

Claims

1. A modified induced pluripotent stem cell iPSC or haemogenic lineage cell comprising at least one heterologous nucleic acid sequence encoding a heterologous T-cell receptor (TCR) integrated at or into a locus in the cell genome.

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2. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to claim 1, wherein the at least one heterologous nucleic acid sequence encoding a heterologous TCR is an expressible heterologous nucleic acid sequence.

10 3. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to either claim 1 or claim 2, wherein the modified induced pluripotent stem cell iPSC or haemogenic lineage cell expresses or presents the at least one heterologous TCR encoded by a heterologous TCR encoding nucleic acid sequence, preferably expressed or presented at the cell surface.

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4. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any previous claim, wherein the modified haemogenic lineage cell is derived from the modified iPSC.

20 5. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any previous claim, wherein the haemogenic lineage cell is selected from any one of;

(a) a mesoderm cell, optionally which may express any one or more of the mesodermal markers, Brachyury, Goosecoid, Mixl1, KDR (also known as FLK1 or VEGFR2), FoxA2, GATA6 or PDGF alpha R,

25 (b) a haemogenic endothelial cell, optionally which may be (a) CD34+ or (b) CD34+ CD73- or (c) CD34+ CD73- CXCR4- (CD184-),

(c) a haematopoietic progenitor cell, optionally which may be (a) CD34+ or (b) CD34+ CD45+ or (c) CD34+ and/or CD45+ in combination with any one or more of CD117+, CD133+, CD45+, FLK+, CD38-, (d) CD34+, CD133+, CD45+, FLK1+,

30 CD38-

- (d) a progenitor T cell, optionally which may be (a) CD5+ and/or CD7+, or (b) CD5+ and/or CD7+ in combination with any one or more of: CD44+, CD25+, CD2+, CD45+, CD3-, CD4-, CD8-,
- 5 (e) a double positive T cell (DP T cell), optionally which may be (a) CD4+ CD8+ (b) CD4+ CD8+ in combination with any one or more of CD3+, CD28+, CD45+,
- (f) a single positive T cells (SP T cell) optionally which may be (a) CD4+ (b) CD8+ (b) CD4+ or CD8+ in combination with any one or more of CD3+, CD28+, CD45+, or
- (g) a mature T cell, optionally alpha beta T cell, gamma delta T cell, NKT cell, T helper cell (TH) which are CD4+, cytotoxic T cell (TC) which are CD8+.

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6. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any previous claim, wherein RAG1 expression is reduced or eliminated.

15 7. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to claim 6, wherein the *rag1* gene is inactivated or knocked-out.

8. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any previous claim, wherein the heterologous nucleic acid sequence encoding a heterologous TCR is integrated at or into one or both alleles of the locus in the cell genome.

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9. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to claim 6, wherein the locus is a gene encoding an endogenous protein of the cell.

25 10. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to claim 7, wherein the heterologous nucleic acid sequence encoding a heterologous TCR is integrated adjacent to or within the gene encoding the endogenous protein

11. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to either of claims 9 or 10, wherein the heterologous nucleic acid sequence encoding a

heterologous TCR is integrated within an intron or exon of the gene encoding the endogenous protein, optionally a 3' exon.

12. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to claim 9, wherein the heterologous nucleic acid sequence encoding a heterologous TCR is integrated within the 3' exon before the TAG stop sequence of the gene or nucleic acid sequence encoding the endogenous protein.

13. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 7 to 10, wherein the integration of the heterologous nucleic acid sequence encoding a heterologous TCR is non-disruptive to the production of the endogenous protein.

14. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 9 to 13, comprising a fusion sequence between the nucleic acid encoding the heterologous TCR and the nucleic acid encoding the endogenous gene, preferably a fusion gene or sequence or multicistronic fusion gene or sequence between the nucleic acid encoding the heterologous TCR and the nucleic acid encoding the endogenous protein.

15. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to claim 14 wherein the nucleic acid encoding the heterologous TCR is connected to the nucleic acid encoding the endogenous protein by a nucleic acid sequence encoding a peptide comprising an enzymatic cleavage site and/or a nucleic acid sequence which mediates ribosome-skipping.

16. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to either of claims 14 or 15, wherein the nucleic acid encoding the heterologous TCR comprises a coding sequence of a TCR α and TCR β chain, optionally with an intervening nucleic acid sequence encoding a peptide comprising an enzymatic cleavage site and/or nucleic acid sequence which mediates ribosome-skipping.

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17. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to either of claims 15 or 16, wherein the nucleic acid sequence which mediates ribosome-skipping is a T2A or P2A skip sequence.
- 5 18. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 15 to 17, wherein the nucleic acid sequence encoding a peptide comprising an enzymatic cleavage site encodes a furin cleavage site, preferably RAKR.
- 10 19. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 9 to 18, wherein the transcription and expression of the heterologous TCR and the endogenous protein is from the same promoter.
- 15 20. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 9 to 19, wherein the heterologous TCR is expressed as a fusion protein with the endogenous protein, optionally connected by a peptide comprising an enzymatic cleavage site, preferably a furin cleavage site, preferably RAKR and/or ribosome skip sequence, preferably T2A or P2A skip sequence.
- 20 21. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 1 to 20, wherein the heterologous TCR is expressed and/or presented in the cell as a nascent heterologous TCR comprising a TCR alpha chain and TCR beta chain, preferably expressed and/or presented at the cell surface.
- 25 22. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 9 to 21, wherein the endogenous protein is a protein that is trafficked to the cell surface, optionally via the secretory pathway and/or to the plasma membrane.
- 30 23. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 3 to 22, wherein the endogenous protein is a membrane protein or transmembrane protein, optionally a receptor protein, preferably a receptor protein tyrosine phosphatase (PTP), preferably CD45 or protein tyrosine phosphatase receptor type C or PTPRC.

24. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any previous claim, wherein the locus is or is in a gene encoding a membrane protein or transmembrane protein, optionally a receptor protein, preferably a receptor protein tyrosine phosphatase (PTP), preferably CD45 or protein tyrosine phosphatase receptor type C or PTPRC.

25. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any previous claim, wherein the locus is or is in the PTPRC (CD45) gene on chromosome 1.

26. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to claim 25, wherein the integration is at exon 33 of the PTPRC (CD45) gene, optionally before the TAG stop codon or immediately adjacent to and/or before the TAG stop codon.

27. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 14 to claim 26, wherein the fusion gene or sequence or multicistronic fusion gene or sequence comprises the nucleic acid encoding the heterologous TCR and nucleic acid encoding PTPRC with an intervening nucleic acid sequence encoding a peptide comprising an enzymatic cleavage site, preferably a furin cleavage site, and nucleic acid sequence which mediates ribosome-skipping, preferably selected from a T2A or P2A skip sequence and wherein the nucleic acid sequence encoding the heterologous TCR comprises the coding sequence of a TCR α and TCR β chain, with an intervening nucleic acid sequence encoding a peptide comprising an enzymatic cleavage site, preferably a furin cleavage site, and nucleic acid sequence which mediates ribosome-skipping, preferably selected from T2A or P2A skip sequence.

28. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any previous claim, wherein the heterologous TCR binds or specifically binds to an antigen or peptide antigen thereof selected from,

- (a) a cancer and/or tumour antigen or peptide antigen thereof, or
- (b) a cancer and/or tumour antigen or peptide antigen thereof associated with a cancerous condition and/or presented by tumour or cancer cell or tissue.

29. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to claim 28 wherein the cancer and/or tumour antigen or peptide antigen thereof is selected from;
- (a) a cancer-testis antigen,
 - 5 (b) a MAGE antigen,
 - (c) MAGE A4 or peptide antigen thereof, optionally a peptide antigen comprising the sequence GGYDGREHTV (SEQ ID NO: 2), or
 - (d) AFP or peptide antigen thereof, optionally a peptide antigen comprising the sequence FMNKFIYEI (SEQ ID No: 21).
- 10 30. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to either one of claims 28 or 29 wherein the cancer and/or tumour antigen or peptide antigen thereof is complexed with a peptide presenting molecule, optionally major histocompatibility complex (MHC) or human leukocyte antigen (HLA), optionally class I or class II, optionally HLA-A2 or HLA-A*02, or HLA-A*0201.
- 15 31. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any previous claim, wherein the heterologous TCR comprises a TCR having;
- (a) an alpha chain variable domain comprising an amino acid sequence that has at least 80%, identity to SEQ ID NO:9 or the sequence of amino acid residues 1-136 of SEQ ID NO:6, and/or the beta chain variable domain comprising an amino acid
 - 20 sequence that has at least 80%, identity to SEQ ID NO:10 or the sequence of amino acid residues 1-133 of SEQ ID NO:7, or
 - (b) an alpha chain variable domain comprising an amino acid sequence that has at least 80%, identity to the sequence of amino acid residues 1-112 of SEQ ID NO:22, and/or the beta chain variable domain comprising an amino acid sequence that has
 - 25 at least 80%, identity to the sequence of amino acid residues 1-112 of SEQ ID NO:23.
32. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any previous claim, wherein the iPSC or haemogenic lineage cell further comprises a nucleic acid encoding and/or expresses or presents a heterologous co-receptor, optionally wherein
- 30 the co-receptor is a CD8 co-receptor.

33. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to claim 32, wherein the heterologous CD8 co-receptor is heterodimer or homodimer, a CD8 $\alpha\beta$ heterodimer or a CD8 $\alpha\alpha$ homodimer.

34. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to
5 either one of claims 32 or 33, wherein the heterologous CD8 co-receptor comprises;

(a) a CDR 1 of at least 80% sequence identity to amino acid sequence VLLSNPTSG, SEQ ID NO:17, CDR 2 of at least 80% sequence identity to amino acid sequence YLSQNKPK SEQ ID NO:18 and CDR 3 of at least 80% sequence identity amino acid sequence LSNSIM SEQ ID NO:19, or

10 (b) an amino acid sequence having at least 80% sequence identity to amino acids number 22 to 235 of SEQ ID NO: 19.

35. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any previous claim, wherein the iPSC or haemogenic lineage cell further comprises a nucleic
15 acid encoding and/or expresses or presents a heterologous co-stimulatory ligand; optionally 4-1BBL or CD80.

36. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 1 to 35, wherein the binding of the iPSC or haemogenic lineage cell and/or
20 heterologous TCR to the cancer and/or tumour antigen or peptide antigen thereof or a cancer and/or tumour cell or tissue expressing or presenting the cancer and/or tumour antigen or peptide antigen thereof according to any of claims 28 to 30 induces activation of the iPSC or haemogenic lineage cell, optionally as determined by any one or more of;

25 (a) up-regulation of T cell activation markers, for example either CD69 and/or CD25 on CD3+ cells,

(b) up-regulation of cytokine production, for example any one or more of IFN gamma, IL-2 or Granzyme B,

(c) induced cell cytotoxic activity in the presence of the antigen or antigen peptide, or

(d) ability to kill tumour cells presenting the antigen or antigen peptide.

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37. A nucleic acid construct or vector comprising a nucleic acid region encoding the heterologous TCR according to any previous claim and at least one homology region comprising a nucleic acid region homologous to a nucleic acid region at the locus in the cell genome for integration of the nucleic acid region encoding the heterologous TCR.

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38. The nucleic acid construct or vector according to claim 37, wherein the locus is or is in a gene encoding a membrane protein or transmembrane protein, optionally a receptor protein, preferably a receptor protein tyrosine phosphatase (PTP), preferably CD45 or protein tyrosine phosphatase receptor type C or PTPRC.

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39. The nucleic acid construct or vector according to either claim 37 or 38, wherein the locus is or is in the PTPRC (CD45) gene on chromosome 1.

40. The nucleic acid construct or vector according to any one of claims 37 to 39, wherein the locus is at exon 33 of the PTPRC (CD45) gene, optionally before the TAG stop codon or immediately adjacent to and/or before the TAG stop codon.

