CLAIMS

1. An isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein said CAR comprises an anti-EGFRvIII binding domain, a transmembrane domain, and an intracellular signaling domain comprising a primary signaling domain, a costimulatory domain or both a primary signaling domain and a costimulatory domain, and wherein the encoded anti-EGFRvIII binding domain comprises:

(a) an amino acid sequence having not more than 10 modifications of the light chain variable region and heavy chain variable region of SEQ ID NO: 68; or

(b) an amino acid sequence with at least 95% sequence identity to the light chain variable region and the heavy chain variable region of SEQ ID NO: 68;

and wherein:

(i) the light chain variable region comprises:

(i)(a) a light chain complementary determining region 1 (LC CDR1) comprising the sequence of SEQ ID NO:26;

(i)(b) a light chain complementary determining region 2 (LC CDR2) comprising the sequence of SEQ ID NO:27; and

(i)(c) a light chain complementary determining region 3 (LC CDR3) comprising the sequence of SEQ ID NO: 28; and

(ii) the heavy chain variable region comprises:

(ii)(a) a heavy chain complementary determining region 1 (HC CDR1) comprising the sequence of SEQ ID NO: 22;

(ii)(b) a heavy chain complementary determining region 2 (HC CDR2) comprising the sequence of SEQ ID NO: 23; and

(ii)(c) and a heavy chain complementary determining region 3 (HC CDR3) comprising the sequence of SEQ ID NO:24.

2. The isolated nucleic acid molecule of claim 1, wherein the encoded EGFRvIII binding domain comprises:

(a) the light chain variable region comprising:

(i) a sequence with 95-99% identity to the amino acid sequence of the light chain variable region of SEQ ID NO:68; or

(iii) the amino acid sequence of the light chain variable region of SEQ ID NO:68; and

(b) the heavy chain variable region comprising:
(i) a sequence with 95-99% identity to the amino acid sequence of the heavy chain variable region of SEQ ID NO:68; or
(ii) the amino acid sequence of the heavy chain variable region of SEQ ID NO: 68.

3. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded anti-EGFRvIII binding domain comprises the amino acid sequence of SEQ ID NO:68.

4. The isolated nucleic acid molecule of any of the preceding claims, wherein the nucleic acid sequence encoding the anti-EGFRvIII binding domain comprises the nucleic acid sequence of SEQ ID NO:69, or a nucleic acid sequence with 95-99% identify thereof.

5. The isolated nucleic acid molecule of any of the preceding claims, wherein:
(i) the encoded CAR includes a transmembrane domain that comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154;
(ii) the encoded transmembrane domain comprises the amino acid sequence of SEQ ID NO: 15, or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:15, ,and/or
(iii) the nucleic acid sequence encoding the transmembrane domain comprises the sequence of SEQ ID NO:8, or a sequence with 95-99% identity thereof.

6. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded anti-EGFRvIII binding domain is connected to the transmembrane domain by a hinge region, optionally wherein:
(a) the encoded hinge region comprises SEQ ID NO:14, or a sequence with 95-99% identity thereof, or (b) the nucleic acid sequence encoding the hinge region comprises the sequence of SEQ ID NO:7, or a sequence with 95-99% identify thereof.

7. The isolated nucleic acid molecule of any of the preceding claims, wherein the encoded intracellular signaling domain comprises a primary signaling domain and/or a

costimulatory domain, e.g., a functional signaling domain, of a protein selected from the group consisting of OX40, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), and 4-1BB (CD137), optionally wherein:
(a) the encoded costimulatory domain comprises the sequence of SEQ ID NO:16, or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:16; or
(b) the nucleic acid sequence encoding the costimulatory domain comprises the sequence of SEQ ID NO:9, or a sequence with 95-99% identify thereof.

