

Virus-Specific Immune Cells Expressing Chimeric Antigen Receptors

This application claims priority from US 63/015,769 filed 27 April 2020, the contents and elements of which are herein incorporated by reference for all purposes.

Technical Field

- 5 The present invention relates to molecular and cell biology, and also relates to methods of medical treatment and prophylaxis.

Background

- 10 Despite the success of autologous chimeric antigen receptor (CAR) T cells in hematologic malignancies, barriers to more widespread use of this potentially curative therapy exist. Manufacture failure, disease progression prior to infusion, and exorbitant cost prove prohibitive for many. There is an urgent need for an immediately available CAR T cell option.

- 15 “Off-the-shelf” T cell products derived from healthy donors that can rapidly be administered, would improve accessibility and reduce the cost of adoptive cellular immunotherapy. However, the development of “off-the-shelf” CAR T cell therapies has been hindered by two major pitfalls: potential for polyclonally activated CAR T cells from unrelated donors to cause graft versus host disease (GVHD), and rejection of allogeneic CAR T cells by recipient alloreactive T cells.

Summary

- 20 In one aspect, the present disclosure refers to use of a virus-specific immune cell in the manufacture of a medicament for reducing the risk of graft-versus-host disease (GVHD) or allogeneic rejection, wherein the virus-specific immune cell comprises a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a
25 transmembrane domain, and (iii) a signalling domain, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD28, and (b) an amino acid sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM).

- In another aspect, the present disclosure refers to an *in vitro* method for producing a virus-specific immune cell, comprising: modifying a virus-specific immune cell to comprise a chimeric antigen receptor
30 (CAR) or nucleic acid encoding a CAR, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD28, and (b) an amino acid sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM).
In another aspect, the present disclosure refers to use of a pharmaceutical composition in the
35 manufacture of a medicament for reducing the risk of graft-versus-host disease (GVHD) or allogeneic rejection, wherein the pharmaceutical composition comprises a virus-specific immune cell according to any one of claims 1 to 18 and a pharmaceutically acceptable carrier, adjuvant, excipient or diluent.

In one example, the present disclosure provides a virus-specific immune cell, comprising a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD28, and (b) an amino acid sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM).

In some examples, the signalling domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:26.

In some examples, the transmembrane domain is derived from the transmembrane domain of CD28.

In some examples, the transmembrane domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:20.

In some examples, the antigen-binding domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:14, and an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:15.

In some examples, the antigen-binding domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:18.

- 5 In some examples, the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD3ζ.

In some examples, the signalling domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:25.

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In some examples, the CAR additionally comprises a hinge region provided between the antigen-binding domain and the transmembrane domain.

- 15 In some examples, the hinge region comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:33.

In some examples, the CAR comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:35 or 36.

- 20 In some examples, the virus-specific immune cell comprises a CAR comprising an antigen-binding domain which binds specifically to a target antigen other than CD30, or nucleic acid encoding a CAR comprising an antigen-binding domain which binds specifically to a target antigen other than CD30.

- 25 In another example, the present disclosure also provides a virus-specific immune cell, comprising: a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain comprising an immunoreceptor tyrosine-based activation motif (ITAM);
wherein the virus-specific immune cell comprises a CAR comprising an antigen-binding domain which binds specifically to a target antigen other than CD30, or nucleic acid encoding a CAR comprising
30 an antigen-binding domain which binds specifically to a target antigen other than CD30.

In some examples, the virus-specific immune cell comprises more than one non-identical CAR, or nucleic acid encoding more than one non-identical CAR.

- 35 In some examples, the target antigen other than CD30 is a cancer cell antigen.

In some examples, the target antigen other than CD30 is selected from CD19, CD20, CD22, ROR1R, CD4, CD7, CD38, BCMA, Mesothelin, EGFR, GPC3, MUC1, HER2, GD2, CEA, EpCAM, LeY and PSCA.

- 40 In some examples, the target antigen other than CD30 is CD19.

In some examples, the virus-specific immune cell is a virus-specific T cell.

In some examples, the virus-specific immune cell is specific for Epstein-Barr virus (EBV).

In one example, the present disclosure also provides a method for producing a virus-specific immune cell,
5 comprising:

modifying a virus-specific immune cell to comprise a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD28, and (b) an amino
10 acid sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM).

In some examples, the signalling domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:26.

15 In some examples, the transmembrane domain is derived from the transmembrane domain of CD28.

In some examples, the transmembrane domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:20.

20 In some examples, the antigen-binding domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:14, and an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:15.

