

Claims

1. An isolated anti-tumor necrosis factor receptor (TNFR) superfamily member antibody, wherein the antibody comprises an E345R mutation, an E345R/E430G mutation or a E345R/E430G/S440Y mutation, residue numbering according to the EU Index, and has enhanced agonistic activity when compared to a parental antibody without the mutation.
2. The antibody of claim 1, comprising the E345R mutation.
3. The antibody of claim 1, comprising the E345R/E430G mutation.
4. The antibody of claim 1, comprising the E345R/E430G/S440Y mutation.
5. The antibody of any one of claims 1-4, wherein the antibody has agonistic activity independent of antibody cross-linking.
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 comprises an amino acid sequence of SEQ ID NOs: 63, 64, 65, 66 or 67.
8. The antibody of any one of claims 1-7, further comprising a second mutation.
9. The antibody of claim 7, 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 all Ig isotypes, 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.
10. The antibody of claim 9, wherein the second mutation is the V234A/G237A/P238S/H268A/V309L/A330S/P331S mutation on IgG2.
11. The antibody of claim 9, wherein the second mutation is the L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation on IgG1.
12. The antibody of claim 9, wherein the second mutation is the S228P/F234A/L235A mutation on IgG4.
13. The antibody of any one of claims 1-12, 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).
14. A pharmaceutical composition comprising the antibody of any one of claims 1-13 and a pharmaceutically acceptable carrier.

15. An engineered antibody specifically binding a TNFR superfamily member for use in enhancing an agonistic activity of an anti-TNFR superfamily member antibody in a subject, wherein an E345R mutation, an E345R/E430G mutation or an E345R/E430G/S440Y mutation is introduced into the antibody, and wherein the engineered antibody is to be administered to the subject.
16. The engineered antibody of claim 15, wherein the antibody further comprises a second mutation.
17. The engineered antibody of claim 16, 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 all Ig isotypes, 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.
18. The engineered antibody of claim 17, wherein the second mutation is the V234A/G237A/P238S/H268A/V309L/A330S/P331S mutation on IgG2.
19. The engineered antibody of claim 17, wherein the second mutation is the L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation on IgG1
20. The engineered antibody of claim 17, wherein the second mutation is the S228P/F234A/L235A mutation on IgG4.
21. The engineered antibody of any one of claims 15-20, wherein the subject has a cancer.
22. The engineered antibody of claim 21, wherein the cancer is a solid tumor.
23. The engineered antibody of claim 22, 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.
24. The engineered antibody of any one claims 15-23, 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).

25. An anti- TNFR superfamily member antibody for use in the treatment of cancer, wherein the antibody comprises an E345R mutation, an E345R/E430G mutation or an E345R/E430G/S440Y mutation for a time sufficient to treat the cancer.
26. Use of an anti- TNFR superfamily member antibody in the manufacture of a medicament for the treatment of cancer, wherein the antibody comprises an E345R mutation, an E345R/E430G mutation or an E345R/E430G/S440Y mutation for a time sufficient to treat the cancer.
27. The use of claim 26, wherein the antibody further comprises a second mutation.
28. The use of claim 27, 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 all Ig isotypes, 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.
29. The use of any one claims 26-28, 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.
30. The use of any one of claims 26-29, 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).
31. An isolated anti-tumor necrosis factor receptor (TNFR) superfamily member antibody comprising an E345R mutation, an E345R/E430G mutation or an E345R/E430G/S440Y mutation, residue numbering according to the EU Index, and has enhanced agonistic activity when compared to a parental antibody without the mutation for use in the treatment of a cancer.
32. The antibody of claim 31 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.

33. Use of an isolated anti-tumor necrosis factor receptor (TNFR) superfamily member antibody comprising an E345R mutation, an E345R/E430G mutation or an E345R/E430G/S440Y mutation, residue numbering according to the EU Index, in the manufacture of a medicament for the treatment of cancer.
34. The use of claim 33, 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.