8. The isolated nucleic acid molecule of any of the preceding claims, wherein:

(i) the encoded intracellular signaling domain comprises a primary signaling domain;

(ii) the encoded intracellular signaling domain comprises a costimulatory signaling domain;

(iii) the encoded intracellular signaling domain comprises a functional signaling domain of 4-1BB and a functional signaling domain of CD3 zeta;

(iv) the encoded costimulatory domain comprises the sequence of SEQ ID NO: 16; or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO: 16, and the encoded primary signaling domain comprises the sequence of SEQ ID NO: 17 or SEQ ID NO:99; or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:17 or SEQ ID NO:99;

(iv) the encoded intracellular signaling domain comprises the sequence of SEQ ID NO: 16 and the sequence of SEQ ID NO: 17 or SEQ ID NO:99, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain; and/or

(v) the nucleic acid sequence encoding the intracellular signaling domain comprises the sequence of SEQ ID NO:9, or a sequence with 95-99% identify thereof, and/or the sequence of SEQ ID NO:10 or SEQ ID NO:100, or a sequence with 95-99% identity thereof.

9. The isolated nucleic acid molecule of any of the preceding claims, further comprising a leader sequence, optionally wherein the leader sequence comprises SEQ ID NO: 13.

10. An isolated polypeptide molecule encoded by the nucleic acid molecule of any of the preceding claims, wherein the isolated polypeptide molecule comprises the sequence SEQ ID NO:73; or a sequence with 95-99% identity thereof.

11. An isolated chimeric antigen receptor (CAR) molecule comprising an anti-EGFRvIII binding domain, a transmembrane domain, and an intracellular signaling domain comprising a primary signaling domain, a costimulatory domain, or both a primary signaling domain and a costimulatory domain, wherein the encoded anti-EGFRvIII binding domain comprises:
(a) an amino acid sequence having not more than 10 modifications of the light chain variable region and heavy chain variable region of SEQ ID NO: 68; or
(b) an amino acid sequence with at least 95% sequence identity to the light chain variable region and the heavy chain variable region of SEQ ID NO:68;
and wherein:

(i) the heavy chain variable region comprises:

(i)(a) a HC CDR1 comprising the sequence of SEQ ID NO: 22;

(i)(b) a HC CDR2 comprising the sequence of SEQ ID NO: 23; and

(i)(c) a HC CDR3 comprising the sequence of SEQ ID NO: 24; and

(ii) the light chain variable region comprises:

(ii)(a) a LC CDR1 comprising the sequence of SEQ ID NO: 26;

(ii)(b) a LC CDR2 comprising the sequence of SEQ ID NO: 27; and

(ii)(c) a LC CDR3 comprising the sequence of SEQ ID NO: 28.

12. The isolated CAR molecule of claim 11, wherein the anti-EGFRvIII binding domain comprises:
(a) the light chain variable region comprising:
(i) a sequence with 95-99% identity to an amino acid sequence of the light chain variable region of SEQ ID NO:68; or
(ii) the amino acid sequence of the light chain variable region of SEQ ID NO:68; and
(b) the heavy chain variable region comprising:
(i) a sequence with 95-99% identity to an amino acid of the heavy chain variable region of SEQ ID NO:68; or
(ii) the amino acid sequence of the heavy chain variable region of SEQ ID NO: 68.

13. The isolated CAR molecule of claims 11 or 12, wherein the anti-EGFRvIII binding domain comprises the amino acid sequence of SEQ ID NO:68.

14. The isolated CAR molecule of any of claims 11-13, wherein:
(i) the CAR molecule comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154; or
(ii), the transmembrane domain comprises the amino acid sequence of SEQ ID NO:15; or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:15.

15. The isolated CAR molecule of any of claims 11-14, wherein the anti-EGFRvIII binding domain is connected to the transmembrane domain by a hinge region, optionally wherein the encoded hinge region comprises SEQ ID NO:14, or a sequence with 95-99% identity thereof.

