

We claim:

1. An isolated engineered anti-tumor necrosis factor receptor (TNFR) superfamily member antibody, wherein the antibody comprises a T437R mutation, a T437R/K248E mutation or a T437R/K338A mutation when compared to a parental wild-type antibody, residue numbering according to the EU Index.
2. The antibody of claim 1, wherein the antibody has enhanced agonistic activity when compared to the parental wild-type antibody.
3. The antibody of claim 2, comprising the T437R mutation.
4. The antibody of claim 3, comprising the T437R/K248E mutation.
5. The antibody of claim 4, comprising the T437R/K338A mutation.
6. The antibody of any one of claims 1-5, wherein the antibody is an IgG1, IgG2, IgG3 or IgG4 isotype.
7. The antibody of any one of claims 1-6, wherein the antibody mediates antibody-dependent cellular cytotoxicity (ADCC).
8. The antibody of any one of claims 1-7, wherein the antibody mediates antibody-dependent cell phagocytosis (ADCP).
9. The antibody of any one of claims 1-8, wherein the antibody mediates complement-dependent cytotoxicity (CDC).
10. The antibody of any one of claims 1-9, wherein the antibody further comprises a second mutation.
11. The antibody of claim 10, wherein the second mutation is a L234A/L235A mutation on IgG1, a V234A/G237A/P238S/H268A/V309L/A330S/P331S mutation on IgG2, a F234A/L235A mutation on IgG4, a S228P/F234A/L235A mutation on IgG4, a N297A mutation on IgG1, IgG2, IgG3 or IgG4, a V234A/G237A mutation on IgG2, a K214T/E233P/L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M mutation on IgG1, a H268Q/V309L/A330S/P331S mutation on IgG2, a L234F/L235E/D265A mutation on IgG1, a L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation on IgG1, a S228P/F234A/L235A/G237A/P238S mutation on IgG4, or a S228P/F234A/L235A/G236-deleted/G237A/P238S mutation on IgG4.

12. The antibody of claim 11, wherein the second mutation is the V234A/G237A/P238S/H268A/V309L/A330S/P331S mutation on IgG2.
13. The antibody of claim 11, wherein the second mutation is the L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation on IgG1.
14. The antibody of claim 11, wherein the second mutation is the S228P/F234A/L235A mutation on IgG4.
15. The antibody of any one of claims 1-14, wherein the TNFR superfamily member is OX40 (SEQ ID NO: 4), CD27 (SEQ ID NO: 8), CD40 (SEQ ID NO: 5), CD137 (SEQ ID NO: 10), or GITR (SEQ ID NO: 23).
16. The antibody of any one of claims 1-15, wherein the antibody has agonistic activity independent of antibody cross-linking.
17. The antibody of any one of claims 1-16, wherein the antibody comprises a heavy chain constant region of SEQ ID NOs: 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73 or 74.
18. A pharmaceutical composition comprising the antibody of any one of claims 1-17 and a pharmaceutically acceptable carrier.
19. A method of enhancing an agonistic activity of an anti-TNFR superfamily member antibody in a subject, comprising providing the anti-TNFR superfamily member antibody, introducing a T437R mutation, a K248E mutation, a K338A mutation, a T437R/K248E mutation or a T437R/K338A mutation into the antibody to generate an engineered antibody specifically binding the TNFR superfamily member, and administering the engineered antibody to the subject.
20. The method of claim 19, wherein the antibody mediates ADCC, ADCP or CDC.
21. The method of claim 19 or 20, wherein the antibody further comprises a second mutation.
22. The method of claim 21, wherein the second mutation is a L234A/L235A mutation on IgG1, a V234A/G237A/P238S/H268A/V309L/A330S/P331S mutation on IgG2, a F234A/L235A mutation on IgG4, a S228P/F234A/L235A mutation on IgG4, a N297A mutation on IgG1, IgG2, IgG3 or IgG4, a V234A/G237A mutation on IgG2, a K214T/E233P/L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M mutation on IgG1, a

