Claims

1. An engineered immune cell expressing at its cell surface membrane an Epidermal Growth Factor Receptor Variant III (EGFRvIII) specific chimeric antigen receptor (CAR) comprising an extracellular ligand-binding domain, a first transmembrane domain, and an intracellular signaling domain, wherein the extracellular ligand-binding domain comprises a single chain Fv fragment (scFv): wherein the scFv comprises:

(a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence of one of SEQ ID NOs: 74-76, (ii) a VH CDR2 comprising the sequence of one of SEQ ID NOs: 77-78, and (iii) a VH CDR3 comprising the sequence of SEQ ID NO: 79; and

a light chain variable (VL) region comprising (i) a VL CDR1 comprising the sequence of SEQ ID NO: 156, (ii) a VL CDR2 comprising the sequence of SEQ ID NO: 157, and (iii) a VL CDR3 comprising the sequence of SEQ ID NO: 158; or

(b) a VH region comprising (i) a VH CDR1 comprising the sequence of one of SEQ ID NOs: 80-82, (ii) a VH CDR2 comprising the sequence of one of SEQ ID NOs: 83-84, and (iii) a VH CDR3 comprising the sequence of SEQ ID NO: 85; and

a VL region comprising (i) a VL CDR1 comprising the sequence of SEQ ID NO: 159, (ii) a VL CDR2 comprising the sequence of SEQ ID NO: 160, and (iii) a VL CDR3 comprising the sequence of SEQ ID NO: 161; or

(c) a VH region comprising (i) a VH CDR1 comprising the sequence of one of SEQ ID NOs: 80-82, (ii) a VH CDR2 comprising the sequence of one of SEQ ID NOs: 86-87, and (iii) a VH CDR3 comprising the sequence of SEQ ID NO: 79; and

a VL region comprising (i) a VL CDR1 comprising the sequence of SEQ ID NO: 162, (ii) a VL CDR2 comprising the sequence of SEQ ID NO: 163, and (iii) a VL CDR3 comprising the sequence of SEQ ID NO: 164; or

(d) a VH region comprising (i) a VH CDR1 comprising the sequence of one of SEQ ID NOs: 88-90, (ii) a VH CDR2 comprising the sequence of one of SEQ ID NOs: 91-92, and (iii) a VH CDR3 comprising the sequence of SEQ ID NO: 85; and

a VL region comprising (i) a VL CDR1 comprising the sequence of SEQ ID NO: 165, (ii) a VL CDR2 comprising the sequence of SEQ ID NO: 163, and (iii) a VL CDR3 comprising the sequence of SEQ ID NO: 161; or

(e) a VH region comprising (i) a VH CDR1 comprising the sequence of one of SEQ ID NOs: 109-111, (ii) a VH CDR2 comprising the sequence of one of SEQ ID NOs: 112-113, and (iii) a VH CDR3 comprising the sequence of SEQ ID NO: 114; and

a VL region comprising (i) a VL CDR1 comprising the sequence of SEQ ID NO: 182, (ii) a VL CDR2 comprising the sequence of SEQ ID NO: 183, and (iii) a VL CDR3 comprising the sequence of SEQ ID NO: 184; or

(f) a VH region comprising (i) a VH CDR1 comprising the sequence of one of SEQ ID NOs: 115-117, (ii) a VH CDR2 comprising the sequence of one of SEQ ID NOs: 118-119, and (iii) a VH CDR3 comprising the sequence of SEQ ID NO: 120; and

a VL region comprising (i) a VL CDR1 comprising the sequence of SEQ ID NO: 185, (ii) a VL CDR2 comprising the sequence of SEQ ID NO: 186, and (iii) a VL CDR3 comprising the sequence of SEQ ID NO: 184; or

(g) a VH region comprising (i) a VH CDR1 comprising the sequence of one of SEQ ID NOs: 121-123, (ii) a VH CDR2 comprising the sequence of one of SEQ ID NOs: 124-125, and (iii) a VH CDR3 comprising the sequence of SEQ ID NO: 126; and

a VL region comprising (i) a VL CDR1 comprising the sequence of SEQ ID NO: 187, (ii) a VL CDR2 comprising the sequence of SEQ ID NO: 188, and (iii) a VL CDR3 comprising the sequence of SEQ ID NO: 189; or

(h) a VH region comprising (i) a VH CDR1 comprising the sequence of one of SEQ ID NOs: 137-139, (ii) a VH CDR2 comprising the sequence of one of SEQ ID