41. The nucleic acid construct or vector according to any one of claims 37 to 40, wherein the nucleic acid encoding the heterologous TCR comprises a coding sequence of a TCR α and TCR β chain, optionally with an intervening nucleic acid sequence encoding a peptide comprising an enzymatic cleavage site and/or nucleic acid sequence which mediates ribosome-skipping, preferably a furin cleavage site, preferably RAKR and/or ribosome skip sequence, preferably T2A or P2A skip sequence.

42. The nucleic acid construct or vector according to any one of claims 37 to 41, wherein the construct or vector comprises a left hand and a right hand homology region each homologous to a nucleic acid region at the locus in the cell genome for integration of the nucleic acid region encoding the heterologous TCR and which flank opposite sides of the integration site.

43. The nucleic acid construct or vector according to any one of claims 37 to 42, wherein the construct or vector further comprises any one or more of:

- (a) a recombination target sequence, preferably loxP (locus of X-over P1) sequence,
- (b) an expressible selection marker sequence, preferably an antibiotic resistance gene, and optionally a neomycin resistance gene, preferably constitutively expressed from a promoter for example from an EF1A promoter.

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44. The nucleic acid construct or vector according to any one of claims 37 to 43, wherein the construct or vector comprises a nucleotide sequence encoding the heterologous TCR comprising the sequence SEQ ID No: 45, homology regions comprising the sequences SEQ ID No: 43 and SEQ ID No: 44, a recombination target sequence comprising SEQ ID No: 48 and an expressible selection marker sequence comprising SEQ ID No: 47 and SEQ ID No: 49.

45. A process of producing a modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 1 to 36 comprising introducing the nucleic acid construct or vector according to any one of claims 37 to 44 into an unmodified induced pluripotent stem cell iPSC or haemogenic lineage cell, optionally as defined in claim 5, under conditions to permit integration of the nucleic acid sequence encoding a heterologous T-cell receptor (TCR) at or into a locus in the cell genome and optionally isolating the modified induced pluripotent stem cell iPSC or haemogenic lineage cell.

46. A pharmaceutical composition comprising the modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 1 to 36 and a pharmaceutically acceptable carrier.

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47. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 1 to 36, or pharmaceutical composition of claim 46, for use in therapy and/or medicine.

48. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 1 to 36, or pharmaceutical composition of claim 46, for use in treatment, prevention or delaying the progression of cancer and/or tumour in an individual or subject optionally wherein the treatment is cancer immunotherapy therapy and/or adoptive T cell therapy, optionally allogenic adoptive T cell therapy.

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49. Use of the modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 1 to 36, or pharmaceutical composition of claim 46, in the manufacture of a medicament for the treatment of cancer and/or tumour in an individual or

subject, optionally wherein the treatment is cancer immunotherapy therapy and/or adoptive T cell therapy, optionally allogenic adoptive T cell therapy.

50. A method of treating, preventing or delaying the progression of cancer and/or tumour in
5 an individual or subject comprising administering to the individual the modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 1 to 34, or pharmaceutical composition of claim 44, optionally wherein the treatment is cancer immunotherapy therapy and/or adoptive T cell therapy, optionally allogenic adoptive T cell therapy.

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51. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 1 to 36, or pharmaceutical composition of claim 46, for use according to any of claims 47 to 49 or in the method of claim 50, wherein the cancer and/or tumour is a solid tumour.

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52. The modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 1 to 36, or pharmaceutical composition of claim 46, for use according to any of claims 47 to 49, or in the method of claim 50, wherein the modified induced pluripotent stem cell iPSC or haemogenic lineage cell, or pharmaceutical composition is for
20 use or used in combination with one or more further therapeutic agent optionally administered or for administration separately, sequentially or simultaneously.

53. A kit comprising,

25 (a) the modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 1 to 36, or pharmaceutical composition of claim 46, and a package insert comprising instructions for use thereof for treating, preventing or delaying the progression of cancer and/or tumour in an individual or subject, or

30 (b) the modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to any one of claims 1 to 36, or pharmaceutical composition of claim 46, and a package insert comprising instructions for use thereof for treating, preventing or delaying the progression of cancer and/or tumour in an individual or subject, in

combination with one or more further therapeutic agent optionally administered or for administration separately, sequentially or simultaneously.