

## SUMMARY

In one aspect, the present invention refers to a chimeric antigen receptor (CAR) comprising an extracellular spacer which comprises at least part of the extracellular domain of human low affinity nerve growth factor (LNGFR), wherein at least part of the LNGFR is suitable for facilitating immunoselection and identification of cells transduced with said CAR. In another aspect, the present invention refers to chimeric antigen receptor (CAR) comprising an extracellular spacer wherein the spacer comprises the sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7 or a sequence at least 90% identical thereto. In another aspect, the present invention refers to a chimeric antigen receptor (CAR) comprising (i) an antigen-specific targeting domain; (ii) an extracellular spacer domain as defined in the present invention; (iii) a transmembrane domain; (iv) optionally at least one costimulatory domain; and (v) an intracellular signaling domain. In another aspect, the present invention refers to polynucleotide encoding a CAR of the present invention. In another aspect, the present invention refers to vector comprising the polynucleotide of the present invention. In another aspect, the present invention refers to cell comprising a CAR according to the present invention, a polynucleotide according to the present invention or a vector according to the present invention. In another aspect, the present invention refers to pharmaceutical composition comprising the cell of the present invention. Also described herein is a chimeric antigen receptor (CAR) comprising an extracellular spacer, which comprises at least part of the extracellular domain of the human low affinity nerve growth factor receptor (LNGFR) or a derivative thereof.

The CAR may comprise at least a fragment of the extracellular domain of the human low affinity nerve growth factor receptor (LNGFR) or a derivative thereof.

Preferably at least part of the LNGFR is suitable for facilitating immunoselection of cells transduced with said CAR.

Preferably the spacer lacks the intracellular domain of LNGFR.

Preferably the extracellular spacer comprises the first three TNFR-Cys domains of LNGFR or fragments or derivatives thereof.

In one example the spacer comprises all four TNFR-Cys domains of LNGFR or fragments or derivatives thereof.

In another example the spacer comprises the fourth TNFR-Cys domain (TNFR-Cys 4) but wherein the following amino acids are removed from said domain: NHVDPCLPCTVCDTERQLRECTRW. Preferably the NHVDPCLPCTVCDTERQLRECTRW sequence is replaced with the following amino acid sequence ARA.

In another example, the spacer comprises the serine /threonine-rich stalk of LNGFR.

In another example, the spacer lacks the serine /threonine-rich stalk of LNGFR.

The spacer may comprise a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7 or a sequence at least 80, 85, 90, 95, 96, 97, 98 or 99% identical thereto.

In another example, the spacer may consist of a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7 or a sequence at least 80, 85, 90, 95, 96, 97, 98 or 99% identical thereto.

SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5 are preferred spacer elements.

According to another example there is provided a chimeric antigen receptor (CAR) comprising

- (i) an antigen-specific targeting domain;
- (ii) an extracellular spacer domain as defined herein;
- (iii) a transmembrane domain;
- (iv) optionally at least one costimulatory domain; and
- (v) an intracellular signaling domain.

Preferably the antigen-specific targeting domain comprises an antibody or fragment thereof, more preferably a single chain variable fragment.

Preferably the antigen-specific targeting domain targets a tumour antigen. Examples of such antigens include CD44, CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, mesothelin, c-Met, PSMA, Her2, GD-2, CEA, MAGE A3 TCR.

Preferably the tumour antigen is isoform 6 of CD44 (CD44v6).

Examples of transmembrane domains include a transmembrane domain of a zeta chain of a T cell receptor complex, CD28 and CD8a.

Examples of costimulatory domains include a costimulating domain from CD28, CD137 (4-1BB), CD134 (OX40), Dap1O, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-II, Fas, CD30 and CD40.

Examples of intracellular signaling domains include human CD3 zeta chain, FcγRIII, FcεRI, a cytoplasmic tail of a Fc receptor and an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptors.

In a preferred example, the antigen-specific targeting domain of the CAR targets CD44v6, the transmembrane domain of the CAR comprises a transmembrane domain of CD28, the intracellular signaling domain of the CAR comprises an intracellular signaling domain of human CD3 zeta chain and the costimulatory domain of the CAR comprises a CD28 endo-costimulating domain.

In another example there is provided a polynucleotide encoding a CAR as defined herein.

Preferably the polynucleotide encodes a spacer domain that comprises the sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8, or a sequence at least 80, 85, 90, 95, 96, 97, 98 or 99% identical thereto.

In one example, the polynucleotide encodes a spacer domain that consists of the sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8, or a sequence at least 80, 85, 90, 95, 96, 97, 98 or 99% identical thereto.

In another example there is provided a vector comprising the polynucleotide as described herein.

In one example, the vector is a viral vector.

In another example there is provided a cell comprising a CAR, a polynucleotide, or a vector as described herein. Preferably the cell is a T-cell.

In another example there is provided a pharmaceutical composition comprising the cell as described herein.

In another example there is provided a CAR, a polynucleotide, a vector or a cell as described herein for use in therapy, preferably cancer therapy.

In another example there is provided a CAR as described herein wherein the antigen-specific targeting domain targets CD44v6 for use in treating tumours that express CD44.