16. The isolated CAR molecule of any of claims 11-15, wherein the intracellular signaling domain comprises a primary signaling domain and/or a costimulatory domain, e.g., a functional signaling domain, of a protein selected from the group consisting of OX40, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18) and 4-1BB (CD137), optionally wherein:
the costimulatory domain comprises the sequence of SEQ ID NO:16; or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:16.

17. The isolated CAR molecule of any of claims 11-16, wherein:
(i) the intracellular signaling domain comprises a primary signaling domain;
(ii) the intracellular signaling domain comprises a costimulatory signaling domain;
(iii) the encoded intracellular signaling domain comprises a functional signaling domain of 4-1BB and/or a functional signaling domain of CD3 zeta;
(iv) the costimulatory domain comprises the sequence of SEQ ID NO: 16; or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO: 16, and the encoded primary signaling domain comprises the sequence of SEQ ID NO: 17 or SEQ ID NO:99; or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:17 or SEQ ID NO:99; or
(v) the encoded intracellular signaling domain comprises the sequence of SEQ ID NO: 16 and the sequence of SEQ ID NO: 17 or SEQ ID NO:99, wherein the sequences comprising the

intracellular signaling domain are expressed in the same frame and as a single polypeptide chain.

18. The isolated CAR molecule of any of claims 11-17, further comprising a leader sequence, optionally wherein the leader sequence comprises the amino acid sequence of SEQ ID NO: 13, or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO: 13.

19. An anti-EGFRvIII binding domain comprising:
(a) an amino acid sequence having not more than 10 modifications of the light chain variable region and heavy chain variable region of SEQ ID NO:68; or
(b) an amino acid sequence with at least 95% sequence identity to the light chain variable region and the heavy chain variable region of SEQ ID NO:68;
and wherein:
(i) the heavy chain variable region comprises:
(i)(a) a HC CDR1 comprising the sequence of SEQ ID NO: 22;
(i)(b) a HC CDR2 comprising the sequence of SEQ ID NO: 23; and
(i)(c) a HC CDR3 comprising the sequence of SEQ ID NO: 24; and
(ii) the light chain variable region comprises:
(ii)(a) a LC CDR1 comprising the sequence of SEQ ID NO: 26;
(ii)(b) a LC CDR2 comprising the sequence of SEQ ID NO: 27; and
(ii)(c) a LC CDR3 comprising the sequence of SEQ ID NO: 28.

20. The anti-EGFRvIII binding domain of claim 19, comprising:
(a) the light chain variable region comprising:
(i) a sequence with 95-99% identity to an amino acid sequence of the light chain variable region of SEQ ID NO:68; or
(ii) the amino acid sequence of the light chain variable region of SEQ ID NO:68; and
(b) the heavy chain variable region comprising:
(i) a sequence with 95-99% identity to an amino acid of the heavy chain variable region of SEQ ID NO:68; or
(ii) the amino acid sequence of the heavy chain variable region of SEQ ID NO: 68.

21. The anti-EGFRvIII binding domain of claim 19 or 20, wherein the anti-EGFRvIII binding domain comprises the amino acid sequence of SEQ ID NO:68.

22. An isolated nucleic acid molecule encoding the anti-EGFRvIII binding domain of claim 21, wherein the nucleic acid comprises the nucleic acid sequence of SEQ ID NO:69, or a nucleic acid sequence with 95-99% identify thereof.

23. A vector comprising a nucleic acid molecule encoding a CAR of any of claims 11-18.

24. The vector of claim 23, wherein the vector is selected from the group consisting of a DNA, a RNA, a plasmid, a lentivirus vector, adenoviral vector, or a retrovirus vector, optionally wherein the vector further comprises a promoter, wherein the promoter is an EF-1 promoter, wherein the EF-1 promoter comprises the sequence of SEQ ID NO: 97.