In some examples, the antigen-binding domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:18.
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In some examples, the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD3 ζ .

30 In some examples, the signalling domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:25.

In some examples, the CAR additionally comprises a hinge region provided between the antigen-binding domain and the transmembrane domain.

35 In some examples, the hinge region comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:33.

In some examples, the CAR comprises an amino acid sequence having at least 80% amino acid
40 sequence identity to SEQ ID NO:35 or 36.

In some examples, the virus-specific immune cell comprises a CAR comprising an antigen-binding domain which binds specifically to a target antigen other than CD30, or nucleic acid encoding a CAR comprising an antigen-binding domain which binds specifically to a target antigen other than CD30.

5 In one example, the present disclosure also provides a method for producing a virus-specific immune cell, comprising:

modifying a virus-specific immune cell to comprise a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain comprising an immunoreceptor
10 tyrosine-based activation motif (ITAM);

wherein the virus-specific immune cell comprises a CAR comprising an antigen-binding domain which binds specifically to a target antigen other than CD30, or nucleic acid encoding a CAR comprising an antigen-binding domain which binds specifically to a target antigen other than CD30.

15 In some examples, the method further comprises:

modifying a virus-specific immune cell to comprise a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR, wherein the CAR comprises an antigen-binding domain which binds specifically to a target antigen other than CD30.

20 In some examples, the virus-specific immune cell comprises more than one non-identical CAR, or nucleic acid encoding more than one non-identical CAR.

In some examples, the target antigen other than CD30 is a cancer cell antigen.

25 In some examples, the target antigen other than CD30 is selected from CD19, CD20, CD22, ROR1R, CD4, CD7, CD38, BCMA, Mesothelin, EGFR, GPC3, MUC1, HER2, GD2, CEA, EpCAM, LeY and PSCA.

In some examples, the target antigen other than CD30 is CD19.

30 In some examples, the virus-specific immune cell is a virus-specific T cell.

In some examples, the virus-specific immune cell is specific for Epstein-Barr virus (EBV).

35 In one example, the present disclosure also provides a virus-specific immune cell obtained or obtainable by the method according to the present disclosure.

In one example, the present disclosure also provides a pharmaceutical composition comprising a virus-specific immune cell according to the present disclosure and a pharmaceutically acceptable carrier, adjuvant, excipient or diluent.

40 The present disclosure also provides a virus-specific immune cell or a pharmaceutical composition according to the present disclosure, for use in a method of medical treatment or prophylaxis. In one example, the present disclosure also provides use of a virus-specific immune cell or a pharmaceutical composition according to the present disclosure, in the manufacture of a medicament for use in a method
45 of medical treatment or prophylaxis.

Claims:

1. Use of a virus-specific immune cell in the manufacture of a medicament for reducing the risk of graft-versus-host disease (GVHD) or allogeneic rejection, wherein the virus-specific immune cell comprises a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD28, and (b) an amino acid sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM).
2. The use according to claim 1, wherein the signalling domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:26.
3. The use according to claim 1 or claim 2, wherein the transmembrane domain is derived from the transmembrane domain of CD28.
4. The use according to any one of claims 1 to 3, wherein the transmembrane domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:20.
5. The use according to any one of claims 1 to 4, wherein the antigen-binding domain comprises an amino acid sequence having at least 90% amino acid sequence identity to SEQ ID NO:14, and an amino acid sequence having at least 90% amino acid sequence identity to SEQ ID NO:15.
6. The use according to any one of claims 1 to 5, wherein the antigen-binding domain comprises an amino acid sequence having at least 90% amino acid sequence identity to SEQ ID NO:18.
7. The use according to any one of claims 1 to 6, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD3 ζ .
8. The use according to any one of claims 1 to 7, wherein the signalling domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:25.
9. The use according to any one of claims 1 to 8, wherein the CAR additionally comprises a hinge region provided between the antigen-binding domain and the transmembrane domain.
10. The use according to claim 9, wherein the hinge region comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:33.
11. The use according to any one of claims 1 to 10, wherein the CAR comprises an amino acid sequence having at least 90% amino acid sequence identity to SEQ ID NO:35 or 36.

12. The use according to any one of claims 1 to 11, wherein the virus-specific immune cell further comprises a CAR comprising an antigen-binding domain which binds specifically to a target antigen other than CD30, or nucleic acid encoding a CAR comprising an antigen-binding domain which binds specifically to a target antigen other than CD30.

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13. The use according to claim 12, wherein the virus-specific immune cell comprises more than one non-identical CAR, or nucleic acid encoding more than one non-identical CAR.

