

CLAIMS:

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

- (a) introducing one or more genetic edits to primary immune cells; 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 stimulating the primary immune cells before the one or more genetic edits is introduced to the primary immune cells.

Claim 3. The method of either claim 1 or claim 2 further comprising stimulating the primary immune cells after the one or more genetic edits is introduced to 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 regulatory factors in the primary immune cells.

Claim 5. The method of claim 4, wherein the endogenous regulatory factor is cyclin-dependent kinase inhibitor 2A (CDKN2A), cyclin-dependent kinase inhibitor 2B (CDKN2B), or S-methyl-5'-thioadenosine phosphorylase (MTAP).

Claim 6. The method of any one of claims 1-5 further comprising inhibiting the expression of one or more endogenous immune related genes in the primary immune cells.

Claim 7. The method of claim 6, wherein the endogenous immune related gene is beta-2 microglobulin (B2M), and/or T-cell receptor α constant (TRAC).

Claim 8. The method of any one of claims 1-7, wherein introducing one or more genetic edits comprise introducing one or more transgenes encoding an anti-apoptotic factor or a virally-derived factor into the primary immune cells.

Claim 9. The method of claim 8, wherein the anti-apoptotic factor is either B-cell lymphoma-extra large (Bcl-xL) or B-cell lymphoma 2 (Bcl-2).

Claim 10. The method of claim 8, wherein the virally-derived factor is any one of *Saimiriine gammaherpesvirus 2 StpA A11*, *Herpesvirus saimiri StpC* *Herpesvirus saimiri Tip*, or a modified *Herpesvirus Ateles-Epstein-Barr virus Tio-LMP1*.

Claim 11. The method of any one of claims 1-10 further comprising inhibiting the expression of cluster of differentiation 38 (CD38).

Claim 12. The method of any one of claims 1-11 further comprising inhibiting the expression of phosphatase and tensin homolog (PTEN).

Claim 13. The method of any one of claims 1-12, wherein the primary immune cells comprise total T cells.

Claim 14. The method of any one of claims 1-12, wherein the primary immune cells comprise CD8⁺ T cells.

Claim 15. The method of any one of claims 1-12, wherein the primary immune cells comprise CD4⁺ T cells.

Claim 16. The method of any one of claims 1-15, 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 17. The method of any one of claims 1-16, wherein the primary immune cells are human.

Claim 18. The method of any one of claims 1-17, further comprising introducing a polynucleotide that encodes a chimeric antigen receptor (CAR).

Claim 19. The method of any one of claims 1-18, wherein the population of primary immune cells can be cultured with or without TCR stimulation for at least 100 days.

Claim 20. The method of any one of claims 1-19, wherein the primary immune cells undergo at least about a 10^6 -fold expansion during culturing.

Claim 21. The method of any one of claims 1-20, wherein the primary immune cells are cultured in a culture medium that does not include a primary immune cell stimulus.

Claim 22. The method of either claim 2 or claim 3 further comprising (c) restimulating the primary immune cells.

Claim 23. The method of claim 22, wherein the primary immune cells undergo at least about a 10^8 -fold expansion during culturing.

Claim 24. The method of any one of claims 1-23, wherein the transgene is introduced using a plasmid-based DNA transposon.

Claim 25. The method of any one of claims 1-23, wherein the transgene is introduced using a lentivirus platform.

Claim 26. The method of any one of claims 1-23, wherein the transgene is introduced using site specific integration via CRISPR.

Claim 27. A method of generating a population of primary immune cells resistant to replicative senescence (RRS), comprising:

(a) inhibiting the expression of one or more endogenous regulatory factors in the primary immune cells,

wherein the endogenous regulatory factor is cyclin-dependent kinase inhibitor 2A (CDKN2A), cyclin-dependent kinase inhibitor 2B (CDKN2B), or S-methyl-5'-thioadenosine phosphorylase (MTAP);

(b) inhibiting the expression of one or more endogenous immune related genes in the primary immune cells,

wherein the endogenous immune related gene is beta-2 microglobulin (B2M), and/or T-cell receptor α constant (TRAC); and

(c) 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 28. A method of generating a population of primary immune cells resistant to replicative senescence (RRS), comprising:

(a) inhibiting expression of one or more endogenous regulatory factors in the primary immune cells,

wherein endogenous regulatory factor is cyclin-dependent kinase inhibitor 2A (CDKN2A), cyclin-dependent kinase inhibitor 2B (CDKN2B), or 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 29. An engineered immune cell population produced according to the method of any one of claims 1-28.

