

CLAIMS

1. 1.A CAR-T cell, which comprises one or more chimeric antigen receptors (CARs) targeting one or more antigens expressed on a malignant T cell, wherein the CAR comprises the amino acid sequence

MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVITISCSASQGISNYLNWYQQ
KPDGTVKLLIYYTSSLHSGVPSRFSGSGSGTDYSLTISNLEPEDIATYYCQQYSKLPY
TFGGGKLEIKRGGGGSGGGSGGGSGGGGSEVQLVESGGGLVKPGGSLKLSCA
ASGLTFSSYAMSWVRQTPEKRLEWVASISSGGFTYYPDSVKGRFTISRDNARNILYL
QMSSLRSEDAMYYCARDEVRGYLDVWGAGTTVTVSPRASTTTPAPRPPTPAPTIA
SQPLSLRPEACRPAAGGAVHTRGLDFACDFWVLVVVGGVLACYSLLVTVAFIIFWV
KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGCELRVKFSRSADAPAYK
QGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA
EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR,

wherein the CAR-T cell is deficient in a subunit of the T cell receptor complex and ~~for~~ is deficient in ~~at least one or more antigens to which the one or more CARs specifically binds~~ CD7.

~~2. A CAR T cell, which comprises one or more chimeric antigen receptors (CARs) targeting one or more antigens, wherein the CAR T cell is deficient in one or more antigens to which the one or more CARs specifically binds.~~

~~3. 2.~~The CAR-T cell as recited in claim 1, wherein the subunit of the T cell receptor complex is chosen from TCR α , TCR β , TCR δ , TCR γ , CD3 ϵ , CD3 γ , CD3 δ , and CD3 ζ .

~~4. The CAR T cell as recited in any of claims 1-2, wherein the chimeric antigen receptor (CAR) specifically binds one or more antigens expressed on a malignant T cell or myeloma cell.~~

~~5. The CAR T cell as recited in any of claims 1-4, wherein endogenous T cell receptor-mediated signaling is blocked in the CAR T cell.~~

~~6. The CAR T cell as recited in any of claims 1-4, wherein the CAR T cell does not induce alloreactivity or graft versus host disease.~~

~~7. The CAR T cell any of claims 1-4, wherein the CAR T cell does not induce fratricide.~~

~~8. A dual or tandem CAR T cell as recited in any of claims 1-7.~~

~~9. The CAR T cell as recited in claim 8, wherein the wherein the CAR(s) specifically bind(s)~~

two different targets chosen from: ~~CD2xCD3ε, CD2xCD4, CD2xCD5, CD2xCD7, CD3εxCD4, CD3εxCD5, CD3εxCD7, CD4xCD5, CD4xCD7, CD5xCD7, TRACxCD2, TRACxCD3ε, TRACxCD4, TRACxCD5, TRACxCD7, TCRβxCD2, TCRβxCD3ε, TCRβxCD4, TCRβxCD7, CD2xCD3ε, CD2xCD4, CD2xCD5, CD2xCD7, CD3εxCD4, CD3εxCD5, CD3εxCD7, CD4xCD5, CD4xCD7, CD5xCD7, TRACxCD2, TRACxCD3ε, TRACxCD4, TRACxCD5, TRACxCD7, TCRβxCD2, TCRβxCD3ε, TCRβxCD4, TCRβxCD5, TCRβxCD7, BCMAxCS1, BCMAxCD19, BCMAxCD38, CS1xCD19, CD19xCD38, APRILxCS1, APRILxBCMA, APRILxCD19, APRILxCD38, CS1xCD38, CD79AxBCMA, CD79AxCS1, CD79AxCD19, CD79AxCD38, CD79AxCD38, CD79AxAPRIL, CD79AxCD79B, CD79BxBCMA, CD79BxCS1, CD79BxCD19, CD79BxCD38, CD79BxAPRIL, CD79BxCD79A, CD138xBCMA, CD138xCS1, CD138xCD19, CD138xCD38, CD138xAPRIL, CD138xCD79A, CD138xCD79B, CD138xBCMA, and CD138xCS1.~~

10. ~~A tandem CAR-T cell as recited in claim 9.~~

11. ~~A tandem chimeric antigen receptor (tCAR) targeting two or more plasma cell antigens.~~

12. ~~The CAR as recited in claim 11, wherein the plasma cell antigen(s) is/are chosen from BCMA, CS1, CD38, CD79A, CD79B, CD138, and CD19.~~