25. The vector of claim 24, wherein the vector is an in vitro transcribed vector.

26. The vector of claim 24 or 25, wherein the nucleic acid sequence in the vector further comprises a poly(A) tail and/or the nucleic acid sequence in the vector further comprises a 3'UTR.

27. A cell comprising the vector of any of claims 23-26, optionally wherein the cell is a T cell, e.g., a CD8+ T cell.

28. The cell of claim 27, wherein the cell is a human cell.

29. An *in vitro* method of making a cell comprising transducing a T cell with a vector of any of claims 23-26.

30. An *in vitro* method of generating a population of RNA-engineered cells comprising introducing an in vitro transcribed RNA or synthetic RNA into a cell, where the RNA comprises a nucleic acid encoding a CAR molecule comprising an anti-EGFRvIII binding domain of any of claims 19-22.

31. Use of an effective amount of a cell expressing a CAR molecule of any of claims 13-23 in the manufacture of a medicament for providing an anti-tumor immunity in a mammal, optionally wherein the cell is an autologous T cell or an allogeneic T cell.

32. The use of the cell of claim 31, wherein the mammal is a human.

33. Use of an effective amount of a cell expressing a CAR molecule of any of claims 11-18 in the manufacture of a medicament for the treatment of a mammal having a disease associated with overexpression of EGFRvIII.

34. Use of an effective amount of a cell expressing a CAR molecule of any of claims 11-18 in the manufacture of a medicament for the treatment of a mammal having a cancer.

35. The use of claim 34, wherein the cancer is selected from the group consisting of glioblastoma, glioblastoma multiforme (GBM), anaplastic astrocytoma, giant cell glioblastoma, gliosarcoma, anaplastic oligodendroglioma, anaplastic ependymoma, choroid plexus carcinoma, anaplastic ganglioglioma, pineoblastoma, medulloepithelioma, ependymoblastoma, medulloblastoma, supratentorial primitive neuroectodermal tumor, atypical teratoid/rhabdoid tumor, lung cancer (e.g., non-small cell lung carcinomas) breast, prostate, ovarian, colorectal and bladder carcinoma; any combination thereof; and metastases of any of the cancers.

36. The use of the cell of claim 35, wherein the cells expressing a CAR molecule are to be administered in combination with:
(a) an agent that increases the efficacy of a cell expressing a CAR molecule;
(b) an agent that ameliorates one or more side effects associated with administration of a cell expressing a CAR molecule; and/or
(c) an agent that treats the disease associated with overexpression of EGFRvIII.

37. The isolated nucleic acid molecule of any of claims 1-9, the isolated polypeptide molecule of claim 10, the isolated CAR of any of claims 11-18, , the vector of any of claims 23-26, or the cell of claim 27 or 28 for use as a medicament.

38. The isolated nucleic acid molecule of any of claims 1-9, the isolated polypeptide molecule of claim 10, the isolated CAR of any of claims 11-18, the vector of any of claims 23-26,or the cell of claim 27 or 28 for use in the treatment of a disease associated with overexpression of EGFRvIII.

39. The isolated nucleic acid molecule of any of claims 1-9, the isolated polypeptide molecule of claim 10, the isolated CAR of any of claims 11-18, the vector of any of claims 23-26,or the cell of claim 27 or 28 for use in the treatment of a cancer.

40. The use of claim 39, wherein the cancer is selected from the group consisting of glioblastoma, glioblastoma multiforme (GBM), anaplastic astrocytoma, giant cell glioblastoma, gliosarcoma, anaplastic oligodendroglioma, anaplastic ependymoma, choroid plexus carcinoma, anaplastic ganglioglioma, pineoblastoma, medulloepithelioma, ependymoblastoma, medulloblastoma, supratentorial primitive neuroectodermal tumor, atypical teratoid/rhabdoid tumor, lung cancer (e.g., non-small cell lung carcinomas) breast, prostate, ovarian, colorectal and bladder carcinoma; any combination thereof; and metastases of any of the cancers.