NOs: 140-141, and (iii) a VH CDR3 comprising the sequence of SEQ ID NO: 142; and

a VL region comprising (i) a VL CDR1 comprising the sequence of SEQ ID NO: 195, (ii) a VL CDR2 comprising the sequence of SEQ ID NO: 196, and (iii) a VL CDR3 comprising the sequence of SEQ ID NO: 197; or

(i) a VH region comprising (i) a VH CDR1 comprising the sequence of one of SEQ ID NOs: 74-76, (ii) a VH CDR2 comprising the sequence of one of SEQ ID NOs: 102-103, and (iii) a VH CDR3 comprising the sequence of SEQ ID NO: 104; and

a VL region comprising (i) a VL CDR1 comprising the sequence of SEQ ID NO: 176, (ii) a VL CDR2 comprising the sequence of SEQ ID NO: 172, and (iii) a VL CDR3 comprising the sequence of SEQ ID NO: 177.

2. The engineered immune cell of claim 1, wherein the scFv comprises:

(a) a VH region comprising the sequence shown in SEQ ID NO: 9, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR; and

a VL region comprising the amino acid sequence shown in SEQ ID NO: 10, or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR; or

(b) a VH region comprising the sequence shown in SEQ ID NO: 11 or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR; and

a VL region comprising the amino acid sequence shown in SEQ ID NO: 12, or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR; or

(c) a VH region comprising the sequence shown in SEQ ID NO: 13, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR; and

a VL region comprising the amino acid sequence shown in SEQ ID NO: 14, or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR; or

(d) a VH region comprising the sequence shown in SEQ ID NO: 15, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR; and

a VL region comprising the amino acid sequence shown in SEQ ID NO: 16, or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR; or

(e) a VH region comprising the sequence shown in SEQ ID NO: 37, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR; and

a VL region comprising the amino acid sequence shown in SEQ ID NO: 38, or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR; or

(f) a VH region comprising the sequence shown in SEQ ID NO: 39, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR; and

a VL region comprising the amino acid sequence shown in SEQ ID NO: 40, or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR; or

(g) a VH region comprising the sequence shown in SEQ ID NO: 41, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR; and

a VL region comprising the amino acid sequence shown in SEQ ID NO: 42, or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR; or

(h) a VH region comprising the sequence shown in SEQ ID NO: 48, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR; and

a VL region comprising the amino acid sequence shown in SEQ ID NO: 49, or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR; or

(i) a VH region comprising the sequence shown in SEQ ID NO: 30, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR; and

a VL region comprising the amino acid sequence shown in SEQ ID NO: 31, or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR.

3. The engineered immune cell of claim 1 or 2, wherein the intracellular signaling domain:

(i) comprises a CD3zeta signalling domain or a 4-1BB signaling domain; and/or

(ii) is a first intracellular signaling domain and the the CAR further comprises a second intracellular signaling domain; optionally wherein the first intracellular signaling domain comprises a CD3zeta signaling domain and the second intracellular signaling domain comprises a 4-1BB signaling domain.

4. The engineered immune cell of any one of claims 1 to 3, further comprising:

(i) a stalk domain between the extracellular ligand-binding domain and the first transmembrane domain; optionally wherein the stalk domain is selected from the group consisting of: a human CD8α hinge, a human CD28 hinge, an IgG1 hinge, and an FcγRIIIα hinge; or

(ii) a second extracellular ligand-binding domain, wherein the second extracellular ligand-binding domain is not specific for EGFRvIII; or

(iii) a second transmembrane domain, wherein the first transmembrane domain and the extracellular ligand-binding domain(s) are on a first polypeptide, and wherein

the second transmembrane domain and the intracellular signaling domain(s) are on a second polypeptide, wherein the first transmembrane domain comprises a transmembrane domain from the α chain of the high-affinity IgE receptor (FcεRI) and the second transmembrane domain comprises a transmembrane domain from the γ or β chain of FcεRI; or

(iv) a third polypeptide comprising a third transmembrane domain fused to an intracellular signaling domain from a co-stimulatory molecule, wherein the third transmembrane domain comprises a transmembrane domain from the γ or β chain of FcεRI.

5. The engineered immune cell of any one of claims 1 to 4, wherein the first transmembrane domain comprises a CD8α chain transmembrane domain; and/or

wherein the extracellular ligand-binding domain(s), the first transmembrane domain, and intracellular signaling domain(s) are on a single polypeptide.

6. The engineered immune cell of any one of claims 1 to 5, further comprising a polynucleotide encoding a suicide polypeptide; optionally:

(i) wherein the suicide polypeptide is RQR8, is R2, or comprises one or more copies of SEQ ID NO: 256; or

(ii) wherein the polynucleotide encoding the suicide polypeptide is part of the same nucleic acid molecule as the polynucleotide comprising a nucleic acid sequence encoding the EGFRvIII specific CAR, and wherein the suicide polypeptide is in the same polypeptide chain as the CAR.

