

CLAIMS

1. A method of making a population of cells that comprise:
a nucleic acid molecule that encodes a controllable chimeric antigen receptor (CCAR), wherein the CCAR is:
 - a fusion polypeptide comprising a degradation polypeptide and a CAR polypeptide; or
 - a fusion polypeptide comprising a degradation domain and a CAR polypeptide;the method comprising:
 - (i) contacting a population of cells with an agent that stimulates a CD3/TCR complex and an agent that stimulates a costimulatory molecule on the surface of the cells, wherein the agent that stimulates a CD3/TCR complex comprises an anti-CD3 antibody and the agent that stimulates a costimulatory molecule comprises an anti-CD28 antibody;
 - (ii) contacting the population of cells with the nucleic acid molecule encoding the CCAR, thereby providing a population of cells comprising the nucleic acid molecule, and
 - (iii) harvesting the population of cells for storage or administration;wherein step (ii) is performed together with step (i) or no later than 20 hours after the beginning of step (i), and step (iii) is performed no later than 48 hours after the beginning of step (i);
wherein the population of cells comprises T cells.
2. The method of claim 1, wherein the storage of step (iii) comprises reformulating the population of cells in cryopreservation media.
3. The method of claim 1 or 2, wherein the agent that stimulates a CD3/TCR complex or the agent that stimulates a costimulatory molecule does not comprise a bead.
4. The method of any one of claims 1-3, wherein the anti-CD3 antibody is covalently attached to a colloidal polymeric nanomatrix and the anti-CD28 antibody is covalently attached to a colloidal polymeric nanomatrix.

5. The method of any one of claims 1-4, wherein the percentage of naïve cells in the population of cells from step (iii) does not decrease, or decreases by no more than 5 or 10%, as compared to the percentage of naïve cells in the population of cells at the beginning of step (i).
6. The method of any one of claims 1-5, wherein the percentage of naïve cells in the population of cells from step (iii) is increased as compared to the percentage of naïve cells in the population of cells at the beginning of step (i).
7. The method of any one of claims 1-6, wherein the percentage of naïve cells in the population of cells from step (iii) is higher than the percentage of naïve cells in cells made by an otherwise similar method in which step (iii) is performed more than 5 day after the beginning of step (i).
8. The method of any one of claims 1-7, wherein the percentage of central memory cells in the population of cells from step (iii) does not increase, or increases by no more than 5 or 10%, as compared to the percentage of central memory cells in the population of cells at the beginning of step (i).
9. The method of any one of claims 1-8, wherein the percentage of central memory cells in the population of cells from step (iii) is reduced by at least 20%, as compared to the percentage of central memory cells in the population of cells at the beginning of step (i).
10. The method of any one of claims 1-9, wherein the percentage of central memory cells in the population of cells from step (iii) is lower than the percentage of central memory cells in cells made by an otherwise similar method in which step (iii) is performed more than 5 days after the beginning of step (i).
11. The method of any one of claims 1-10, wherein the percentage of stem memory T cells, in the population of cells from step (iii) is increased, as compared to the percentage of stem memory T cells in the population of cells at the beginning of step (i).

12. The method of any one of claims 1-11, wherein the population of cells from step (iii) are not expanded.

13. The method of any one of claims 1-12, further comprising step (vi):

culturing a portion of the population of cells from step (iii) for at least 2 days and, and measuring CAR expression level or percentage of viable, CAR-expressing cells in the portion.

14. The method of any one of claims 1-13, wherein the CCAR comprises an antigen binding domain, a transmembrane domain, and/or an intracellular signaling domain.

15. The method of claim 14, wherein the antigen binding domain binds to CD19.

16. The method of claim 14 or 15, wherein the intracellular signaling domain comprises a primary signaling domain.

17. The method of claim 16, wherein the primary signaling domain comprises a CD3 zeta functional signaling domain.

18. The method of any one of claims 14-17, wherein the intracellular signaling domain comprises a costimulatory signaling domain.