- H268Q/V309L/A330S/P331S mutation on IgG2, a L234F/L235E/D265A mutation on IgG1, a L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation on IgG1, a S228P/F234A/L235A/G237A/P238S mutation on IgG4, or a S228P/F234A/L235A/G236-deleted/G237A/P238S mutation on IgG4.
23. The method of claim 21, wherein the second mutation is the V234A/G237A/P238S/H268A/V309L/A330S/P331S mutation on IgG2.
24. The method of claim 21, wherein the second mutation is the L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation on IgG1
25. The method of claim 21, wherein the second mutation is the S228P/F234A/L235A mutation on IgG4.
26. The method of any one of claims 19-25, wherein the antibody comprises the heavy chain constant region of SEQ ID NOs: 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73 or 74.
27. The method of any one of claims 19-26, wherein the subject has a cancer.
28. The method of claim 27, wherein the cancer is a solid tumor.
29. The method of claim 28, wherein the solid tumor is a melanoma, a lung cancer, a squamous non-small cell lung cancer (NSCLC), a non-squamous NSCLC, a colorectal cancer, a prostate cancer, a castration-resistant prostate cancer, a stomach cancer, an ovarian cancer, a gastric cancer, a liver cancer, a pancreatic cancer, a thyroid cancer, a squamous cell carcinoma of the head and neck, a carcinoma of the esophagus or gastrointestinal tract, a breast cancer, a fallopian tube cancer, a brain cancer, an urethral cancer, a genitourinary cancer, an endometriosis, a cervical cancer or a metastatic lesion of the cancer.
30. The method of any one of claims 19-29, wherein the TNFR superfamily member is OX40 (SEQ ID NO: 4), CD27 (SEQ ID NO: 8), CD40 (SEQ ID NO: 5), CD137 (SEQ ID NO: 10), or GITR (SEQ ID NO: 23).
31. A method of treating a cancer in a subject, comprising administering to the subject an antibody specifically binding a TNFR superfamily member comprising a T437R mutation, a T437R/K248E mutation or a T437R/K338A mutation when compared to a parental wild-type antibody, residue numbering according to the EU Index, for a time sufficient to treat the cancer.

32. The method of claim 31, wherein the antibody comprises the T347R mutation.
33. The method of claim 31, wherein the antibody comprises the T437R/K248E mutation.
34. The antibody of claim 31, wherein the antibody comprises the T437R/K338A mutation.
35. The method of any one of claims 31-34, wherein the antibody has enhanced agonistic activity when compared to the parental antibody.
36. The method of any one of claims 31-35, wherein the antibody further comprises a second mutation.
37. The method of claim 36, wherein the second mutation is a L234A/L235A mutation on IgG1, a V234A/G237A/ P238S/H268A/V309L/A330S/P331S mutation on IgG2, a F234A/L235A mutation on IgG4, a S228P/F234A/ L235A mutation on IgG4, a N297A mutation on IgG1, IgG2, IgG3 or IgG4, a V234A/G237A mutation on IgG2, a K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M mutation on IgG1, a H268Q/V309L/A330S/P331S mutation on IgG2, a L234F/L235E/D265A mutation on IgG1, a L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation on IgG1, a S228P/F234A/L235A/G237A/P238S mutation on IgG4, or a S228P/F234A/L235A/G236-deleted/G237A/P238S mutation on IgG4.
38. The method of any one of claims 31-36, wherein the antibody comprises the heavy chain constant region of SEQ ID NOs: 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73 or 74.
39. The method of any one of claims 31-38, wherein the cancer is a melanoma, a lung cancer, a squamous non-small cell lung cancer (NSCLC), a non-squamous NSCLC, a colorectal cancer, a prostate cancer, a castration-resistant prostate cancer, a stomach cancer, an ovarian cancer, a gastric cancer, a liver cancer, a pancreatic cancer, a thyroid cancer, a squamous cell carcinoma of the head and neck, a carcinoma of the esophagus or gastrointestinal tract, a breast cancer, a fallopian tube cancer, a brain cancer, an urethral cancer, a genitourinary cancer, an endometriosis, a cervical cancer or a metastatic lesion of the cancer.