13. ~~The CAR as recited in claim 12, wherein the CAR(s) specifically bind(s) two different targets chosen from: BCMAxCS1, BCMAxCD19, BCMAxCD38, CS1xCD19, CD19xCD38, APRILxCS1, APRILxBCMA, APRILxCD19, APRILxCD38, CS1xCD38, CD79AxBCMA, CD79AxCS1, CD79AxCD19, CD79AxCD38, CD79AxCD38, CD79AxAPRIL, CD79AxCD79B, CD79BxBCMA, CD79BxCS1, CD79BxCD19, CD79BxCD38, CD79BxAPRIL, CD79BxCD79A, CD138xBCMA, CD138xCS1, CD138xCD19, CD138xCD38, CD138xAPRIL, CD138xCD79A, CD138xCD79B, CD138xBCMA, and CD138xCS1.~~

14. ~~A tandem chimeric antigen receptor (tCAR) targeting two or more leukemia cell antigens.~~

15. ~~The CAR as recited in claim 14, wherein the leukemia cell antigen(s) is/are chosen from CD123, CLEC12A, CD117, FLT3, CD7 and Tim3.~~

16. ~~The CAR as recited in claim 15, wherein the CAR(s) specifically bind(s) two different targets chosen from: CD123xCD371, CD123xCLEC12A, CD123xCD117, CD123xFLT3, CD123xCD7, CD123xTim3, CD371xCLEC12A, CD371xCD117, CD371xFLT3, CD371xCD7, CD371xTim3, CLEC12AxCD117, CLEC12AxFLT3, CLEC12AxCD7,~~

~~CLEC12A $\times$ Tim3, CD117 $\times$ FLT3, CD117 $\times$ CD7, CD117 $\times$ Tim3, FLT3 $\times$ CD7, FLT3 $\times$ Tim3, and CD7 $\times$ Tim3.~~

~~17. A tandem chimeric antigen receptor (tCAR) targeting two or more T-cell antigens.~~

~~18. The tCAR as recited in claim 17, wherein the T-cell antigens chosen from CD5, CD7, CD2, CD4, and CD3.~~

~~19. The tCAR as recited in claim 18, targeting a pair of (i.e., two) antigens.~~

~~20. The tCAR as recited in claim 19, wherein the antigen pair is chosen from CD2 $\times$ CD3 ϵ , CD2 $\times$ CD4, CD2 $\times$ CD5, CD2 $\times$ CD7, CD3 ϵ $\times$ CD4, CD3 ϵ $\times$ CD5, CD3 ϵ $\times$ CD7, CD4 $\times$ CD5, CD4 $\times$ CD7, CD5 $\times$ CD7, TRAC $\times$ CD2, TRAC $\times$ CD3 ϵ , TRAC $\times$ CD4, TRAC $\times$ CD5, TRAC $\times$ CD7, TCR β $\times$ CD2, TCR β $\times$ CD4, TCR β $\times$ CD7, CD2 $\times$ CD3 ϵ , CD2 $\times$ CD4, CD2 $\times$ CD5, CD2 $\times$ CD7, CD3 ϵ $\times$ CD4, CD3 ϵ $\times$ CD5, CD4 $\times$ CD5, CD4 $\times$ CD7, CD5 $\times$ CD7, TRAC $\times$ CD2, TRAC $\times$ CD3 ϵ , TRAC $\times$ CD4, TRAC $\times$ CD5, TRAC $\times$ CD7, TCR β $\times$ CD2, TCR β $\times$ CD3 ϵ , TCR β $\times$ CD4, TCR β $\times$ CD5, and TCR β $\times$ CD7.~~

~~21. The tCAR as recited in any of claims 17-20, wherein the CAR construct is a hairpin tCAR construct.~~

~~22. The tCAR as recited in any of claims 17-21, wherein each of the V_H and V_L chains is derived from an scFv that recognizes a different antigen chosen from CD5, CD7, CD2, CD4, and CD3.~~

~~23. The tCAR as recited in claim 22, wherein each of the V_H and V_L chains is different and is a sequence chosen from SEQ ID NO:12 to SEQ ID NO:31.~~

~~24. 3. The genome-edited CAR-T cell, population of genome-edited CAR-T cells, dual CAR-T cells, or tandem CAR-T as recited in any of any of claims 1, 2, 10-13 1-10, or the population of CAR-T cells comprising CAR(s) as recited in any of claims 11-23, for use in the treatment of cancer.~~

