

CLAIMS

1. A multispecific polypeptide comprising a first binding domain, designated B1, which is capable of specifically binding to GITR, and a second binding domain, designated B2,
5 which is capable of specifically binding to CTLA-4.
2. A polypeptide according to Claim 1, wherein the first and/or second binding domains are selected from the group consisting of: antibodies or antigen-binding fragments thereof.
- 10 3. A polypeptide according to Claim 2 wherein the antigen-binding fragment is selected from the group consisting of: an Fv fragment (such as a single chain Fv fragment, or a disulphide-bonded Fv fragment), a Fab-like fragment (such as a Fab fragment; a Fab' fragment or an F(ab)₂ fragment) and domain antibodies.
- 15 4. A polypeptide according to any one of the preceding claims wherein the polypeptide is a bispecific antibody.
- 20 5. A polypeptide according to any one of the preceding claims wherein:
 - (a) B1 comprises or consists of an IgG1 antibody and B2 comprises or consists of an scFv, or *vice versa*; or
 - (b) B1 comprises or consists of at least one scFv and B2 comprises or consists of at least one scFv.
- 25 6. A polypeptide according to any one of claims 1 to 3 wherein the first and/or second binding domains is non-antibody polypeptide.
7. A polypeptide according to claim 6 wherein B1 comprises or consists of an IgG1
30 antibody and B2 comprises or consists of a non-immunoglobulin polypeptide, or *vice versa*.
8. A polypeptide according to claim 6 or 7 wherein B2 comprises or consists of a CD86 domain or variant thereof capable of binding to CTLA-4.
- 35 9. A polypeptide according to any one of the preceding claims in which B1 comprises at least one heavy chain (H) and/or at least one light chain (L) and B2 is attached to said at least one heavy chain (H) or least one light chain (L).

10. A polypeptide according to claim 9 in which B1 comprises:
- (a) at least one heavy chain (H) and at least one light chain (L) and B2 is attached
5 to either the heavy chain or the light chain; or
- (b) two identical heavy chains (H) and two identical light chains (L) and B2 is attached to both heavy chains or to both light chains.
11. A polypeptide according to claim 9 or 10 which comprises or consists of a polypeptide chain arranged according to any one of the following formulae, written in the direction N-C:
- (a) L-(X)_n-B2;
(b) B2-(X)_n-L;
15 (c) B2-(X)_n-H; or
(d) H-(X)_n-B2;
- wherein X is a linker and n is 0 or 1.
12. A polypeptide according to claim 12, wherein X is a peptide with the amino acid sequence SGGGGSGGGGS (SEQ ID NO: 47), SGGGGSGGGGSAP (SEQ ID NO: 48), NFSQP (SEQ ID NO:49), KRTVA (SEQ ID NO: 50), GGGGSGGGGSGGGGS (SEQ ID NO: 51) or (SG)_m, where m = 1 to 7.
13. A polypeptide according to any one of the preceding claims comprising a human Fc region or a variant of a said region, where the region is an IgG1, IgG2, IgG3 or IgG4 region, preferably an IgG1 or IgG4 region.
14. A polypeptide according to Claim 13 wherein the Fc region is a naturally occurring
30 (*i.e.* wildtype) human Fc region.
15. A polypeptide according to Claim 13 wherein the Fc region is a non-naturally occurring (*e.g.* mutated) human Fc region.
16. A polypeptide according to any one of Claims 13 to 15 wherein the Fc region is afucosylated.