40. The method of any one of claims 31-39, wherein the receptor of the TNFR family is OX40 (SEQ ID NO: 4), CD27 (SEQ ID NO: 8), CD40 (SEQ ID NO: 5), CD137 (SEQ ID NO: 10), or GITR (SEQ ID NO: 23).
41. An isolated Fc domain containing molecule comprising a T437R mutation, a T437R/K248E mutation or a T437R/K338A mutation in the Fc domain.
42. The Fc domain containing molecule of claim 41, comprising the T437R mutation.
43. The Fc domain containing molecule of claim 41, comprising a T437R/K248E mutation.
44. The Fc domain containing molecule of claim 41, comprising a T437R/K338A mutation.
45. The Fc domain containing molecule of any one of claims 41-44, wherein the Fc domain is an IgG1, IgG2, IgG3 or IgG4 isotype.
46. The Fc domain containing molecule of any one of claims 41-45, wherein the Fc domain containing molecule is a monoclonal antibody.
47. The Fc domain containing molecule of any one of claims 41-46, comprising the amino acid sequence of SEQ ID NOs: 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73 or 74.
48. An isolated polynucleotide
- a. encoding the Fc domain containing molecule comprising a T437R mutation, a T437R/K248E mutation or a T437R/K338A mutation in the Fc domain;
 - b. encoding the Fc domain of SEQ ID NOs: 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73 or 74; or
 - c. comprising the polynucleotide sequence of SEQ ID NOs: 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, or 86.
49. A vector comprising the polynucleotide of claim 48.
50. The vector of claim 49, which is an expression vector.
51. A host cell comprising the vector of claim 49 or 50.
52. A method of producing the Fc domain containing molecule of claim 41, comprising culturing the host cell of claim 51 in conditions wherein the Fc domain

containing molecule is expressed, and isolating the Fc domain containing molecule.

53. An isolated anti-tumor necrosis factor receptor (TNFR) superfamily member antibody comprising a T437R mutation, a T437R/K248E mutation or a T437R/K338A mutation when compared to a parental wild-type antibody, residue numbering according to the EU Index, for use in the treatment of a cancer.
54. The antibody of claim 53 for use in the treatment of the cancer, wherein the cancer is a melanoma, a lung cancer, a squamous non-small cell lung cancer (NSCLC), a non-squamous NSCLC, a colorectal cancer, a prostate cancer, a castration-resistant prostate cancer, a stomach cancer, an ovarian cancer, a gastric cancer, a liver cancer, a pancreatic cancer, a thyroid cancer, a squamous cell carcinoma of the head and neck, a carcinoma of the esophagus or gastrointestinal tract, a breast cancer, a fallopian tube cancer, a brain cancer, an urethral cancer, a genitourinary cancer, an endometriosis, a cervical cancer or a metastatic lesion of the cancer.
55. Use of an isolated anti-tumor necrosis factor receptor (TNFR) superfamily member antibody comprising a T437R mutation, a T437R/K248E mutation or a T437R/K338A mutation, residue numbering according to the EU Index, in the manufacture of a medicament for the treatment of cancer.
56. The use of claim 35, wherein the cancer is a melanoma, a lung cancer, a squamous non-small cell lung cancer (NSCLC), a non-squamous NSCLC, a colorectal cancer, a prostate cancer, a castration-resistant prostate cancer, a stomach cancer, an ovarian cancer, a gastric cancer, a liver cancer, a pancreatic cancer, a thyroid cancer, a squamous cell carcinoma of the head and neck, a carcinoma of the esophagus or gastrointestinal tract, a breast cancer, a fallopian tube cancer, a brain cancer, an urethral cancer, a genitourinary cancer, an endometriosis, a cervical cancer or a metastatic lesion of the cancer.