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 stimulatory domain, and wherein said anti-EGFRvIII binding domain comprises one or more of light chain complementary determining region 1 (LC CDR1), light chain complementary determining region 2 (LC CDR2), and light chain complementary determining region 3 (LC CDR3) of any anti-EGFRvIII light chain binding domain amino acid sequence listed in Table 2 or SEQ ID NO:11, and one or more of heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of any anti-EGFRvIII heavy chain binding domain amino acid sequence listed in Table 2 or SEQ ID NO:11.

2. The isolated nucleic acid molecule of claim 1, comprising: (a) LC CDR1, LC CDR2 and LC CDR3 of any anti-EGFRvIII light chain binding domain amino acid sequence listed in Table 2 or SEQ ID NO:11; (b) HC CDR1, HC CDR2, and HC CDR3 of any anti-EGFRvIII heavy chain binding domain amino acid sequence listed in Table 2 or SEQ ID NO:11; or (c) (a) and (b).

3. The isolated nucleic acid molecule of claim 1, comprising: (a) any light chain variable region listed in Table 2 or SEQ ID NO:11; (b) any heavy chain variable region listed in Table 2 or SEQ ID NO:11; or (c) (a) and (b).

4. The isolated nucleic acid molecule of any of the preceding claims, wherein the EGFRvIII binding domain is a scFv, optionally wherein: (a) the light chain variable region comprises an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of an amino acid sequence of a light chain variable region provided in Table 2 or SEQ ID NO:11, or a sequence with 95-99% identity to an amino acid sequence provided in Table 2 or SEQ ID NO:11; (b) the heavy chain variable region comprises an amino acid sequence having at least one, two or three modifications but

not more than 30, 20 or 10 modifications of an amino acid sequence of a heavy chain variable region provided in Table 2 or SEQ ID NO:11, or a sequence with 95-99% identity to an amino acid sequence provided in Table 2 or SEQ ID NO:11; or (c) both (a) and (b).

5. The isolated nucleic acid molecule of any of the preceding claims, wherein the anti-EGFRvIII binding domain comprises a sequence selected from a group consisting of SEQ ID NO:38, SEQ ID NO:44, SEQ ID NO:50, SEQ ID NO:56, SEQ ID NO:62, SEQ ID NO:68, SEQ ID NO:74, SEQ ID NO:80, and SEQ ID NO:86, or a sequence with 95-99% identify thereof, optionally wherein the nucleic acid sequence encoding the anti-EGFRvIII binding domain comprises a sequence selected from a group consisting of SEQ ID NO:39, SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57, SEQ ID NO:63, SEQ ID NO:69, SEQ ID NO:75, SEQ ID NO:81 and SEQ ID NO:98, or a sequence with 95-99% identify thereof.

6. The isolated nucleic acid molecule of any of the preceding claims, wherein 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, optionally wherein the encoded transmembrane domain comprises a sequence of SEQ ID NO: 15, or an amino acid sequence comprising at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:15, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:15, preferably wherein the nucleic acid sequence encoding the transmembrane domain comprises a sequence of SEQ ID NO:8, or a sequence with 95-99% identify thereof.

7. 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 a sequence of SEQ ID NO:7, or a sequence with 95-99% identify thereof.

8. The isolated nucleic acid molecule of any of the preceding claims, further comprising a sequence encoding a costimulatory domain, optionally wherein the encoded costimulatory domain comprises 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), preferably wherein: (a) the encoded costimulatory domain comprises a sequence of SEQ ID NO:16; (b) the encoded costimulatory domain comprises an amino acid sequence comprising at least one, two or three modifications (e.g., substitutions) but not more than 20, 10 or 5 modifications (e.g., substitutions) of an amino acid sequence of SEQ ID NO:16, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:16; or (c) the nucleic acid sequence encoding the costimulatory domain comprises a sequence of SEQ ID NO:9, or a sequence with 95-99% identify thereof.

9. The isolated nucleic acid molecule of any of the preceding claims, further comprising a sequence encoding an intracellular signaling domain, optionally wherein the encoded intracellular signaling domain comprises a functional signaling domain of 4-1BB and/or a functional signaling domain of CD3 zeta, preferably wherein the encoded intracellular signaling domain comprises the sequence of SEQ ID NO: 16 and/or the sequence of SEQ ID NO:17 or SEQ ID NO:99.

10. The isolated nucleic acid molecule of any of the preceding claims, wherein the intracellular signaling domain comprises an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:16 and/or an amino acid sequence of SEQ ID NO:17 or SEQ ID NO:99, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:16 and/or an amino acid sequence of SEQ ID NO:17 or SEQ ID NO:99, optionally wherein 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 and the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain, preferably wherein the nucleic acid sequence encoding the intracellular signaling domain comprises a sequence of SEQ ID NO:9, or a sequence with 95-99% identify thereof, and/or a sequence of SEQ ID NO:10 or SEQ ID NO:100, or a sequence with 95-99% identity thereof.

