

making and using these CD19-specific CARs, and immune cells comprising these CD19-specific CARs.

[007] In one [aspectexample](#), the disclosure provides an isolated polynucleotide encoding a polypeptide comprising an anti-CD19 chimeric antigen receptor (CAR) that is at least 70% identical to SEQ ID NO: 9, wherein the polypeptide does not comprise a rituximab binding site, and wherein the polynucleotide comprises a short EF1a promoter that is capable of expressing the anti-CD19 chimeric antigen receptor (CAR) in a mammalian T cell.

[007a] In one aspect, the present disclosure refers to an isolated polynucleotide encoding a polypeptide comprising an anti-CD19 chimeric antigen receptor (CAR) that is identical to SEQ ID NO: 9, wherein the polypeptide does not comprise a rituximab binding site, and wherein the polynucleotide comprises a short EF1a promoter that is capable of expressing the anti-CD19 chimeric antigen receptor (CAR) in a mammalian T cell, wherein the short EF1a promoter comprises the nucleic acid sequence of SEQ ID NO: 16 and does not comprise the nucleic acid sequence of SEQ ID NO: 39.

[008] In some [embodimentexamples](#), the short EF1a promoter does not comprise an intron sequence within the nucleic acid sequence of SEQ ID NO:15. In some [embodimentexamples](#), the intron comprises the nucleic acid sequence of SEQ ID NO:39.

[009] In some [embodimentexamples](#), the promoter comprises the nucleic acid sequence of SEQ ID NO:16,

[010] In some [embodimentexamples](#), the promoter is a full length EF1a promoter comprising the nucleic acid sequence of SEQ ID NO:15.

[011] In some [embodimentexamples](#), the promoter comprises the nucleic acid sequence of SEQ ID NO:15, and the polynucleotide encodes a polypeptide that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to any one of SEQ ID NO: 8-14.

[012] In some [embodimentexamples](#), the polypeptide further comprises a safety switch.

[013] In some [embodimentexamples](#), the safety switch is linked to the CD19 CAR using a linker peptide.

[014] In some [embodimentexamples](#), the safety switch is linked to the anti-CD19 CAR using a T2A linker.

[015] In some [embodimentexamples](#), the safety switch comprises an antibody binding site.

[016] In some [embodimentexamples](#), the safety switch comprises a mutated CD20 mimotope.

[017] In some [embodimentexamples](#), the polypeptide further comprises a CD8 hinge/transmembrane domain.

[018] In some [embodimentexamples](#), the polypeptide comprises a CD34 epitope.

[019] In some [embodimentexamples](#), the CD34 epitope is a QBEND-10 epitope.

[020] In some [embodimentexamples](#), the isolated polynucleotide comprises a nucleic acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to any one of SEQ ID NO: 1-7.

[021] In some [embodimentexamples](#), the isolated polynucleotide encodes a polypeptide that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to any one of SEQ ID NO: 8-14.

[022] In another aspect, the disclosure provides a vector comprising the isolated polynucleotide described herein.

[023] In some [embodimentexamples](#), the vector is a retroviral vector, a DNA vector, a plasmid, an RNA vector, an adenoviral vector, an adenovirus associated vector, a lentiviral vector, or any combination thereof.

[024] In one [aspectexample](#), the disclosure provides an engineered immune cell comprising the isolated polynucleotide described herein.

[024a] In another aspect, the present disclosure refers to an engineered immune cell comprising the isolated polynucleotide as disclosed herein, wherein the engineered immune cell does not express a rituximab binding site. In another aspect, the present disclosure refers to an engineered immune cell comprising the vector as disclosed herein, wherein the engineered immune cell does not express a rituximab binding site.

[025] In some [embodimentexamples](#), the disclosure provides an engineered immune cell comprising a polynucleotide that comprises a nucleic acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to SEQ ID NO: 3.

[026] In some [embodimentexamples](#), the disclosure provides an engineered immune cell comprising a polynucleotide that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the anti-CD19 CAR v1.2, v1.3, v1.4, v1.5 or v1.6 lentiviral construct as shown in Table 1.

[027] In some [embodimentexamples](#), the disclosure provides an engineered immune cell comprising a polynucleotide that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to the anti-CD19 CAR v1.2 lentiviral construct as shown in Table 1.

[028] In some [embodimentexamples](#), the disclosure provides an engineered immune cell comprising a polynucleotide that encodes a polypeptide that is at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100% identical to SEQ ID NO:9 or SEQ ID NO:10, with or without the signal sequence.

[029] In some [embodimentexamples](#), the disclosure provides an engineered immune cell comprising a polynucleotide that comprises the nucleic acid sequence of SEQ ID NO:3, driven by an EF1a short promoter comprising the nucleic acid of SEQ ID NO:16.

