Claims

1. A polynucleotide, ~~the polynucleotide~~ comprising:

   a. an immune cell survival-enhancing gene comprising a nucleic acid encoding a protein selected from:~~a first nucleotide sequence encoding a chimeric antigen receptor comprising an extracellular domain that specifically binds to a CD19 antigen; and~~

      i. CD28, wherein the CD28 comprises one or more of the following activating mutations: D124E, D124V, T195I, or T195P; or

      ii. An inhibitor of apoptosis selected from Survivin, Bcl2, Bcl6, or Bcl-XL,

      wherein the immune cell survival-enhancing gene is operably linked to a first heterologous regulatory sequence effective for expression of the protein within an immune cell thereby enhancing survival of the immune cell; and

   b. a gene encoding a receptor capable of binding to an antigen, wherein the gene is operably linked to a second heterologous regulatory sequence effective for expression of the receptor within the immune cell.

~~a second nucleotide sequence encoding either an apoptosis inhibitor selected from the group consisting of SEQ ID NOs: 237 and 238 or an activating mutant consisting of SEQ ID NO. 251.~~

2. A transposon comprising ~~T~~the polynucleotide according to claim 1~~, further comprising a transposon~~.

3. An immune cell ~~modified T-cell expressing a~~ whose genome comprises the polynucleotide according to claim 1 ~~or 2~~.

~~4. The modified T-cell according to claim 3, wherein:~~

~~a persistence of the modified T-cell is increased relative to a persistence of a T-cell that does not comprise the first and second nucleotide sequences; and/or~~

~~a proliferation rate of the modified T-cell is increased relative to a proliferation rate of a T-cell that does not comprise the first and second nucleotide sequences.~~

~~5.~~4. A method for preparing an immune cell ~~modified T-cell~~ according to claim 3 ~~or 4~~, the method comprising the step of introducing the polynucleotide into an immune cell ~~T-cell ex-vivo~~.

~~6.~~5. The method of claim ~~5~~4, wherein~~, when~~ the polynucleotide further comprises ~~a~~ transposon ends, and wherein the method further comprises the step of introducing into the immune cell a corresponding transposase, such that the gene encoding the protein and the gene encoding the receptor are transposed into the genome of the immune cell.~~:~~

~~(A) the second nucleotide sequence is operably linked to a heterologous promoter; and~~

~~(B) the polynucleotide is flanked by a pair of transposon ends,~~

~~wherein a transposase is introduced into the T-cell, and~~

~~wherein the polynucleotide is transposable by the transposase.~~

~~7. The method of claim 6, wherein the transposon ends have flanking sequences selected from the group consisting of SEQ ID NOs: 6 and 7, SEQ ID NOs: 10 and 11, SEQ ID NOs:~~

~~14 and 15, SEQ ID NOs: 20 and 21, SEQ ID NOs: 16 and 17, a sequence at least 90% identical to SEQ ID NO: 24 and a sequence at least 90% identical to SEQ ID NO: 25, or a sequence at least 90% identical to SEQ ID NO: 397 and a sequence at least 90% identical to SEQ ID NO: 398; and~~

~~(1) where, when the pair of flanking sequences is SEQ ID NOs: 6 and 7 or SEQ ID NOs: 10 and 11, the transposase is represented by a sequence at least 90% identical to SEQ ID NO: 31 or 32;~~

~~(2) where, when the pair of flanking sequences is SEQ ID NOs: 14 and 15, the transposase is represented by a sequence at least 90% identical to SEQ ID NO: 64;~~

~~(3) where, when the pair of flanking sequences is SEQ ID NOs: 20 and 21, the transposase is represented by a sequence at least 90% identical to SEQ ID NO: 29;~~

~~(4) where, when the pair of flanking sequences is SEQ ID NOs: 16 and 17, the transposase is represented by a sequence at least 90% identical to SEQ ID NO: 30;~~

~~(5) where, when the pair of flanking sequences is a sequence at least 90% identical to SEQ ID NO: 24 and a sequence at least 90% identical to SEQ ID NO: 25, the transposase is represented by a sequence at least 90% identical to SEQ ID NO: 28; and~~

~~(6) where, when the pair of flanking sequences is a sequence at least 90% identical to SEQ ID NO: 397 and a sequence at least 90% identical to SEQ ID NO: 398, the transposase is represented by a sequence at least 90% identical to SEQ ID NO: 401.~~

~~8.~~6. The method of claim ~~6 or claim 7~~5, wherein the transposase is introduced as a nucleic acid encoding the transposase.

~~9.~~7. The method of claim ~~8~~6, wherein the nucleic acid encoding the transposase is an mRNA.

~~10.~~8. The method of claim ~~5~~7, wherein the ~~polynucleotide is integrated into the genome of the modified T cell~~ nucleic acid encoding the transposase is operably linked to a promoter that is active in the immune cell.

~~11. The modified T cell of claim 3 for use in the treatment of cancer.~~

~~12. A pharmaceutical composition comprising a plurality of modified T cells according to claim 3.~~

~~13. The pharmaceutical composition according to claim 12 for use in the treatment of a B cell malignancy.~~

~~14. A kit comprising:~~

~~(A) the polynucleotide of claim 2;~~

~~(B) a transposase capable of transposing the transposon or a nucleic acid encoding a transposase capable of transposing the transposon; and, optionally,~~

~~(C) an anti-hCD19-CD3 bispecific T cell engager.~~

~~15. The kit according to claim 14, wherein, when the kit comprises a nucleic acid encoding the transposase, the nucleic acid encoding the transposase is an mRNA.~~

9. The method of claim 8, wherein the immune cell is a T-cell, wherein binding of the receptor to a target cell which displays the antigen on its surface causes the T-cell to kill the target cell.

10. A lentiviral vector comprising the polynucleotide according to claim 1.

11. The immune cell of claim 3, wherein the immune cell is a T-cell.

12. The immune cell of claim 11, wherein the survival upon repeated antigen challenge of the T-cell is increased by at least 25% relative to the survival of an immune cell whose genome does not comprise the polynucleotide.

13. The polynucleotide of claim 1, wherein the gene encoding the receptor encodes a CD 19-binding chimeric antigen receptor, a polypeptide with sequence given by SEQ ID NO: 229, and is operably linked to either a PGK promoter or a GAPDH promoter; and the immune cell survival-enhancing gene has a sequence given by SEQ ID NO: 226 encoding Survivin and is operably linked to a PGK promoter.

14. The polynucleotide of claim 1, wherein the gene encoding the receptor encodes a CD 19-binding chimeric antigen receptor, a polypeptide with sequence given by SEQ ID NO: 229, and is operably linked to either a PGK promoter or a GAPDH promoter; and the immune cell survival-enhancing gene has a sequence given by SEQ ID NO: 227 encoding CD28-D124E-T195P and is operably linked to a PGK promoter.

15. The polynucleotide of claim 1, wherein the gene encoding the receptor encodes a CD 19 binding chimeric antigen receptor, a polypeptide with sequence given by SEQ ID NO:

229, and is operably linked to either a PGK promoter or a GAPDH promoter; and the immune cell survival-enhancing gene encodes a polypeptide with sequence given by SEQ ID NO: 238 and is operably linked to a PGK promoter.