

Claims

1. A modified $\gamma\delta$ T cell comprising a chimeric antigen receptor wherein the chimeric antigen receptor comprises an extracellular antigen binding domain with binding specificity to a disease antigen, a hinge, a transmembrane domain, and
5 (i) one or more co-stimulatory signalling regions wherein a CD3 zeta domain is absent (tuneable), or
(ii) one or more co-stimulatory signalling regions and a non-functional CD3 zeta domain (tuneable), or
10 (iii) a CD3 zeta domain, or
(iv) one or more co-stimulatory signalling regions and a functional CD3 zeta domain.
2. A modified $\gamma\delta$ T cell comprising a chimeric antigen receptor wherein the
15 chimeric antigen receptor comprises an extracellular antigen binding domain with binding specificity to a disease antigen, a hinge, a transmembrane domain, one or more co-stimulatory signalling regions and a non-functional CD3 zeta domain.
3. The modified $\gamma\delta$ T cell of claim 1 or claim 2 wherein the non-functional CD3
20 zeta domain is provided by the absence of a CD3 zeta domain in the chimeric antigen receptor.
4. The modified $\gamma\delta$ T cell of any preceding claim comprising at least two chimeric antigen receptors wherein the chimeric antigen receptors have binding
25 specificities to different disease antigens.
5. The modified $\gamma\delta$ T cell of any preceding claim further comprising an inhibitory chimeric antigen receptor (ICAR), wherein the binding of an antigen by the inhibitory chimeric antigen receptor will cause the signal provided by any binding
30 to a disease antigen of a chimeric antigen receptor comprising an extracellular antigen binding domain with binding specificity to the disease antigen, a hinge, a transmembrane domain, and

- (i) one or more co-stimulatory signalling regions wherein a CD3 zeta domain is absent (tuneable), or
- (ii) one or more co-stimulatory signalling regions and a non-functional CD3 zeta domain (tuneable), or
- 5 (iii) a CD3 zeta domain, or
- (iv) one or more co-stimulatory signalling regions and a functional CD3 zeta domain,
- to be inhibited to minimise activation of the $\gamma\delta$ T cell in off-target cells.
- 10 6. The $\gamma\delta$ T cell of any preceding claim wherein the $\gamma\delta$ pairing is from V γ 1 to 9 and V δ 1 to 8.
7. The $\gamma\delta$ T cell of any preceding claim wherein the $\gamma\delta$ T cell is of the V γ 9V δ 2 subtype.
- 15 8. The $\gamma\delta$ T cell of any preceding claim wherein the antigen recognition domain of the chimeric antigen receptor is capable of binding to a cell surface target or natural ligand found in or associated with cell infection, bacterial infection, fungal infection or protozoan infection; an active or inactivated viral fragment; a peptide;
- 20 a protein; an antigenic segment from a virus; a tumour-specific antigen or tumour associated antigen.
9. A modified $\gamma\delta$ T cell of any of claims 1 to 8 for use in treatment of cancer or infection.
- 25 10. A modified $\gamma\delta$ T cell of any of claims 2 to 8 for use in treatment of cancer or infection.
11. A modified $\gamma\delta$ T cell for use in treatment of cancer or infection of claim 9
- 30 wherein the modified $\gamma\delta$ T cell is provided to a subject by allogeneic transfer.
12. A modified $\gamma\delta$ T cell for use in treatment of cancer or infection of claim 10 wherein the modified $\gamma\delta$ T cell is provided to a subject by allogeneic transfer.

13. A modified $\gamma\delta$ T cell for use in the treatment of an infection as claimed in any one of claims 9 to 12 wherein the infection is selected from a viral, bacterial, fungal or protozoan infection.

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14. A modified $\gamma\delta$ T cell for use in the treatment of cancer or infection as claimed in claim 9 wherein the modified $\gamma\delta$ T cell is provided to a subject by autologous transfer.

10 15. A modified $\gamma\delta$ T cell for use in the treatment of cancer or infection as claimed in claim 10 wherein the modified $\gamma\delta$ T cell is provided to a subject by autologous transfer.

15 16. A modified $\gamma\delta$ T cell for use in the treatment of an infection as claimed in any one of claims 14 or 15 wherein the infection is selected from a viral, bacterial, fungal or protozoan infection.

20 17. A modified $\gamma\delta$ T cell for use in the treatment of cancer or infection as claimed in any one of claims 9 to 15 wherein the modified $\gamma\delta$ T cell is co-administered to a subject with an aminobisphosphonate, or substitute thereof, wherein the substitute is capable of enhancing the levels of phosphoantigens present on the target cells *via* dysregulation of the physiological mevalonate pathway.

25 18. A nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an extracellular antigen recognition domain, a hinge, a transmembrane domain, and
(i) one or more co-stimulatory signalling regions wherein a CD3 zeta domain is absent (tuneable), or
(ii) one or more co-stimulatory signalling regions and a non-functional CD3 zeta
30 domain (tuneable), or
(iii) a CD3 zeta domain, or
(iv) one or more co-stimulatory signalling regions and a functional CD3 zeta domain.