11. 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.

12. An isolated polypeptide molecule encoded by the nucleic acid molecule of claim 11, optionally wherein the isolated polypeptide molecule comprises a sequence selected from the group consisting of SEQ ID NO:43, SEQ ID NO:49, SEQ ID NO:55, SEQ ID NO:61, SEQ ID NO:67, SEQ ID NO:73, SEQ ID NO:79, SEQ ID NO:85 and SEQ ID NO:90, or a sequence with 95-99% identify thereof, preferably wherein the isolated polypeptide molecule comprises a sequence of SEQ ID NO:73, or a sequence with 95-99% identify thereof or a sequence of SEQ ID NO:79, or a sequence with 95-99% identify thereof.

13. An isolated chimeric antigen receptor (CAR) molecule comprising an anti-EGFRvIII binding domain, a transmembrane domain, and an intracellular signaling domain.

14. The isolated CAR molecule of claim 13, wherein the intracellular signaling domain comprises a costimulatory domain and/or a primary signaling domain, optionally wherein the anti-EGFRvIII binding domain comprises an antibody or antibody fragment which includes an anti-EGFRvIII binding domain.

15. The isolated CAR molecule of claim 13 or 14, wherein the anti-EGFRvIII binding domain comprises one or more light chain complementary determining region 1 (LC CDR1), light chain complementary determining region 2 (LC CDR2), and light chain complementary determining region 3 (LC CDR3) of any anti-EGFRvIII binding domain listed in Table 2 or SEQ ID NO:11, and one or more heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of any anti-EGFRvIII binding domain listed in Table 2 or SEQ ID NO:11, optionally wherein the isolated CAR molecule comprises: (a) LC CDR1, LC CDR2, and LC CDR3 of any anti-EGFRvIII light chain binding domain amino acid sequence listed in Table 2 or SEQ ID NO: 11; (b) HC CDR1, HC CDR2, and HC CDR3 of any anti-EGFRvIII heavy chain binding domain amino acid sequence listed in Table 2 or SEQ ID NO:11; or (c) (a) and (b).

16. The isolated CAR molecule of any of claims 13-15, wherein the anti-EGFRvIII binding domain is a scFv, optionally wherein the anti-EGFRvIII binding domain comprises: (a) a light chain and a heavy chain of an amino acid sequence listed in Table 2 or SEQ ID NO:11; (b) a light chain variable region comprising an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of an amino acid sequence of a light chain variable region provided in Table 2 or SEQ ID NO:11, or a sequence with 95-99% identity with an amino acid sequence provided in Table 2 or SEQ ID NO:11; and/or (c) a heavy chain variable region comprising an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of an amino acid sequence of a heavy chain variable region provided in Table 2 or SEQ ID NO:11, or a sequence with 95-99% identity to an amino acid sequence provided in Table 2 or SEQ ID NO:11.

17. The isolated CAR molecule of any of claims 13-16, wherein the anti-EGFRvIII binding domain comprises a sequence selected from a group consisting of SEQ ID NO:38, SEQ ID NO:44, SEQ ID NO:50, SEQ ID NO:56, SEQ ID NO:62, SEQ ID NO:68, SEQ ID NO:74, SEQ ID NO:80, and SEQ ID NO:86, or a sequence with 95-99% identify thereof.

18. The isolated CAR molecule of any of claims 13-17, further comprising 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, optionally wherein the transmembrane domain comprises: (a) a sequence of SEQ ID NO:15; and/or (b) an amino acid sequence having at least one, two, or three modifications, but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:15, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:15.

19. The isolated CAR molecule of any of claims 13-18, 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.

20. The isolated CAR molecule of any of claims 13-19 further comprising a sequence encoding a costimulatory domain, optionally wherein the costimulatory domain comprises 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), preferably wherein the costimulatory domain comprises: (a) a sequence of SEQ ID NO:16 or; (b) an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:16, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:16.

21. The isolated CAR molecule of any of claims 13-20, further comprising a sequence encoding an intracellular signaling domain, optionally wherein the intracellular signaling domain comprises a functional signaling domain of 4-1BB and/or a functional signaling domain of CD3 zeta, preferably wherein the intracellular signaling domain comprises: (a) the sequence of SEQ ID NO: 16 and/or the sequence of SEQ ID NO: 17; or (b) the sequence of SEQ ID NO: 16 and/or the sequence of SEQ ID NO: 99.

