

What is claimed is:

1. An isolated multispecific polypeptide that binds glucocorticoid-induced TNFR-related protein (GITR) and a second antigen, comprising two or more GITR-binding domain (GITR-BD) that specifically binds GITR, further wherein the multispecific polypeptide is capable of enhanced clustering of TNFRSF members and induces enhanced TNFR-dependent signaling compared to non-crosslinked bivalent antibodies.
2. The multispecific polypeptide of claim 1, wherein the polypeptide comprises at least two copies of the same GITR-BD.
3. The multispecific polypeptide of claim 1, wherein the polypeptide comprises at least three copies of the same GITR-BD.
4. The multispecific polypeptide of any one of claims 1-3, wherein two or more GITR-binding domains are operably linked via a linker polypeptide.
5. The multispecific polypeptide of any one of claims 1-4, wherein each GITR-BD comprises a single domain antibody (sdAb) 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.
6. The multispecific polypeptide of any one of claims 1-4 wherein each GITR-binding domain (GITR-BD) comprises a single domain antibody (sdAb), wherein at least one GITR-BD comprises 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. The multispecific polypeptide of any one of claims 5 and 6, wherein the sdAb 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;

8. The multispecific polypeptide of any one of claims 1 to 7, 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.

9. The multispecific polypeptide of any one of claims 1 to 8, wherein the GITR-BD comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 42- 62.

10. The multispecific polypeptide of any one of claims 1 to 8, wherein the GITR-BD comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 63- 80.

11. The multispecific polypeptide of any one of claims 1 to 10, wherein the polypeptide comprises an immunoglobulin Fc region polypeptide.

12. The multispecific polypeptide of claim 11, wherein the immunoglobulin Fc region polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6.

13. The multispecific polypeptide of any one of claims 1 to 12, 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.

14. The multispecific polypeptide of any one of claims 1 to 13, wherein the polypeptide is tetravalent for binding GITR wherein the polypeptide comprises two copies of a fusion protein that comprises the structure: (GITR-BD)-Linker-(GITR-BD)-Linker- Hinge-Fc.

15. The multispecific polypeptide of any one of claims 1 to 13, wherein the polypeptide is hexavalent for binding GITR, 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.

16. The multispecific polypeptide of any one of claims 14 and 15, 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.

17. The multispecific polypeptide of claim 16, wherein each GITR-BD comprises the amino acid sequence selected from SEQ ID NOs: 50-59 and 61-62.

18. The multispecific polypeptide of claim 16 or claim 17, wherein each GITR-BD comprises the amino acid sequence set forth in SEQ ID NO:59.

19. The multispecific polypeptide of any one of claims 15-18, wherein the polypeptide comprises the amino acid sequence SEQ ID NO:93.

20. The multispecific polypeptide of any one of claims 1-19, wherein the polypeptide comprises at least a second binding domain that binds a second Tumor Necrosis Factor receptor superfamily (TNFRSF) member.

21. The multispecific polypeptide of claim 20, 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.

22. The multispecific polypeptide of any one of claims 1 to 19, wherein the polypeptide comprises at least a second binding domain that binds a non-TNFRSF-member.

23. A nucleic acid encoding the multispecific polypeptide of any one of claims 1 to 22.

24. A host cell comprising the nucleic acid of claim 23.

25. A pharmaceutical composition comprising the polypeptide of any one of claims 1 to 24.

26. The pharmaceutical composition of claim 25 for use in treating cancer in a subject in need thereof.

27. Use of the multispecific polypeptide of any one of claims 1 to 22 or the pharmaceutical composition of claim 25 in the manufacture of a medicament for treating cancer in a subject in need thereof.

28. The pharmaceutical composition of claim 26 or use of claim 27, wherein said subject is a human.

29. The pharmaceutical composition or use of any one of claims 26 to 28, wherein the cancer is a metastatic cancer, refractory cancer, or recurrent cancer.

30. The pharmaceutical composition or use of any one of claims 26 to 29, 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.

31. A combination of the pharmaceutical composition or use of any one of claims 26 to 30 and an anti-PD1 or anti-PDL1 antibody.

32. The combination of claim 31, wherein the anti-PD1 antibody is BMS-936558.

33. The pharmaceutical composition of claim 25 for use in inhibiting tumor growth, reducing the frequency of intratumoral regulatory T cells (Treg), inducing activation and proliferation of CD8+ T cells, or reducing the frequency of Treg cells and inducing activation and proliferation of CD8+ T cells, in a subject.

34. Use of the multispecific polypeptide of any one of claims 1 to 22 or the pharmaceutical composition of claim 25 in the manufacture of a medicament for inhibiting tumor growth, for reducing the frequency of intratumoral regulatory T cells (Treg), for inducing activation and proliferation of CD8+ T cells, or for reducing the frequency of Treg cells and inducing activation and proliferation of CD8+ T cells, in a subject.