

Claims

1. A bicistronic retroviral vector comprising:

(a) a first polynucleotide encoding an anti-CD7 chimeric antigen receptor (CAR) comprising at least 90% sequence identity to the amino acid sequence of any one of SEQ ID

NOS:28-31; and wherein the anti-CD7 CAR comprises an antigen-binding domain comprising

(i) a heavy chain complementarity-determining region 1 (HC CDR1) of the amino acid sequence of SEQ ID NO: 47, HC CDR2 of the amino acid sequence of SEQ ID NO: 48, HC CDR3 of SEQ ID NO: 49, and a light chain (LC) CDR1 of the amino acid sequence of SEQ ID NO: 44, LC CDR2 of the amino acid sequence of SEQ ID NO: 45, and LC CDR3 of the amino acid sequence of SEQ ID NO: 46 of the amino acid sequence of SEQ ID NO: 28 or SEQ ID NO: 29, or

(ii) a HC CDR1 of the amino acid sequence of SEQ ID NO: 53, HC CDR2 of the amino acid sequence of SEQ ID NO: 54, HC CDR3 of the amino acid sequence of SEQ ID NO: 55, and a LC CDR1 of the amino acid sequence of SEQ ID NO: 50, LC CDR2 of the amino acid sequence of SEQ ID NO: 51, and LC CDR3 of the amino acid sequence of SEQ ID NO: 52 of the amino acid sequence of SEQ ID NO: 30 or SEQ ID NO: 31;

(b) a second polynucleotide encoding ~~an Internal Ribosome Entry Site (IRES) or a ribosomal codon skipping site~~ a 2A self-cleaving peptide; and

(c) a third polynucleotide encoding an anti-CD7 protein expression blocker (PEBL) comprising at least 90% sequence identity to the amino acid sequence of SEQ ID

NOS:24-27; and wherein the anti-CD7 PEBL comprises an antigen-binding domain comprising

(i) a heavy chain complementarity-determining region 1 (HC CDR1) of the amino acid sequence of SEQ ID NO: 47, HC CDR2 of the amino acid sequence of SEQ ID NO: 48, HC CDR3 of SEQ ID NO: 49, and a light chain (LC) CDR1 of the amino acid sequence of SEQ ID NO: 44, LC CDR2 of the amino acid sequence of SEQ ID NO: 45, and LC CDR3 of the amino acid sequence of SEQ ID NO: 46 of the amino acid sequence of SEQ ID NO: 24 or SEQ ID NO: 25, or

(ii) a HC CDR1 of the amino acid sequence of SEQ ID NO: 53, HC CDR2 of the amino acid sequence of SEQ ID NO: 54, HC CDR3 of the amino acid sequence of SEQ ID NO: 55, and a LC CDR1 of the amino acid sequence of SEQ ID NO: 50, LC CDR2 of the amino acid

sequence of SEQ ID NO: 51, and LC CDR3 of the amino acid sequence of SEQ ID NO: 52 of the amino acid sequence of SEQ ID NO: 26 or SEQ ID NO: 27;

wherein the first polynucleotide is operably linked to the second polynucleotide which is operably linked to the third polynucleotide.

2. The bicistronic retroviral vector of claim 1, wherein the anti-CD7 CAR comprises the amino acid sequence of any one of SEQ ID NOS:28-31.

3. The bicistronic retroviral vector ~~composition~~ of claim 1 or 2, wherein the anti-CD7 PEBL comprises the amino acid sequence of any one of SEQ ID NOS:24-27.

4. The bicistronic retroviral vector ~~composition~~ of any one of claims 1 to 3, wherein the anti-CD7 CAR comprises the amino acid sequence of SEQ ID NO:29 and the anti-CD7 PEBL comprises the amino acid sequence of SEQ ID NO:25.

5. The bicistronic retroviral vector ~~composition~~ of any one of claims 1 to 3, wherein the anti-CD7 CAR comprises the amino acid sequence of SEQ ID NO:31 and the anti-CD7 PEBL comprises the amino acid sequence of SEQ ID NO:27.

~~6. The bicistronic retroviral vector of any one of claims 1 to 5, wherein the IRES is derived from *Encephalomyocarditis virus* (EMCV) or an *Enterovirus*.~~

~~7. The bicistronic retroviral vector of any one of claims 1 to 5, wherein the ribosomal codon skipping site comprises a 2A self-cleaving peptide.~~

~~8~~6. The bicistronic retroviral vector of claim ~~7~~1, wherein the 2A self-cleaving peptide is selected from the group consisting of a F2A peptide, an E2A peptide, a P2A peptide, and a T2A peptide.