22. The isolated CAR molecule of claim 21, wherein the intracellular signaling domain comprises an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO:16 and/or an amino acid sequence of SEQ ID NO:17 or SEQ ID NO:99, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:16 and/or an amino acid sequence of SEQ ID NO:17 or SEQ ID NO:99, optionally wherein the intracellular signaling domain comprises the sequence of SEQ ID NO: 16 and the sequence of SEQ ID NO: 17 or SEQ ID NO:99, preferably wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain.

23. The isolated CAR molecule of any of claims 13-22, further comprising a leader sequence, optionally wherein the leader sequence comprises an amino acid sequence of SEQ ID NO: 13, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO: 13.

24. An anti-EGFRvIII binding domain comprising one or more light chain complementary determining region 1 (LC CDR1), light chain complementary determining region 2 (LC CDR2), and light chain complementary determining region 3 (LC CDR3) of an anti-EGFRvIII binding domain in SEQ ID NO:38, 44, 50, 56, 62, 68, 74 or 80, and one or more heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of an anti-EGFRvIII binding domain in SEQ ID NO:38, 44, 50, 56, 62, 68, 74 or 80.

25. The anti-EGFRvIII binding domain of claim 24, wherein the anti-EGFRvIII binding domain comprises: (a) LC CDR1, LC CDR2 and LC CDR3 of an anti-EGFRvIII light chain binding domain amino acid sequence in SEQ ID NO: 38, 44, 50, 56, 62, 68, 74 or 80; (b) HC CDR1, HC CDR2, and HC CDR3 of an anti-EGFRvIII heavy chain binding domain amino acid sequence in SEQ ID NO:38, 44, 50, 56, 62, 68, 74 or 80; or (c) (a) and (b) .

26. The anti-EGFRvIII binding domain of claim 24 or 25, comprising a light chain variable region in Table 2 and a heavy chain variable region in SEQ ID NO: 38, 44, 50, 56, 62, 68, 74 or 80, optionally wherein the anti-EGFRvIII binding domain comprises an amino acid sequence having at least one, two or three modifications, but not more than 30, 20 or 10 modifications of an amino acid sequence of a light chain variable region provided in SEQ ID NO:38, 44, 50, 56, 62, 68, 74 or 80, or a sequence with 95-99% identity with an amino acid sequence in SEQ ID NO:38, 44, 50, 56, 62, 68, 74 or 80; and/or a heavy chain variable region comprising an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of an amino acid sequence of a heavy chain variable region provided in SEQ ID NO:38, 44, 50, 56, 62, 68, 74 or 80, or a sequence with 95-99% identity to an amino acid sequence in SEQ ID NO:38, 44, 50, 56, 62, 68, 74 or 80, preferably wherein the anti-EGFRvIII binding domain comprises a sequence selected from a group consisting of SEQ ID NO:38, SEQ ID NO:44, SEQ ID NO:50, SEQ ID NO:56, SEQ ID NO:62, SEQ ID NO:68, SEQ ID NO:74, SEQ ID NO:80, and SEQ ID NO:86, or a sequence with 95-99% identify thereof.

27. A vector comprising a nucleic acid molecule encoding a CAR of any of the preceding claims.

28. The vector of claim 27, 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, preferably wherein the promoter is an EF-1 promoter, such as the EF-1 promoter comprises a sequence of SEQ ID NO: 97.

29. The vector of claim 28, wherein the vector is an in vitro transcribed vector.

30. The vector of claim 28 or 29, 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.

31. A cell comprising the vector of any of claims 27-30, optionally wherein the cell is a T cell, preferably wherein the T cell is a CD8+ T cell.

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

33. A method of making a cell comprising transducing a T cell with a vector of any of claims 27-32.

34. A 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 described herein.

35. 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.

36. The use of the cell of claim 35, wherein the mammal is a human.

37. 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 the treatment of a mammal having a disease associated with expression of EGFRvIII, optionally wherein the disease associated with EGFRvIII is a glioblastoma, preferably wherein the disease associated with EGFRvIII is a cancer selected from the group consisting of 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 and any combination thereof, and metastases of any of the cancers.

38. The use of the cell of claim 37, 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 EGFRvIII.

39. The isolated nucleic acid molecule of any of claims 1-11, the isolated polypeptide molecule of claim 12, the isolated CAR of any of claims 13-23, the anti-EGFRvIII binding domain of any of claims 24-26, the vector of any of claims 27-30, or the cell of claim 31 or 32 for use as a medicament.

40. The isolated nucleic acid molecule of any of claims 1-11, the isolated polypeptide molecule of claim 12, the isolated CAR of any of claims 13-23, the anti-EGFRvIII binding domain of any of claims 24-26, the vector of any of claims 27-30,or the cell of claim 31 or 32 for use in the treatment of a disease expressing EGFRvIII.