17. A polypeptide according to any of the preceding claims, wherein the polypeptide is capable of inducing antibody dependent cell cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), and/or apoptosis.
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18. A polypeptide according to any of the preceding claims, wherein the polypeptide is capable of inducing tumour immunity.
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19. A polypeptide according to any one of the preceding claims, which binds to human GITR with a K_d of less than $10 \times 10^{-9}M$, $4 \times 10^{-9}M$, or $1 \times 10^{-9}M$ and/or which binds to human CTLA-4 with a K_d value which is less than $60 \times 10^{-9}M$, $25 \times 10^{-9}M$, or $10 \times 10^{-9}M$.
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20. A polypeptide according to any one of the preceding claims, which induces an increase in the activity of an effector T cell, optionally wherein said increase is at least 1.5-fold, 4.5-fold or 7-fold higher than the increase in activity of an effector T cell induced by a combination of the first and second binding domains administered to the T cell as separate molecules.
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21. A polypeptide according to any one of the preceding claims, wherein the polypeptide is capable of:
- i) Killing GITR expressing tumour cells; and
 - ii) Activating the immune system via activation of effector T cells
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22. A polypeptide according to claim 20, wherein said increase in T cell activity is an increase in proliferation and/or IFN γ or IL-2 production by the T cell.
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23. A polypeptide according to any of the preceding claims wherein B1 is an antibody, or antigen binding fragment thereof, specific for GITR; and B2 is a polypeptide binding domain specific for CTLA-4, which comprises or consists of:
- (a) the amino acid sequence of SEQ ID NO: 3; or
 - (b) an amino acid sequence in which at least one amino acid is changed when
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- compared to the amino acid sequence of SEQ ID NO: 3 provided that said binding domain binds to human CTLA-4 with higher affinity than wild-type human CD86.

24. A polypeptide according to any one of claims 13 to 20, wherein 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids in said amino acid sequence of B2 (ii) are substituted when compared to the amino acid sequence of SEQ ID NO: 3; optionally wherein there are no insertions or deletions compared to the amino acid sequence of SEQ ID NO: 3.

25. A polypeptide according to claim 23, wherein at least one of said amino acid substitutions in said amino acid sequence of B2 is at position 122, and optionally wherein said amino acid sequence is also substituted in at least one of positions 107, 121 and 125.

26. A polypeptide according to any one of the preceding claims wherein said amino acid sequence of B2 comprises or consists of an amino acid sequence selected from any one of SEQ ID NOs 6 to 24.

27. A polypeptide according to any one of the preceding claims wherein the GITR binding domain (B1) is capable of competitively inhibiting the binding to human GITR of an antibody comprising a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NOs: 61, 63, 65 and 67 and a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NOs: 52, 54, 56 and 58.

28. A polypeptide according to claim 26 wherein B1 comprises a light chain variable region amino acid sequence comprising the CDRs of SEQ ID NOs: 88, 89 and 90 **and/or** a heavy chain variable region amino acid sequence comprising the CDRs of SEQ ID NOs: 76, 77 and 78.

29. A polypeptide according to claim 26 wherein B1 comprises a light chain variable region amino acid sequence comprising the CDRs of SEQ ID NOs: 91, 92 and 93 and/or a heavy chain variable region amino acid sequence comprising the CDRs of SEQ ID NOs: 79, 80 and 81.

30. A polypeptide according to claim 26 wherein B1 comprises a light chain variable region amino acid sequence comprising the CDRs of SEQ ID NOs: 94, 89 and 95 and/or a heavy chain variable region amino acid sequence comprising the CDRs of SEQ ID NOs: 82, 83 and 84.

31. A polypeptide according to claim 26 wherein B1 comprises a light chain variable region amino acid sequence comprising the CDRs of SEQ ID NOs: 94, 89 and 96 **and/or** a heavy chain variable region amino acid sequence comprising the CDRs of SEQ ID NOs:
5 85, 86 and 87.
32. A polypeptide according to any one of the preceding claims wherein the GITR binding domain (B1) comprises a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NOs: 61, 63, 65 and 67 and/or a heavy chain variable
10 region amino acid sequence selected from the group consisting of SEQ ID NOs: 52, 54, 56 and 58.
33. A polypeptide according to claim 31 wherein B1 comprises a light chain variable region amino acid sequence of SEQ ID NO: 61 and/or a heavy chain variable region amino
15 acid sequence of SEQ ID NO: 52.
34. A polypeptide according to claim 31 wherein B1 comprises a light chain variable region amino acid sequence of SEQ ID NO: 63 and/or a heavy chain variable region amino acid sequence of SEQ ID NO: 54.
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35. A polypeptide according to claim 31 wherein B1 comprises a light chain variable region amino acid sequence of SEQ ID NO: 65 and/or a heavy chain variable region amino acid sequence of SEQ ID NO: 56.
- 25 36. A polypeptide according to claim 31 wherein B1 comprises a light chain variable region amino acid sequence of SEQ ID NO: 67 and/or a heavy chain variable region amino acid sequence of SEQ ID NO: 58.
37. A polypeptide according to any one of claims 31 to 35 wherein B1 comprises or
30 consists of:
- (a) a light chain variable region amino acid sequence of SEQ ID NO: 61 and a heavy chain variable region amino acid sequence of SEQ ID NO: 52;
 - (b) a light chain variable region amino acid sequence of SEQ ID NO: 63 and a
35 heavy chain variable region amino acid sequence of SEQ ID NO: 54;