~~9. The bicistronic retroviral vector of any one of claims 1 to 6, comprising at least 90% sequence identity to SEQ ID NO:12.~~

~~10~~7. The bicistronic retroviral vector of any one of claims 1 to ~~5 and~~ 6, comprising at least 90% sequence identity to SEQ ID NO:13.

~~118~~. The bicistronic retroviral vector of any one of claims 1 to 6~~claim 10~~, comprising SEQ ID NO:13.

~~129~~. The bicistronic retroviral vector of any one of claims 1 to ~~118~~, further comprising a promoter element, wherein the promoter element is selected from the group consisting of a CMV promoter, a EF1 α promoter, a EFS promoter, a MSCV promoter, and a PGK promoter. ~~—~~

~~1310~~. The bicistronic retroviral vector of claim ~~129~~, wherein the promoter element comprises at least 90% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOS:6-10.

~~1411~~. The bicistronic retroviral vector of claim ~~139~~, wherein the promoter element comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOS:6-10.

~~1512~~. The bicistronic retroviral vector of any one of claims ~~12-9~~ to ~~1411~~, ~~comprising wherein the bicistronic retroviral vector comprises a nucleic acid sequence having~~ at least 90% sequence identity to ~~the a~~ nucleic acid sequence selected from the group consisting of SEQ ID NOS:14-16.

~~1613~~. The bicistronic retroviral vector of any one of claims ~~12-9~~ to ~~1511~~, ~~comprising wherein the bicistronic retroviral vector comprises~~ a nucleic acid sequence selected from the group consisting of SEQ ID NOS:14-16.

~~1714~~. The bicistronic retroviral vector of any one of claims 1 to ~~1613~~, wherein the retroviral vector is a lentiviral vector.

~~1815~~. An engineered immune cell comprising the bicistronic retroviral vector of any one of claims 1 to ~~1714~~.

~~1916~~. The engineered immune cell of claim ~~1815~~, wherein the engineered immune cell is ~~an allogeneica~~ T cell.

~~2017~~. The engineered immune cell of claim ~~1915 or 16~~, wherein the engineered immune cell is ~~an autologous a~~ CD4⁺ or a CD8⁺ T cell.

~~21~~18. The engineered immune cell of any one of claims 19 or 20~~15 to 17~~, wherein the engineered immune cell expresses the anti-CD7 CAR and has reduced CD7 surface expression compared to ~~a corresponding~~an otherwise identical immune cell that does not express the anti-CD7 PEBL and expresses the anti-CD7 CAR.

~~22~~19. A pharmaceutical composition comprising a T cell that is the engineered immune cell of any one of claims 15 to 18 and a pharmaceutically effective carrier.

~~23~~20. A therapeutically effective amount of T cells, wherein each T cell is an~~the~~ engineered immune cell of any one of claims 15 to 18 ~~to 20~~, or the pharmaceutical composition of claim ~~22~~19, for use in the treatment of cancer.

~~24~~21. Use of a therapeutically effective amount of T cells, wherein each T cell is an~~the~~ engineered immune cell of any one of claims 15 to 18 ~~to 21~~, or the pharmaceutical composition of claim 19, in the preparation of a medicament for the treatment of cancer.

~~25~~22. An in vitro method of producing an engineered immune cell comprising transducing an immune cell with the bicistronic retroviral vector of any one of claims 1 to ~~14~~14, and recovering the engineered immune cell.

~~26~~23. The in vitro method of claim ~~25~~22, wherein the immune cell is selected from the group consisting of a peripheral blood mononuclear cell, an isolated CD4+ T cell, an isolated CD8+ T cell, and an isolated CD3+ T cell.

~~27~~24. The in vitro method of claim ~~25~~22 or ~~26~~23, wherein the engineered immune cell has reduced CD7 surface expression compared to ~~a corresponding~~an otherwise identical immune cell that (i) does not express the anti-CD7 PEBL and (ii) expresses the anti-CD7 CAR.