14. The use according to claim 12 or claim 13, wherein the target antigen other than CD30 is a cancer cell antigen.

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15. The use according to any one of claims 12 to 14, wherein the target antigen other than CD30 is selected from CD19, CD20, CD22, ROR1R, CD4, CD7, CD38, BCMA, Mesothelin, EGFR, GPC3, MUC1, HER2, GD2, CEA, EpCAM, LeY and PSCA.

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16. The use according to any one of claims 12 to 15, wherein the target antigen other than CD30 is CD19.

17. The use according to any one of claims 1 to 12, wherein the virus-specific immune cell is a virus-specific T cell.

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18. The use according to any one of claims 1 to 17, wherein the virus-specific immune cell is specific for Epstein-Barr virus (EBV).

19. An *in vitro* method for producing a virus-specific immune cell, comprising:

modifying a virus-specific immune cell to comprise a chimeric antigen receptor (CAR) or nucleic acid encoding a CAR, wherein the CAR comprises: (i) an antigen-binding domain which binds specifically to CD30, (ii) a transmembrane domain, and (iii) a signalling domain, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD28, and (b) an amino acid sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM).

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20. The method according to claim 19, wherein the signalling domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:26.

21. The method according to claims 19 or claim 20, wherein the transmembrane domain is derived from the transmembrane domain of CD28.

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22. The method according to any one of claims 19 to 21, wherein the transmembrane domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:20.

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23. The method according to any one of claims 19 to 22, wherein the antigen-binding domain comprises an amino acid sequence having at least 90% amino acid sequence identity to SEQ ID NO:14, and an amino acid sequence having at least 90% amino acid sequence identity to SEQ ID NO:15.
- 5 24. The method according to any one of claims 19 to 23, wherein the antigen-binding domain comprises an amino acid sequence having at least 90% amino acid sequence identity to SEQ ID NO:18.
25. The method according to any one of claims 19 to 24, wherein the signalling domain comprises: (a) an amino acid sequence derived from the intracellular domain of CD3ζ.
- 10 26. The method according to any one of claims 19 to 25, wherein the signalling domain comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:25.
27. The method according to any one of claims 19 to 26, wherein the CAR additionally comprises a hinge
- 15 region provided between the antigen-binding domain and the transmembrane domain.
28. The method according to any one of claims 19 to 27, wherein the hinge region comprises an amino acid sequence having at least 80% amino acid sequence identity to SEQ ID NO:33.
- 20 29. The method according to any one of claims 19 to 28, wherein the CAR comprises an amino acid sequence having at least 90% amino acid sequence identity to SEQ ID NO:35 or 36.
30. The method according to any one of claims 19 to 29, wherein the virus-specific immune cell further comprises a CAR comprising an antigen-binding domain which binds specifically to a target antigen other
- 25 than CD30, or nucleic acid encoding a CAR comprising an antigen-binding domain which binds specifically to a target antigen other than CD30.
31. The method according to any one of claims 19 to 30, wherein the method comprises:
modifying a virus-specific immune cell to further comprise a chimeric antigen receptor (CAR) or
- 30 nucleic acid encoding a CAR, wherein the CAR comprises an antigen-binding domain which binds specifically to a target antigen other than CD30.
32. The method according to claim 30 or claim 31, wherein the virus-specific immune cell comprises more than one non-identical CAR, or nucleic acid encoding more than one non-identical CAR.
- 35 33. The method according to any one of claims 30 to 32, wherein the target antigen other than CD30 is a cancer cell antigen.
34. The method according to any one of claims 30 to 34, wherein the target antigen other than CD30 is
- 40 selected from CD19, CD20, CD22, ROR1R, CD4, CD7, CD38, BCMA, Mesothelin, EGFR, GPC3, MUC1, HER2, GD2, CEA, EpCAM, LeY and PSCA.

35. The method according to any one of claims 30 to 35, wherein the target antigen other than CD30 is CD19.

5 36. The method according to any one of claims 19 to 35, wherein the virus-specific immune cell is a virus-specific T cell.

37. The method according to any one of claims 19 to 36, wherein the virus-specific immune cell is specific for Epstein-Barr virus (EBV).

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38. Use of a pharmaceutical composition in the manufacture of a medicament for reducing the risk of graft-versus-host disease (GVHD) or allogeneic rejection, wherein the pharmaceutical composition comprises a virus-specific immune cell according to any one of claims 1 to 18 and a pharmaceutically acceptable carrier, adjuvant, excipient or diluent.

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