[030] In some [embodimentexamples](#), the disclosure provides an engineered immune cell comprising a polynucleotide that encodes a polypeptide sequence of SEQ ID NO:9 or 10, with or without the signal sequence, driven by an EF1a short promoter comprising the nucleic acid of SEQ ID NO:16. In some [embodimentexamples](#), the promoter does not comprise the first intron of the EF1a gene.

[031] In some [embodimentexamples](#), the engineered immune cell does not comprise a rituximab mimotope.

[032] In some [embodimentexamples](#), the engineered immune cell comprises a polynucleotide comprising the CD19 CAR v1.2 lentiviral construct as shown in FIG. 1 (may also be referred to as ALLO-501A).

[033] In some [embodimentexamples](#), the engineered immune cell comprises a vector described herein.

[034] In some [embodimentexamples](#), the immune cell is a T cell, tumor infiltrating lymphocyte (TIL), NK cell, TCR-expressing cell, dendritic cell, or NK-T cell.

[035] In some [embodimentexamples](#), the cell is an autologous T cell.

[036] In some [embodimentexamples](#), the cell is an allogeneic T cell.

[037] In one [aspectexample](#), the disclosure provides an engineered immune cell described herein, wherein the cell is resistant to rituximab.

[038] In another aspect, the disclosure provides a pharmaceutical composition comprising the engineered immune cell described herein.

[039] In one aspectexample, the disclosure provides a method of treating a disease or disorder in a subject in need thereof comprising administering to the subject the engineered immune cell described herein, or the pharmaceutical composition described herein.

[039a] In another aspect, the present disclosure refers to use of the engineered immune cell as disclosed herein in the manufacture of a medicament for treating a cancer in a subject in need thereof. In another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a cancer in a subject in need thereof.

[040] In some embodimentexamples, the disease or disorder is Non-Hodgkin lymphoma (NHL).

[041] In some embodimentexamples, the subject has been treated or is currently being treated with rituximab.

[042] In one aspectexample, the disclosure provides an article of manufacture comprising the engineered immune cell or the pharmaceutical composition comprising the engineered immune cell expressing a chimeric antigen receptors described herein.

[042a] In another aspect, the present disclosure refers to an article of manufacture comprising the engineered immune cell as disclosed herein, or a pharmaceutical composition thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[043] FIG. 1 shows schematic representations of rituximab-resistant CD19 chimeric antigen receptors.

[044] FIG. 2A and 2B show flow cytometry plots demonstrating CAR expression on day 5 from Pan T cells transduced with the rituximab resistant CAR expression vectors. FIG. 2A shows CAR expression on cells from donor 541 and 604. FIG. 2B shows CAR and CD34 expression on cells from donor 410. A similar profile was observed with donor 2593 (data not shown).

[045] FIG. 3 shows normalized cell expansion and final CAR expression from all four donors on day 13.

[046] FIG. 4A-4D show cell expansion and CAR expression over time of Pan T cells from donor 541 (FIG. 4A), 604 (FIG. 4B), 410 (FIG. 4C), 2593 (FIG. 4D) transduced with rituximab resistant CAR expression vectors.

[047] FIGs. 5A-5D show CD4/CD8 ratios on day 5, 9, and 13 of Pan T cells from donor 541 (FIG. 5A), 604 (FIG. 5B), 410 (FIG. 5C), 2593 (FIG. 5D) transduced with rituximab resistant CAR expression vectors.

[048] FIGs. 6A-6D show phenotype and activation on day 9 of Pan T cells from donor 541 (FIG. 6A), 604 (FIG. 6B), 410 (FIG. 6C), 2593 (FIG. 6D) transduced with rituximab resistant CAR expression vectors.

[049] FIG. 7 shows phenotype, activation %CD8+, and anergy measured using TIM3 and PD1 staining averaged from all four donors on day 9.

[050] FIGs. 8A-8D show phenotype and activation on day 13 of Pan T cells from donor 541 (FIG. 8A), 604 (FIG. 8B), 410 (FIG. 8C), 2593 (FIG. 8D) transduced with rituximab resistant CAR expression vectors.

WHAT IS CLAIMED IS

1. An isolated polynucleotide encoding a polypeptide comprising an anti-CD19 chimeric antigen receptor (CAR) that is ~~at least 70%~~ identical to SEQ ID NO: 9, wherein the polypeptide does not comprise a rituximab binding site, and wherein the polynucleotide comprises a short EF1a promoter that is capable of expressing the anti-CD19 chimeric antigen receptor (CAR) in a mammalian T cell, wherein the short EF1a promoter comprises the nucleic acid sequence of SEQ ID NO: 16 and does not comprise the nucleic acid sequence of SEQ ID NO: 39.