~~25. Use of the genome-edited CAR-T cell, population of genome-edited CAR-T cells, dual CAR-T cells, or tandem CAR-T as recited in any of any of claims 1-10, or the population of CAR-T cells comprising CAR(s) as recited in any of claims 11-23 in the manufacture of a medicament for the treatment of cancer.~~

~~26. 4. The use CAR-T cell as recited in claim 25 3, wherein the cancer is a hematologic malignancy.~~

~~27. 5. The use CAR-T cell as recited in claim 26 4, wherein the hematologic malignancy is a T-cell malignancy.~~

- ~~28.~~ 6. The ~~use~~ CAR-T cell as recited in claim ~~27~~5, wherein the T cell malignancy is T-cell acute lymphoblastic leukemia (T-ALL).
- ~~29.~~ 7. The ~~use~~ CAR-T cell as recited in claim ~~27~~5, wherein the T cell malignancy is non-Hodgkin's lymphoma.
- ~~30.~~ 8. The ~~use~~ CAR-T cell as recited in claim ~~27~~5, wherein the T cell malignancy is T-cell chronic lymphocytic leukemia (T-CLL).
- ~~31.~~ 9. A chimeric antigen receptor (CAR)-comprising T cell (CAR-T cell) that is deficient in a subunit of the T cell receptor complex and is deficient in CD7, comprising a CAR that specifically binds to CD7 antigen, wherein the CAR comprises:
- a CD8 α signal peptide comprising SEQ ID NO:1;
 - a light chain variable region comprising SEQ ID NO: 21;
 - a peptide linker comprising SEQ ID NO:9;
 - a heavy chain variable region comprising SEQ ID NO: 20;
 - a CD8 α hinge comprising SEQ ID NO:2;
 - a CD28 transmembrane domain comprising SEQ ID NO:3;
 - ~~a~~ the costimulatory domain selected from 4-1BB, comprising SEQ ID NO: 5 and CD28, comprising SEQ ID NO: 6 and not a CD28 costimulatory domain; and
 - a CD3 zeta signaling domain comprising SEQ ID NO:7.
- ~~32.~~ The CAR-T cell as recited in claim 1 or 31, wherein the subunit of the T cell receptor complex is chosen from TCR α , TCR β , TCR δ , TCR γ , CD3 ϵ , CD3 γ , CD3 δ , and CD3 ζ .
- ~~33.~~ The CAR-T cell as recited in claim 1 or 31, wherein the chimeric antigen receptor (CAR) specifically binds one or more antigens expressed on a malignant T cell or myeloma cell.
- ~~34.~~ The CAR-T cell as recited in claim 1 or 31, wherein endogenous T cell receptor mediated signaling is blocked in the CAR-T cell.
- ~~35.~~ The CAR-T cell as recited in claim 1 or 31, wherein the CAR-T cell does not induce alloreactivity or graft-versus-host disease.
- ~~36.~~ The CAR-T cell as recited in claim 1 or 31, wherein the CAR-T cell does not induce fratricide.
- ~~37.~~ The CAR-T cell according to claim 1 or 31, further comprising hCD34.
- ~~38.~~ The CAR-T cell according to claim 31, wherein the hCD34 comprises SEQ ID NO. 10.

~~39. The CAR-T cell according to claim 1 or 31, wherein the cell is engineered to be deficient in CD7 and TRAC by deletion or disruption of the genes encoding CD7 and TRAC using CRISPR.~~

10. The CAR-T cell as recited in claim 1, wherein the CAR is an amino acid sequence shown in SEQ ID NO. 32.

11. The CAR-T cell as recited in any of claims 1 or 2, wherein the chimeric antigen receptor (CAR) displays at least 95% sequence identity to an amino acid sequence chosen from SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38 or SEQ ID NO:39.

12. The CAR-T cell as recited in claim 1, wherein the antigen expressed on a malignant T cell is chosen from CD2, CD3, CD4, CDS, CD7, TCR α , and TCR β .

13. A therapeutic composition comprising a population of CAR-T cells as recited in any of any of claims 1, 3, 10, 11, or 12 and at least one therapeutically acceptable carrier and/or adjuvant.

14. The CAR-T cell or therapeutic composition for use in the method as recited in claim 5, wherein the hematologic malignancy is acute myeloid leukemia (AML).

15. A therapeutic composition comprising a population of CAR-T cells as recited in any of claim 9 and at least one therapeutically acceptable carrier and/or adjuvant.

16. The CAR-T cell as recited in claim 9 or the therapeutic composition as recited in claim 15 for use in a method of treatment of cancer in a patient in need thereof.