

CLAIMS:

Claim 1. A method of generating a population of primary immune cells resistant to replicative senescence (RRS), comprising:

(a) inhibiting expression of at least three endogenous regulatory factors in the primary immune cells, wherein the endogenous regulatory factors are at least cyclin-dependent kinase inhibitor 2A (CDKN2A), cyclin-dependent kinase inhibitor 2B (CDKN2B), and S-methyl-5'-thioadenosine phosphorylase (MTAP); and

(b) culturing the primary immune cells in a culture medium;
wherein the culturing induces proliferation of the primary immune cells to yield a population of primary immune cells resistant to replicative senescence (RRS).

Claim 2. The method of claim 1 further comprising inhibiting the expression of the at least three endogenous regulatory factors in the primary immune cells after stimulating the primary immune cells.

Claim 3. The method of either claim 1 further comprising inhibiting the expression of the at least three endogenous regulatory factors in the primary immune cells before stimulating the primary immune cells.

Claim 4. The method of any one of claims 1-3 further comprising inhibiting the expression of one or more endogenous immune related genes in the primary immune cells.

Claim 5. The method of claim 4, wherein the endogenous immune related gene is beta-2 microglobulin (B2M), and/or T-cell receptor α constant (TRAC).

Claim 6. The method of any one of claims 1-5 further comprising inhibiting the expression of cluster of differentiation 38 (CD38).

Claim 7. The method of any one of claims 1-6 further comprising inhibiting the expression of phosphatase and tensin homolog (PTEN).

Claim 8. The method of any one of claims 1-7, wherein the primary immune cells comprise total T cells.

Claim 9. The method of any one of claims 1-7, wherein the primary immune cells comprise CD8⁺ T cells.

Claim 10. The method of any one of claims 1-7, wherein the primary immune cells comprise CD4⁺ T cells.

Claim 11. The method of any one of claims 1-7, wherein the primary immune cells comprise gamma-delta T cells, mucosal associated invariant T (MAIT) T cells, natural killer (NK) cells, and/or natural killer T (NKT) cells.

Claim 12. The method of any one of claims 1-11, wherein the primary immune cells are human.

Claim 13. The method of any one of claims 1-12, further comprising introducing a polynucleotide that encodes a chimeric antigen receptor (CAR).

Claim 14. The method of any one of claims 1-13, wherein the population of primary immune cells can be cultured with or without TCR stimulation for at least 100 days.

Claim 15. The method of any one of claims 1-14, wherein the primary immune cells undergo at least a 10⁶-fold expansion during culturing.

Claim 16. The method of any one of claims 1-15, wherein the primary immune cells are cultured in a culture medium that does not include a primary immune cell stimulus.

Claim 17. The method of either claim 2 or claim 3 further comprising (c) restimulating the primary immune cells.

Claim 18. The method of claim 17, wherein the primary immune cells undergo at least a 10^8 -fold expansion during culturing.

Claim 19. An engineered immune cell population produced according to the method of any one of claims 1-18.

Claim 20. A pharmaceutical composition comprising the engineered immune cell population of claim 19 and a pharmaceutically acceptable carrier.

Claim 21. The pharmaceutical composition of claim 20 for use in treating a cancer in a subject in need thereof.

Claim 22. An engineered T cell that does not express cyclin-dependent kinase inhibitor 2A (CDKN2A), cyclin-dependent kinase inhibitor 2B (CDKN2B), and S-methyl-5'-thioadenosine phosphorylase (MTAP).

Claim 23. The engineered T cell of claim 22, wherein the engineered T cell further comprises a transgene encoding either B-cell lymphoma-extra large (Bcl-xL) or B-cell lymphoma 2 (Bcl-2).

Claim 24. The engineered T cell of either claim 22 or 23, wherein the engineered T cell does not express one or more endogenous immune related genes.

Claim 25. The engineered T cell of claim 24, wherein the endogenous immune related gene is beta-2 microglobulin (B2M), and/or T-cell receptor α constant (TRAC).

Claim 26. The engineered T cell of any one of claims 22-25, wherein the engineered T cell does not express cluster of differentiation 38 (CD38).

Claim 27. The engineered T cell of any one of claims 22-26 further comprising a polynucleotide that encodes a chimeric antigen receptor (CAR).

Claim 28. The engineered T cell of claim 22, wherein the engineered T cell is a CD8⁺ T cell, a CD4⁺ T cell, a gamma-delta T cell, a mucosal associated invariant T (MAIT) T cell, a natural killer (NK) cell, a natural killer T (NKT) cell, or a combination thereof.

Claim 29. The engineered T cell of claim 22, wherein the engineered T cell is a CD8⁺ T cell.

Claim 30. The engineered T cell of claim 22, wherein the engineered T cell is a CD4⁺ T cell.

Claim 31. The engineered T cell of any one of claims 22-30, wherein the engineered T cell is human.

Claim 42. A pharmaceutical composition comprising the engineered T cell of any one of claims 22-31.

Claim 43. The pharmaceutical composition of claim 42 for use in treating a cancer in a subject in need thereof.