19. The method of claim 18, wherein the costimulatory signaling domain comprises a 4-1BB functional signaling domain or a CD28 functional signaling domain.

20. The method of any one of claims 1-19, wherein the method further comprises formulating the harvested cells in a pharmaceutical composition in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents, or excipients.

21. A population of cells that comprise the nucleic acid molecule, wherein the cells were made by the method of any one of claims 1-20.
22. The method of any one of claims 1-21 or the population of cells of claim 21, wherein the CCAR is a fusion polypeptide comprising a degradation polypeptide and a CAR polypeptide.
23. The method of claim 22 or the population of cells of claim 22, wherein the degradation polypeptide comprises 45 to 65 amino acid residues of IKZF3 comprising SEQ ID NO: 328, or a sequence having at least 95% identity thereto.
24. The method of claim 22 or 23 or the population of cells of claim 22 or 23, wherein the degradation polypeptide comprises the amino acid sequence of SEQ ID NO: 312.
25. The method of any one of claims 1-21 or the population of cells of claim 21, wherein the CCAR is a fusion polypeptide comprising a degradation domain and a CAR polypeptide.
26. The method of claim 25 or the population of cells of claim 25, wherein the degradation domain is separated from the CAR polypeptide by a heterologous protease cleavage site.
27. The method of claim 26 or the population of cells of claim 26, wherein the heterologous protease cleavage site comprises a sequence having a cleavage motif selected from the group consisting of RX(K/R)R consensus motif (X can be any amino acid; SEQ ID NO: 348), RXXX[KR]R consensus motif (X can be any amino acid; SEQ ID NO: 349), RRX consensus motif (SEQ ID NO : 350), I-E-P-D-X consensus motif (SEQ ID NO: 351), Ile-Glu/Asp-Gly-Arg (SEQ ID NO : 352), Asp-Asp-Asp-Asp-Lys (SEQ ID NO: 353), Pro-Gly-Ala-Ala-His-Tyr (SEQ ID NO: 354), LPXTG/A consensus motif (SEQ ID NO: 355), Leu-Glu-Val-Phe-Gln-Gly-Pro (SEQ ID NO: 356), Leu-Val-Pro-Arg-Gly-Ser (SEQ ID NO: 357), E-N-L-Y-F-Q-G (SEQ ID NO: 358), and [AGSV]-X (X can be any amino acid; SEQ ID NO: 359).

28. The method of claim 26 or the population of cells of claim 26, wherein the heterologous protease cleavage site comprises a furin cleavage site selected from the group consisting of RTKR (SEQ ID NO: 378); GTGAEDPRPSRKRRSLGDVG (SEQ ID NO: 379); GTGAEDPRPSRKRR (SEQ ID NO: 381); LQWLEQQVAKRRTKR (SEQ ID NO: 383); GTGAEDPRPSRKRRSLGG (SEQ ID NO: 385); GTGAEDPRPSRKRRSLG (SEQ ID NO: 387); SLNLTESHNSRKKR (SEQ ID NO: 389); CKINGYPKRGRKRR (SEQ ID NO: 391); and SARNRQKR (SEQ ID NO: 336).

29. The method of any one of claims 26-28 or the population of cells of claim 26-28, wherein, the degradation domain is an estrogen receptor (ER) domain; an FKB protein (FKBP) domain; or a dihydrofolate reductase (DHFR) domain.

30. A pharmaceutical composition comprising the population of cells of any one of claims 21-29 and a pharmaceutically acceptable carrier.

31. Use of the population of cells of any one of claims 21-29 or the pharmaceutical composition of claim 30 in the manufacture of a medicament for treating a hematological cancer in a subject.

32. The use of claim 31, wherein the hematological cancer is chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), diffuse large B cell lymphoma (DLBCL), or relapsed and/or refractory DLBCL.