~~2. The isolated polynucleotide of claim 1, wherein the short EF1a promoter does not comprise an intron comprised by the nucleic acid sequence of SEQ ID NO:15.~~

~~3. The isolated polynucleotide of claim 2, wherein the intron comprises the nucleic acid sequence of SEQ ID NO:39.~~

~~4.2.~~ The isolated polynucleotide of ~~any one of the preceding claims~~ claim 1, wherein the short EF1a promoter ~~comprises~~ is according to the nucleic acid sequence of SEQ ID NO:16.

~~5.3.~~ The isolated polynucleotide of ~~any one of the preceding claims~~ claim 1 or 2, wherein the polypeptide further comprises a safety switch.

~~6.4.~~ The isolated polynucleotide of claim ~~5.3~~, wherein the safety switch is linked to the CD19 CAR using a linker peptide.

~~7.5.~~ The isolated polynucleotide of claim ~~5.3~~, wherein the safety switch is linked to the anti-CD19 CAR using a T2A linker.

~~8.6.~~ The isolated polynucleotide of any one of claims ~~5-7.3-5~~, wherein the safety switch comprises an antibody binding site.

~~9.7.~~ The isolated polynucleotide of any one of claims ~~5-7.3-5~~, wherein the safety switch comprises a mutated CD20 mimotope.

~~10.8.~~ The isolated polynucleotide of any one of claims ~~5-9.3-7~~, wherein the polypeptide comprises a CD34 epitope.

~~11.9.~~ The isolated polynucleotide of claim ~~10.8~~, wherein the CD34 epitope is a QBEND-10 epitope.

12.10. The isolated polynucleotide of any one of claims ~~5-11~~3-9, wherein the polypeptide further comprises a CD8 hinge/transmembrane domain.

13.11. The isolated polynucleotide of any one of the preceding claims, comprising a nucleic acid sequence that is ~~at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100%~~ identical to any one of the nucleic acid sequences of SEQ ID NOs: ~~12~~7.

14.12. The isolated polynucleotide of any one of the preceding claims, wherein the polynucleotide encodes a polypeptide that is ~~at least about 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100%~~ identical to any one of the amino acid sequences of SEQ ID NOs: ~~89~~14.

15.13. A vector comprising the isolated polynucleotide of any one of the preceding claims.

16.14. The vector of claim ~~15~~13, wherein the vector is a retroviral vector, a DNA vector, a plasmid, an RNA vector, an adenoviral vector, an adenovirus associated vector, a lentiviral vector, or any combination thereof.

17.15. An engineered immune cell comprising the isolated polynucleotide of any one of claims 1-~~14~~12, wherein the engineered immune cell does not express a rituximab binding site.

18.16. An engineered immune cell comprising the vector of claim ~~15~~13 or ~~16~~14, wherein the engineered immune cell does not express a rituximab binding site.

19.17. The engineered immune cell of claim ~~17~~15 or ~~18~~16, wherein the immune cell is a T cell, tumor infiltrating lymphocyte (TIL), NK cell, TCR-expressing cell, dendritic cell, or NK-T cell.

~~20.18.~~ The engineered immune cell of claim ~~19~~17, wherein the cell is an autologous T cell.

21.19. The engineered immune cell of claim ~~19~~17, wherein the cell is an allogeneic T cell.

22.20. The engineered immune cell of any one of claims ~~17-21~~15-21, wherein the cell comprises the polynucleotide that is ~~at least 80%, 85%, 90%, 95%, 96%, 98%, 99% or 100%~~ identical to the nucleic acid sequence of SEQ ID NO: 3.

23.21. The engineered immune cell of any one of claims ~~17-22~~15-22, wherein the cell is resistant to rituximab.

24.22. A pharmaceutical composition comprising the engineered immune cell of any one of claims ~~17-23~~15-23.

25.23. Use of the engineered immune cell of any one of claims 1715-23-21 in the manufacture of a medicament for treating a ~~disease or disorder~~cancer in a subject in need thereof.

26.24. Use of the pharmaceutical composition of claim 24-22 in the manufacture of a medicament for treating a ~~disease or disorder~~cancer in a subject in need thereof.

5 27.25. The use of claim 25-23 or 26-24, wherein the ~~disease or disorder~~cancer is Non-Hodgkin lymphoma (NHL).

28.26. The use of any one of claims 25-23-27-25, wherein the subject is one that has been treated or is currently being treated with rituximab.

10 29.27. An article of manufacture comprising the engineered immune cell of any one of claims 1715-23-21, or a pharmaceutical composition thereof.