- (c) a light chain variable region amino acid sequence of SEQ ID NO: 65 and a heavy chain variable region amino acid sequence of SEQ ID NO: 56; or
- (d) a light chain variable region amino acid sequence of SEQ ID NO: 67 and a heavy chain variable region amino acid sequence of SEQ ID NO: 58.

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38. A polypeptide according to any one of the preceding claims, wherein the G1TR binding domain comprises a human Fc region or a variant of a said region, where the region is an IgG1, IgG2, IgG3 or IgG4 region, preferably an IgG1 or IgG4 region.

10 39. A polypeptide according to any of the preceding claims comprising or consisting of:

- (a) a light chain amino acid sequence selected from SEQ ID NOs: 69, 71, 73 and 75; and

- (b) a heavy chain variable region amino acid sequence selected from the group
15 consisting of 52, 54, 56 and 58.

40. A polypeptide according to any of the preceding claims comprising or consisting of the amino acid sequences:

- 20 (a) SEQ ID NOs: 52 and 69; or
- (b) SEQ ID NOs: 54 and 71; or
- (c) SEQ ID NO: 56 and 73; or
- (d) SEQ ID NOs: 58 and 75.

25 41. A polypeptide according to any of the preceding claims further comprising at least one further binding domain.

42. A polypeptide according to Claim 40 wherein the at least one further binding domain is an antigen-binding fragment selected from the group consisting of: an
30 Fv fragment (such as a single chain Fv fragment, or a disulphide-bonded Fv fragment), a Fab-like fragment (such as a Fab fragment; a Fab' fragment or a F(ab)₂ fragment) and domain antibodies

43. A polypeptide according to Claim 40 or 41 wherein the at least one further binding
35 domain.

44. A polypeptide according to any one of the preceding claims further comprising an additional therapeutic moiety.
45. A composition comprising a bispecific polypeptide according to any one of claims 1 to 38 and at least one pharmaceutically acceptable diluent or carrier.
46. An antibody specific for GITR which is as defined in any one of claims 26 to 37.
47. A polynucleotide encoding a bispecific polypeptide according to any one of claims 1 to 42, or a component polypeptide chain thereof.
48. A bispecific polypeptide according to any one of the preceding claims for use in a method for treating or preventing a neoplastic disease or condition in an individual.
49. A bispecific polypeptide according to claim 48 wherein the disease or condition is a cancer.
50. A bispecific polypeptide according to claim 49 wherein the cancer is selected from the groups consisting of prostate cancer, breast cancer, colorectal cancer, pancreatic cancer, ovarian cancer, lung cancer, cervical cancer, rhabdomyosarcoma, neuroblastoma, multiple myeloma, leukemia, acute lymphoblastic leukemia, melanoma, bladder cancer, gastric cancer, head and neck cancer, liver cancer, skin cancer, lymphoma and glioblastoma.
51. Use of a bispecific polypeptide according to any one of claims 1 to 43 in the preparation of a medicament for treating or preventing a neoplastic disease or condition in an individual.
52. A method of treating or preventing a neoplastic disease or condition in an individual, the method comprising administering to an individual a bispecific polypeptide according to any one of claims 1 to 43.
53. A method according to claim 51, wherein the method comprises administering the bispecific antibody systemically or locally, such as at the site of a tumour or into a tumour draining lymph node.
54. A bispecific polypeptide substantially as herein described with reference to the description.