

Claims

What is claimed:

1. A nucleic acid encoding a chimeric antigen receptor (CAR) for binding HER2, said CAR comprising:

- a) an antigen-specific targeting region (ASTR) that binds to HER2 protein;
- b) a transmembrane domain; and
- c) an intracellular activating domain, wherein the transmembrane domain is located between the ASTR and the intracellular activating domain, and wherein the ASTR comprises a heavy chain variable region comprising three complementarity determining regions (CDRs), said CDRs having sequences HCDR1, HCDR2, and HCDR3, wherein:

the HCDR1 sequence is GFNIKDTYIH (SEQ ID NO:131);

the HCDR2 sequence is X₁IYPTNGYTX₂YADSVKG (SEQ ID NO:137); and

the HCDR3 sequence is WGGDGFYAMDY (SEQ ID NO:133), wherein amino acid 11 of SEQ ID NO:133 is Y or V;

and the ASTR comprises a light chain variable region including three CDRs having sequences LCDR1, LCDR2, and LCDR3, wherein:

the LCDR1 sequence is RASQDVNTX₃VA (SEQ ID NO:142);

the LCDR2 sequence is SASFLYS (SEQ ID NO:135), wherein amino acid 6 of SEQ ID NO:135 is Y or E; and

the LCDR3 sequence is QQX₄YTPPT (SEQ ID NO:143),

wherein X₁ is R or K, X₂ is R or E, X₃ is A or D, and X₄ is H, D or E; and

wherein the combination of X₁, X₂, X₃, and X₄ in the ASTR is other than R, R, A, and H, respectively.

2. A chimeric antigen receptor (CAR) for binding HER2, wherein the CAR is encoded by the nucleic acid of claim 1.

3. A modified T cell or NK cell, wherein the nucleic acid of claim 1 is operably linked to a promoter active in T cells and/or NK cells, and wherein:

a) the modified T cell or NK cell comprises the nucleic acid of claim 1, or
b) wherein a recombinant nucleic acid vector is associated with the modified T cell or NK cell, wherein the recombinant nucleic acid vector comprises the nucleic acid of claim 1.

4. A delivery suspension, wherein the delivery suspension comprises the modified T cell or NK cell of claim 3.

5. The nucleic acid of claim 1, the CAR of claim 2, the modified T cell or NK cell of claim 3, or the delivery suspension of claim 4, wherein the combination of X₁, X₂, X₃, and X₄ is R, R, D, and H, respectively; R, R, A, and D, respectively; R, R, A, and E, respectively; K, R, A, and H, respectively; or R, E, A, and H, respectively, wherein the heavy chain variable region and the light chain variable region are separated by a linker between 5 and 50 amino acids in length, and wherein the sequence of the heavy chain variable region is at least 90% identical to SEQ ID NO: 119, and wherein the sequence of the light chain variable region is at least 90% identical to SEQ ID NO: 122.

6. The nucleic acid of claim 1, the CAR of claim 2, the modified T cell or NK cell of claim 3, or the delivery suspension of claim 4, wherein said CAR is a conditionally active CAR having increased anti-HER2 CAR activity at a pH of 6.7 compared to a pH of 7.4.

7. The nucleic acid, the CAR, the modified T cell or NK cell, or the delivery suspension of claim 6, wherein the sequence of the heavy chain variable region is at least 90% identical to SEQ ID NO:119, and wherein the sequence of the light chain variable region is at least 90% identical to SEQ ID NO:122.

8. The nucleic acid of claim 1, the CAR of claim 2, the modified T cell or NK cell of claim 3, or the delivery suspension of claim 4, wherein the sequence of the heavy

chain variable region is at least 90% identical to SEQ ID NO: 119, and wherein the sequence of the light chain variable region is at least 90% identical to SEQ ID NO:122.

9. The nucleic acid of claim 1, the CAR of claim 2, the modified T cell or NK cell of claim 3, or the delivery suspension of claim 4, wherein the ASTR binds to the same epitope of HER2 as an antibody or a single-chain variable antibody fragment comprising the antibody heavy chain variable region of SEQ ID NO: 119 and the antibody light chain variable region of SEQ ID NO: 122.

10. The nucleic acid of claim 1, the CAR of claim 2, the modified T cell or NK cell of claim 3, or the delivery suspension of claim 4, wherein the ASTR is an antibody selected from a single-chain antibody, an Fab fragment, an Fab' fragment, an (Fab')₂ fragment, an Fv fragment, and a divalent single-chain antibody or a diabody.

11. The nucleic acid of claim 1, the CAR of claim 2, the modified T cell or NK cell of claim 3, or the delivery suspension of claim 4, wherein the ASTR is a single-chain variable fragment comprising a heavy chain variable region and a light chain variable region.

12. The nucleic acid of claim 1, the CAR of claim 2, the modified T cell or NK cell of claim 3, or the delivery suspension of claim 4, wherein the heavy and light chains are separated by a linker, and wherein the linker is between 5 and 50 amino acids in length.

13. The nucleic acid of claim 1, the CAR of claim 2, the modified T cell or NK cell of claim 3, or the delivery suspension of claim 4, wherein the heavy chain variable region and the light chain variable region are separated by a linker, and wherein the linker comprises one of the sequences of SEQ ID NOs:1, 63-71, 144, 152, or 249.

14. The nucleic acid, the CAR, the modified T cell or NK cell, or the delivery suspension of claim 5, wherein the heavy chain is N-terminal to the light chain.

15. The nucleic acid of claim 1, the CAR of claim 2, the modified T cell or NK cell of claim 3, or the delivery suspension of claim 4, wherein the light chain is N-terminal to the heavy chain.

16. The nucleic acid, the CAR, the modified T cell or NK cell, or the delivery suspension of claim 5, wherein the light chain is N-terminal to the heavy chain.

17. The nucleic acid of claim 1, the CAR of claim 2, the modified T cell or NK cell of claim 3, or the delivery suspension of claim 4, wherein the CAR further comprises a stalk domain, and a co-stimulatory domain, and wherein the CAR comprises from amino terminus to carboxy terminus, the ASTR, the stalk domain, the transmembrane domain, the co-stimulatory domain, and the intracellular activating domain.

18. The nucleic acid, the CAR, the modified T cell or NK cell, or the delivery suspension of claim 17, wherein the intracellular activating domain is a CD3Z activating domain and wherein the co-stimulatory domain is an ICA co-stimulatory domain, a CD28 co-stimulatory domain, a CD137 co-stimulatory domain, or comprises both a ICA co-stimulatory domain and a CD137 co-stimulatory domain, or a CD28 co-stimulatory domain and a CD137 co-stimulatory domain.

19. The nucleic acid, the CAR, the modified T cell or NK cell, or the delivery suspension of claim 17, wherein the stalk domain is a CD8 stalk domain or a CD28 stalk domain, wherein the transmembrane domain is a CD8 transmembrane domain or a CD28 transmembrane domain, wherein the intracellular activating domain is a CD3Z activating domain, and wherein the co-stimulatory domain is a CD137 co-stimulatory domain, a CD28 co-stimulatory domain, or an ICA co-stimulatory domain.

20. The nucleic acid of claim 1, the CAR of claim 2, the modified T cell or NK cell of claim 3, or the delivery suspension of claim 4, wherein the CAR further comprises a recognition domain.