Claim 30. A pharmaceutical composition comprising the engineered immune cell population of claim 29 and a pharmaceutically acceptable carrier.

Claim 31. The pharmaceutical composition of claim 30 for use in treating a cancer in a subject in need thereof.

Claim 32. An engineered T cell that does not express cyclin-dependent kinase inhibitor 2A (CDKN2A), cyclin-dependent kinase inhibitor 2B (CDKN2B), and/or S-methyl-5'-thioadenosine phosphorylase (MTAP).

Claim 33. The engineered T cell of claim 32, 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 34. The engineered T cell of either claim 32 or 33, wherein the engineered T cell does not express of one or more endogenous immune related genes in the primary immune cells.

Claim 35. The engineered T cell of claim 34, wherein the endogenous immune related gene is beta-2 microglobulin (B2M), and/or T-cell receptor α constant (TRAC).

Claim 36. The engineered T cell of any one of claims 32-35, wherein the engineered T cell does not express cluster of differentiation 38 (CD38).

Claim 37. The engineered T cell of any one of claims 32-36 further comprising a polynucleotide that encodes a chimeric antigen receptor (CAR).

Claim 38. The engineered T cell of claim 32, 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 39. The engineered T cell of claim 32, wherein the engineered T cell is a CD8⁺ T cell.

Claim 40. The engineered T cell of claim 32, wherein the engineered T cell is a CD4⁺ T cell.

Claim 41. The engineered T cell of any one of claims 32-40, wherein the engineered T cell is human.

Claim 42. An engineered T cell that does not express cyclin-dependent kinase inhibitor 2A (CDKN2A), cyclin-dependent kinase inhibitor 2B (CDKN2B), S-methyl-5'-thioadenosine phosphorylase (MTAP), beta-2 microglobulin (B2M), and/or T-cell receptor α constant (TRAC).

Claim 43. The engineered T cell of claim 42, wherein the engineered T cell does not express cluster of differentiation 38 (CD38).

Claim 44. The engineered T cell of either claim 42 or claim 43 further comprising a polynucleotide that encodes a chimeric antigen receptor (CAR).

Claim 45. The engineered T cell of claim 42, wherein the engineered T cell is 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 46. The engineered T cell of claim 42, wherein the engineered T cell is a CD8⁺ T cell.

Claim 47. The engineered T cell of claim 42, wherein the engineered T cell is a CD4⁺ T cell.

Claim 48. The engineered T cell of any one of claims 42-47, wherein the engineered T cell is human.

Claim 49. An engineered T cell expressing a transgene encoding a B-cell lymphoma-extra large (Bcl-XL), wherein the engineered T cell does not express cyclin-dependent kinase inhibitor 2A (CDKN2A), cyclin-dependent kinase inhibitor 2B (CDKN2B), S-methyl-5'-thioadenosine phosphorylase (MTAP), and/or phosphatase and tensin homolog (PTEN).

Claim 50. The engineered T cell of claim 49, wherein the engineered T cell does not express of one or more endogenous immune related genes in the primary immune cells.

Claim 51. The engineered T cell of claim 50, wherein the endogenous immune related gene is beta-2 microglobulin (B2M), or T-cell receptor α constant (TRAC).

Claim 52. The engineered T cell of any one of claims 49-51, wherein the engineered T cell does not express cluster of differentiation 38 (CD38).

Claim 53. The engineered T cell of any one of claims 49-52 further comprising a polynucleotide that encodes a chimeric antigen receptor (CAR).

Claim 54. The engineered T cell of claim 49, wherein the engineered T cell is a CD8⁺ T cell, a CD4⁺ T cell, a delta gamma T cell, a mucosal associated invariant T (MAIT) T cell, a natural killer (NK) T cell, or a combination thereof.

Claim 55. The engineered T cell of claim 49, wherein the engineered T cell is a CD8⁺ T cell.

Claim 56. The engineered T cell of claim 49, wherein the engineered T cell is a CD4⁺ T cell.

Claim 57. The engineered T cell of any one of claims 49-56, wherein the engineered T cell is human.