7. The engineered immune cell of any one of claims 1 to 6:

(i) further comprising another CAR which is not specific for EGFRvIII; and/or

(ii) wherein the immune cell is derived from an inflammatory T-lymphocyte, a cytotoxic T-lymphocyte, a regulatory T- lymphocyte, a memory T-lymphocyte, a helper T-lymphocyte, a natural killer T-lymphocyte, or a natural killer cell; and/or

(iii) further comprising a disruption of one or more endogenous genes, wherein the endogenous gene encodes TCRα, TCRβ, CD52, glucocorticoid receptor (GR), deoxycytidine kinase (dCK), or programmed death-1 (PD-1); and/or

(iv) wherein the immune cell is obtained from a healthy donor or is obtained from a patient.

8. A pharmaceutical composition comprising the engineered immune cell of any one of claims 1 to 7.

9. The engineered immune cell of any one of claims 1 to 7, or the pharmaceutical compostion of claim 8, for use as a medicament.

10. The engineered immune cell of any one of claims 1 to 7, or the pharmaceutical compostion of claim 8, for use in treatment of: cancer; tumor growth, progression, or metastasis in a subject who has malignant cells expressing EGFRvIII; or an EGFRvIII related cancer selecting from the group consisting of glioblastoma multiforme, anaplastic astrocytoma, giant cell glioblastoma, gliosarcoma, anaplastic oligodendroglioma, anaplastic ependymoma, anaplastic oligoastrocytoma, choroid plexus carcinoma, anaplastic ganglioglioma, pineoblastoma, pineocytoma, meningioma, medulloepithelioma, ependymoblastoma, medulloblastoma, supraentorial primitive neuroectodermal tumor, atypical teratoid/rhabdoid tumor, mixed glioma, head and neck cancer, non-small cell lung cancer, breast cancer, ovarian cancer, prostate cancer, medullobastoma, colorectal cancer, anal cancer, cervical cancer, renal cancer, skin cancer, pancreatic cancer, liver cancer, bladder cancer, gastric cancer, thyroid cancer, mesothelioma, uterine cancer, lymphoma, and leukemia.

11. Use of the engineered immune cell of any one of claims 1 to 7, or the pharmaceutical compostion of claim 8, in the manufacture of a medicament for the treatment of: cancer; tumor growth, progression, or metastasis in a subject who has malignant cells expressing EGFRvIII; or an EGFRvIII related cancer selecting from the group consisting of glioblastoma multiforme, anaplastic astrocytoma, giant cell glioblastoma, gliosarcoma, anaplastic oligodendroglioma, anaplastic ependymoma, anaplastic oligoastrocytoma, choroid plexus carcinoma, anaplastic ganglioglioma, pineoblastoma, pineocytoma, meningioma, medulloepithelioma, ependymoblastoma, medulloblastoma, supraentorial primitive neuroectodermal tumor, atypical

teratoid/rhabdoid tumor, mixed glioma, head and neck cancer, non-small cell lung cancer, breast cancer, ovarian cancer, prostate cancer, medullobastoma, colorectal cancer, anal cancer, cervical cancer, renal cancer, skin cancer, pancreatic cancer, liver cancer, bladder cancer, gastric cancer, thyroid cancer, mesothelioma, uterine cancer, lymphoma, and leukemia.

12. An Epidermal Growth Factor Receptor Variant III (EGFRvIII) specific chimeric antigen receptor (CAR) comprising an extracellular ligand-binding domain, a first transmembrane domain, and an intracellular signaling domain, wherein the extracellular ligand-binding domain comprises a single chain Fv fragment (scFv): wherein the scFv comprises a heavy chain variable (VH) region comprising (i) a VH CDR1 comprising the sequence of one of SEQ ID NOs: 74-76, (ii) a VH CDR2 comprising the sequence of one of SEQ ID NOs: 102-103, and (iii) a VH CDR3 comprising the sequence of SEQ ID NO: 104; and a light chain variable (VL) region comprising (i) a VL CDR1 comprising the sequence of SEQ ID NO: 176, (ii) a VL CDR2 comprising the sequence of SEQ ID NO: 172, and (iii) a VL CDR3 comprising the sequence of SEQ ID NO: 177.

13. The EGFRvIII specific CAR of claim 12, wherein the scFv comprises a VH region comprising the sequence shown in SEQ ID NO: 30, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR; and a VL region comprising the amino acid sequence shown in SEQ ID NO: 31, or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR.