SEQ ID NOS: 138, 139 and 116, respectively;
the amino acid sequences set forth in SEQ ID NOS: 106, 139 and 116, respectively;
the amino acid sequences set forth in SEQ ID NOS: 138, 139 and 140, respectively;
the amino acid sequences set forth in SEQ ID NOS: 109, 110 and 111, respectively;
the amino acid sequences set forth in SEQ ID NOS: 138, 141 and 116, respectively;
the amino acid sequences set forth in SEQ ID NOS: 138, 141 and 142, respectively;
the amino acid sequences set forth in SEQ ID NOS: 143, 144 and 145, respectively;
the amino acid sequences set forth in SEQ ID NOS: 143, 144 and 146, respectively;

or

the amino acid sequences set forth in SEQ ID NOS: 143, 147 and 146, respectively;

10. The polypeptide of any one of claims 1 to 9, wherein the GITR-BD comprises a single domain antibody (sdAb).

11. The polypeptide of any one of claims 1 to 10, wherein the GITR-BD comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 19-80, or a sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 19-80.

12. The polypeptide of any one of claims 1 to 11, wherein the GITR-BD comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 42-62.

13. The polypeptide of any one of claims 1 to 11, wherein the GITR-BD comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 63-80.

14. The polypeptide of any one of claims 1 to 13, wherein the polypeptide comprises an immunoglobulin Fc region polypeptide.

15. The polypeptide of claim 14, wherein the immunoglobulin Fc region polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6.

16. The polypeptide of any one of claims 1 to 15, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 81-105, or an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 81-105.

17. The polypeptide of any one of claims 1 to 16, wherein the polypeptide is tetravalent for binding GITR.

18. The polypeptide of claim 17, wherein the polypeptide comprises two copies of a fusion protein that comprises the structure: (GITR-BD)-Linker-(GITR-BD)-Linker-Hinge-Fc.

19. The polypeptide of any one of claims 1 to 16, wherein the polypeptide is hexavalent for binding GITR.

20. The polypeptide of claim 19, wherein the polypeptide comprises two copies of a fusion protein that comprises the structure: (GITR-BD)-Linker-(GITR-BD)-Linker-(GITR-BD)-Linker-Hinge-Fc.

21. The polypeptide of claim 18 or claim 20, wherein the GITR-BD is a sdAb.

22. The polypeptide of any one of claims 18, 20 and 21, wherein each GITR-BD comprises a complementarity determining region 1 (CDR1) comprising the amino acid sequence of SEQ ID NO: 138; a complementarity determining region 2 (CDR2) comprising the amino acid sequence of SEQ ID NO: 141; and a complementarity determining region 3 (CDR3) comprising the amino acid sequence of SEQ ID NO: 116.

23. The polypeptide of claim 22, wherein each GITR-BD comprises the amino acid sequence selected from SEQ ID NOs: 50-59 and 61-62.

24. The polypeptide of claim 22 or claim 23, wherein each GITR-BD comprises the amino acid sequence set forth in SEQ ID NO:59.

25. The polypeptide of any one of claims 19-24, wherein the polypeptide comprises the amino acid sequence SEQ ID NO:93.

26. The polypeptide of any one of claims 7 to 25, wherein the one or more additional binding domain binds to a target other than GITR.

27. The polypeptide of claim 26, wherein the one or more additional binding domains binds a Tumor Necrosis Factor receptor superfamily (TNFRSF).

28. The polypeptide of claim 27, wherein the TNFRSF member is selected from the group consisting of OX40, CD27, herpesvirus entry mediator (HVEM), CD40, lymphotoxin beta receptor (LTBR), ectodysplasin A2 receptor (ED2R), ectodysplasin A receptor (EDAR), TweakR, B cell maturation antigen (BCMA), B cell-activating factor receptor (BAFFR), death receptor 3 (DR3), death receptor 6 (DR6), and CD137.

29. The polypeptide of any one of claims 7 to 28, wherein the one or more additional binding domains binds a non-TNFRSF-member.

30. The polypeptide of any one of claims 7 to 29, wherein the one or more additional binding domain comprises an antibody or antigen-binding fragment thereof.

31. The polypeptide of claim 30, wherein the antibody or antigen-binding fragment thereof is a scFv, a Fab, a single domain antibody (sdAb), a V_{NAR} , or a VHH.

32. The polypeptide of claim 30 or claim 31, wherein the antibody or antigen-binding fragment is a sdAb.

33. A nucleic acid encoding the polypeptide of any one of claims 1 to 32.
34. A host cell comprising the nucleic acid of claim 33.
35. A pharmaceutical composition comprising the polypeptide of any one of claims 1 to 32.
36. A pharmaceutical composition of claim 35 for use in treating cancer in a subject in need thereof.
37. Use of the polypeptide of any one of claims 1 to 32 or the pharmaceutical composition of claim 35 in the manufacture of a medicament for treating cancer in a subject in need thereof.
38. The pharmaceutical composition of claim 36 or use of claim 37, wherein said subject is a human.
39. The pharmaceutical composition or use of any one of claims 36 to 38, wherein the cancer is a metastatic cancer, refractory cancer, or recurrent cancer.
40. The pharmaceutical composition or use of any one of claims 36 to 39, wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, uterine cancer, cervical cancer, ovarian cancer, prostate cancer, testicular cancer, esophageal cancer, gastrointestinal cancer, pancreatic cancer, colorectal cancer, colon cancer, kidney cancer, head and neck cancer, lung cancer, stomach cancer, germ cell cancer, bone cancer, liver cancer, thyroid cancer, skin cancer, neoplasm of the central nervous system, lymphoma, leukemia, myeloma, sarcoma, and virus-related cancer.
41. The pharmaceutical composition or use of any one of claims 36 to 40 for combination with an anti-PD1 or anti-PDL1 antibody.

42. The pharmaceutical composition or use of claim 41, wherein the antibody is an anti-PD1 antibody and the anti-PD1 antibody is BMS-936558.

43. A pharmaceutical composition of claim 35 for use in inhibiting tumor growth, reducing the frequency of intratumoral regulatory T cells (T_{reg}), inducing activation and proliferation of CD8⁺ T cells, or reducing the frequency of T_{reg} cells and inducing activation and proliferation of CD8⁺ T cells, in a subject.

44. Use of the polypeptide of any one of claims 1 to 32 or the pharmaceutical composition of claim 35 in the manufacture of a medicament for inhibiting tumor growth, for reducing the frequency of intratumoral regulatory T cells (T_{reg}), for inducing activation and proliferation of CD8⁺ T cells, or for reducing the frequency of T_{reg} cells and inducing activation and proliferation of CD8⁺ T cells, in a subject.