19. The nucleic acid sequence of claim 18 encoding a chimeric antigen receptor (CAR) comprising an extracellular antigen binding domain with binding specificity to a disease antigen, a hinge, a transmembrane domain, one or more co-stimulatory signalling regions and a non-functional CD3 zeta domain.
20. The nucleic acid sequence of claim 18 or claim 19 wherein the non-functional CD3 zeta domain is provided by the absence of a CD3 zeta domain in the chimeric antigen receptor.
21. The nucleic acid sequence as claimed in claim 18 comprising or consisting of the nucleotide sequence SEQ ID NO: 1, capable of encoding the amino acid sequences SEQ ID NOS: 2 to 6.
22. The nucleic acid sequence of any of claims 18 to 20 encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an extracellular antigen recognition domain wherein the extracellular antigen recognition domain is a scFv recognising a B cell protein CD19, a hinge, a transmembrane domain, one or more co-stimulatory signalling regions, but in which a functional CD3 zeta domain is absent.
23. The nucleic acid sequence of claim 18 comprising or consisting of the nucleotide sequence SEQ ID NO: 7 capable of encoding the amino acid sequences SEQ ID NOS: 8 to 11.
24. A process to provide a CAR modified $\gamma\delta$ T cell comprising a step of incorporating or transducing a nucleic acid of any one of claims 18 to 23 into a $\gamma\delta$ T cell to genetically modify the $\gamma\delta$ T cell.
25. The process of claim 24 wherein the step of transducing uses a lentiviral CAR construct.

26. The process of claim 24 or 25 comprising a step of simultaneously transducing a nucleic acid of any one of claims 18 to 23 into a cell, and selectively expanding CAR-transduced $\gamma\delta$ T cells.

- 5 27. A method to detect between a CAR comprising one or more co-stimulatory signalling regions and a CD3 zeta domain and a CAR in which a CD3 zeta domain is absent comprising a step of binding a primer pair selected from the group SEQ ID NO: 12 and SEQ ID NO: 13, and SEQ ID NO:14 and SEQ ID NO: 15 to a nucleic acid encoding a CAR to be tested

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Forward (SEQ ID NO: 12)	5'-GCTCCTGCACAGTGACTACAT-3'
Reverse (SEQ ID NO: 13)	5'-GGAGTTTCTTTCTGCCCCGT-3'

Forward (SEQ ID NO: 14)	5'-CTGTAGCTGCCGATTTCAGA-3'
Reverse (SEQ ID NO: 15)	5'-CATCGTACTCCTCTCTTCGTCC-3'

- 15 28. A method of treatment comprising; administering to a recipient subject in need thereof donor cells collected from a donor subject and processed to allow allogeneic administration of a therapeutically effective amount of $\gamma\delta$ T cells to the recipient subject, wherein said processing includes the step of modifying the $\gamma\delta$ T cells to incorporate a chimeric antigen receptor.
- 20 29. A method of treatment comprising administering to a donor subject in need thereof, donor cells collected from the donor subject and processed to allow administration of a therapeutically effective amount of $\gamma\delta$ T cells to the donor subject, wherein said processing includes the step of modifying the $\gamma\delta$ T cells to incorporate a chimeric antigen receptor wherein said chimeric antigen receptor
- 25 comprises an extracellular antigen binding domain with binding specificity to a disease antigen, a hinge, a transmembrane domain, one or more co-stimulatory signalling regions and a non-functional CD3 zeta domain.

30. A method of treatment as claimed in claim 28 wherein the chimeric antigen receptor comprises an extracellular antigen binding domain with binding specificity to a disease antigen, a hinge, a transmembrane domain, one or more co-stimulatory signalling regions and a non-functional CD3 zeta domain.

31. A method of treatment of claims 29 or 30 wherein the non-functional CD3 zeta domain is provided by the absence of a CD3 zeta domain in the chimeric antigen receptor.

32. The method of treatment of any one of claims 28 to 31 wherein the subject being administered $\gamma\delta$ T cells has a viral, bacterial, fungal or protozoan infection or cancer.

33. The method of treatment of any one of claims 28 to 32 wherein the subject being administered $\gamma\delta$ T cells is simultaneously, sequentially or separately administered with immunosuppressive drugs.

34. A kit comprising a $\gamma\delta$ T cell of any one of claims 1 to 8 and a pyrophosphate/phosphonate drug for use in combination to treat a subject by autologous or allogeneic transfer.

35. A kit comprising a $\gamma\delta$ T cell of any one of claims 2 to 8 and a pyrophosphate/phosphonate drug for use in combination to treat a subject by autologous or allogeneic transfer.

36. A pharmaceutical composition comprising a $\gamma\delta$ T cell of any one of claims 1 to 8.

37. A pharmaceutical composition comprising a $\gamma\delta$ T cell of any one of claims 2 to 8.

38. A pharmaceutical composition of claim 36 or 37 wherein the composition comprises a unified dose of $\gamma\delta$ T cells suitable to provide to an individual to provide a therapeutic effect.