25. The bicistronic retroviral vector of any one of claims 9-14, wherein the promoter element is a MSCV promoter.

26. The bicistronic retroviral vector of any one of claims 9-14, wherein the promoter element is a EFS promoter.

~~28. A recombinant retroviral vector comprising:~~

~~(a) a first promoter element operably linked to a first polynucleotide encoding an anti-CD7 chimeric antigen receptor (CAR) comprising at least 90% sequence identity to the amino acid sequence of SEQ ID NO:28 or SEQ ID NO:30; and~~

~~(b) a second promoter element operably linked to a second polynucleotide encoding an anti-CD7 protein expression blocker (PEBL) comprising at least 90% sequence identity to the amino acid sequence of SEQ ID NO:24 or SEQ ID NO:26.~~

~~29.—The recombinant retroviral vector of claim 28, wherein the anti-CD7 CAR comprises the amino acid sequence of SEQ ID NO:28 or SEQ ID NO:30.~~

~~30.—The recombinant retroviral vector of claim 28 or 29, wherein the anti-CD7 PEBL comprises the amino acid sequence of SEQ ID NO:24 or SEQ ID NO:26.~~

~~31.—The recombinant retroviral vector of any one of claims 28 to 30, wherein the first promoter element and/or the second promoter element are selected from the group consisting of a CMV promoter, a EF1 α promoter, a EFS promoter, a MSCV promoter, and a PGK promoter.~~

~~32.—The recombinant retroviral vector of any one of claims 28 to 31, wherein the first promoter element and/or the second promoter element comprises at least 90% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOS:6-10.~~

~~33.—The recombinant retroviral vector of any one of claims 28 to 32, wherein the first promoter element and/or the second promoter element comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOS:6-10.~~

~~34.—The recombinant retroviral vector of any one of claims 28 to 33, wherein the first promoter and the second promoter share less than 95% sequence identity.~~

~~35.—The recombinant retroviral vector of any one of claims 28 to 34, wherein the first promoter element operably linked to the first polynucleotide, is 5' of the second promoter element operably linked to the second polynucleotide.~~

~~36.—The recombinant retroviral vector of any one of claims 28 to 34, wherein the second promoter element operably linked to the second polynucleotide, is 5' of the first promoter element operably linked to the first polynucleotide.~~

~~37. The recombinant retroviral vector of any one of claims 28 to 35, comprising at least 90% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOS:18-23.~~

~~38. The recombinant retroviral vector of any one of claims 28 to 37, wherein the retroviral vector is a lentiviral vector.~~

~~39~~27. An engineered immune cell comprising the recombinant retroviral vector of claim 25 or 26~~any one of claims 28 to 38~~.

~~40. The engineered immune cell of claim 39, wherein the engineered immune cell is an allogenic T cell.~~

~~41. The engineered immune cell of claim 40, wherein the engineered immune cell is an autologous T cell.~~

~~42~~28. The engineered immune cell of claim ~~40~~27, wherein the engineered immune cell has reduced CD7 surface expression compared to an otherwise identical immune cell that (i) does not express the anti-CD7 PEBL and (ii) a corresponding immune cell and expresses the anti-CD7 CAR.

~~43~~29. A pharmaceutical composition comprising a T cell that is the engineered immune cell of claim 27 or 28~~any one of claim 39 to 42~~ and a pharmaceutically effective carrier.

~~44~~30. A therapeutically effective amount of T cells, wherein each T cell is an ~~The~~ engineered immune cell of claim 27 or 28~~any one of claims 39 to 41~~, or the pharmaceutical composition of claim ~~43~~29 for use in the treatment of cancer.

~~45~~31. Use of a therapeutically effective amount of T cells, wherein each T cell is an ~~the~~ engineered immune cell of claim 27 or 28~~any one of claims 39 to 41~~, or the pharmaceutical composition of claim ~~43~~29, in the preparation of a medicament for the treatment of cancer.

~~46~~32. An in vitro method of producing an engineered immune cell comprising transducing an immune cell with the recombinant retroviral vector of claim 25 or 26~~any one of claims 28 to 38~~, and recovering the engineered immune cell.

4733. The in vitro method of claim 4632, wherein the immune cell is selected from the group consisting of a peripheral blood mononuclear cell, an isolated CD4+ T cell, an isolated CD8+ T cell, and an isolated CD3+ T cell.

4834. The in vitro method of claim 4632 or 4733, wherein the engineered immune cell has reduced CD7 surface expression compared to an otherwise identical immune cell that (i) does not express the anti-CD7 PEBL and (ii) a corresponding immune cell and expresses the anti-CD7 CAR.