5 39. A pharmaceutical composition of one of claims 36 to 38 and an antibody immunotherapy for use in the treatment of cancer.

40. A pharmaceutical composition of any one of claims 36 to claim 39 and a cytokine, interferon gamma, IL-2, chemotherapeutic, biologic or combination
10 thereof for use in the treatment of cancer.

41. A pharmaceutical composition of any one of claims 36 to 38 and a therapeutic for use in the treatment of virus.

15 42. A method of preparing a treatment comprising; processing donor cells collected from a donor subject to allow allogeneic administration of a therapeutically effective amount of $\gamma\delta$ T cells to a recipient subject, wherein said processing includes the step of modifying the $\gamma\delta$ T cells to incorporate a chimeric antigen receptor and optionally administration of the $\gamma\delta$ T cells to the recipient
20 subject.

43. A method of preparing a treatment comprising processing donor cells collected from a donor subject to allow administration of a therapeutically effective amount of $\gamma\delta$ T cells to the donor subject, wherein said processing
25 includes the step of modifying the $\gamma\delta$ T cells to incorporate a chimeric antigen receptor wherein said chimeric antigen receptor comprises an extracellular antigen binding domain with binding specificity to a disease antigen, a hinge, a transmembrane domain, one or more co-stimulatory signalling regions and a non-functional CD3 zeta domain and optionally administration of the $\gamma\delta$ T cells to
30 the donor subject.

44. A method of preparing a treatment as claimed in claim 42 or claim 43 wherein the non-functional CD3 zeta domain is provided by the absence of a CD3 zeta domain in the chimeric antigen receptor.

5 45. A lentiviral CAR construct comprising a nucleic acid as claimed by any one of claims 18 to 23.

46. A kit to detect between a CAR comprising one or more co-stimulatory signalling regions and a CD3 zeta domain and a CAR in which a CD3 zeta domain is absent the kit comprising at least one primer pair selected from the group SEQ ID NO: 12 and SEQ ID NO: 13, and SEQ ID NO:14 and SEQ ID NO: 15

Forward (SEQ ID NO: 12)	5'-GCTCCTGCACAGTGACTACAT-3'
Reverse (SEQ ID NO: 13)	5'-GGAGTTTCTTTCTGCCCCGT-3'

Forward (SEQ ID NO: 14)	5'-CTGTAGCTGCCGATTTCAGA-3'
Reverse (SEQ ID NO: 15)	5'-CATCGTACTCCTCTCTTCGTCC-3'

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47. A modified $\gamma\delta$ T cell comprising a chimeric antigen receptor wherein the chimeric antigen receptor comprises an extracellular antigen binding domain with binding specificity to a disease antigen, a hinge, a transmembrane domain, one or more co-stimulatory signalling regions and absence of a functional signal 1 providing domain.

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48. A $\gamma\delta$ T-like cell, wherein the $\gamma\delta$ T-like cell is a T cell genetically engineered to express a functional gamma delta T cell receptor comprising a chimeric antigen receptor wherein the chimeric antigen receptor comprises an extracellular antigen binding domain with binding specificity to a disease antigen, a hinge, a transmembrane domain, and

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- (i) one or more co-stimulatory signalling regions wherein a CD3 zeta domain is absent (tuneable), or
- (ii) one or more co-stimulatory signalling regions and a non-functional CD3 zeta domain (tuneable), or
- 5 (iii) a CD3 zeta domain, or
- (iv) one or more co-stimulatory signalling regions and a functional CD3 zeta domain.
49. A $\gamma\delta$ T-like cell of claim 48, wherein the $\gamma\delta$ T-like cell is an $\alpha\beta$ T cell
- 10 genetically engineered to express a functional gamma delta T cell receptor.
50. A T cell or T cell-like cell of defined antigen specificity, comprising a chimeric antigen receptor wherein the chimeric antigen receptor comprises an extracellular antigen binding domain with binding specificity to a disease antigen,
- 15 a hinge, a transmembrane domain, and
- (i) one or more co-stimulatory signalling regions wherein a CD3 zeta domain is absent (tuneable), or
- (ii) one or more co-stimulatory signalling regions and a non-functional CD3 zeta domain (tuneable), or
- 20 (iii) a CD3 zeta domain, or
- (iv) one or more co-stimulatory signalling regions and a functional CD3 zeta domain.
51. A T cell or T cell-like cell of defined antigen specificity as claimed in claim 50
- 25 selected from a NKT cell with specificity towards α -GalCer, and a mucosal-associated invariant T (MAIT) cell with specificity for vitamin B-related antigens.
52. A method of treatment comprising administering a cell of any one of claims 1 to 8 or 47 to 51 to a subject in need thereof.
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53. A method of treatment as claimed in claim 52 wherein said administering is by autologous transfer.

54. A method of treatment as claimed in claim 52 wherein said administering is by allogeneic transfer.