In another example there is provided a method of treatment comprising administering a CAR, a polynucleotide, a vector or a cell as described herein to a subject in need of the same.

Exemplary CARs are shown in Figures 10 to 17.

## DESCRIPTION OF THE DRAWINGS

**Figure 1. Rationale of generating different LNGFR-spaced CD44v6-CAR.28z constructs.** **A.** Scheme explaining the limitations of CAR T cells carrying the IgG1 CH2CH3 spacer. **B.** Structure of the extracellular portion of the low-affinity nerve growth factor receptor (LNGFR) and of the 4 new CAR constructs that have been generated. The CD44v6-CAR.28z carrying the wild-type or the mutated IgG1 CH2CH3 spacer (mCH2CH3) are also included. CHW: CD44v6-CAR.28z carrying the wild-type CH2CH3 spacer. CHM:

## Claims

1. A chimeric antigen receptor (CAR) comprising an extracellular spacer which comprises at least part of the extracellular domain of human low affinity nerve growth factor (LNGFR), wherein at least part of the LNGFR is suitable for facilitating immunoselection and identification of cells transduced with said CAR.
2. The CAR according to claim 1 wherein the spacer lacks the intracellular domain of LNGFR.
3. The CAR according to any preceding claim wherein the extracellular spacer comprises the first three TNFR-Cys domains of LNGFR.
4. The CAR according to claim 3 wherein spacer comprises all four TNFR-Cys domains of LNGFR.
5. The CAR according to any preceding claim wherein the spacer comprises the fourth TNFR-Cys domain (TNFR-Cys 4) but wherein the following amino acid sequence is removed from said domain: NHVDPCLPCTVCEDTERQLRECTRW and replaced with the following amino acid sequence: ARA.
6. The CAR according to any preceding claim wherein the spacer comprises the serine/threonine-rich stalk of LNGFR.
7. The CAR according to claims 1 to 5 wherein the spacer lacks the serine/threonine-rich stalk of LNGFR.
8. The CAR according to claim 1 or 2 wherein said spacer comprises the entire extracellular domain of LNGFR.
9. The CAR according to claim 1 or 2 wherein the spacer comprises the extracellular domain of LNGFR with the exception of the serine /threonine-rich stalk of said domain.
10. A chimeric antigen receptor (CAR) comprising an extracellular spacer wherein the spacer comprises the sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7 or a sequence at least 90% identical thereto.
11. The CAR according to claim 10 wherein the spacer consists of a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7 or a sequence at least 90% identical thereto.

12. A chimeric antigen receptor (CAR) comprising
- (i) an antigen-specific targeting domain;
  - (ii) an extracellular spacer domain as defined in any one of claims 1 to 11;
  - (iii) a transmembrane domain;
  - (iv) optionally at least one costimulatory domain; and
  - (v) an intracellular signaling domain.
13. The CAR according to claim 12 wherein the antigen-specific targeting domain comprises an antibody.
14. The CAR according to claim 13 wherein the antigen-specific targeting domain is a single chain variable fragment.
15. The CAR according to any one of claims 12 to 14 wherein the antigen-specific targeting domain targets a tumour antigen.
16. The CAR according to claim 15 wherein the tumour antigen is selected from the group consisting of CD44, CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, mesothelin, c-Met, PSMA, Her2, GD-2, CEA, MAGE A3 TCR and combinations thereof.
17. The CAR according to claim 15 wherein the tumour antigen is isoform 6 of CD44 (CD44v6).
18. The CAR according to any one of claims 12 to 17 wherein the transmembrane domain comprises any one or more of a transmembrane domain of a zeta chain of a T cell receptor complex, CD28, CD8a, and combinations thereof.
19. The CAR according to claim any one of claims 12 to 18 wherein the costimulatory domain comprises a costimulating domain from any one or more of CD28, CD137 (4-1BB), CD134 (OX40), Dap10, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-II, Fas, CD30, CD40 and combinations thereof.
20. The CAR according to any one of claims 12 to 19 wherein the intracellular signaling domain comprises an intracellular signaling domain of one or more of a human CD3 zeta chain, FcγRIII, FcεRI, a cytoplasmic tail of a Fc receptor, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptors, and combinations thereof.

21. The CAR according to any one of claims 12 to 20 wherein the antigen-specific targeting domain targets CD44v6, the transmembrane domain comprises a transmembrane domain of CD28, the intracellular signaling domain comprises an intracellular signaling domain of human CD3 zeta chain and the costimulatory domain comprises a CD28 endo-costimulating domain.
22. A polynucleotide encoding a CAR of any one of claims 1 to 21.
23. A vector comprising the polynucleotide of claim 22.
24. The vector according to claim 23 wherein the vector is a viral vector.
25. A cell comprising a CAR according to any one of claims 1 to 21, a polynucleotide according to claim 22 or a vector according to claim 23 or 24.
26. The cell according to claim 25 wherein the cell is a T-cell.
27. A pharmaceutical composition comprising the cell of claim 25 or 26.
28. The CAR according to any one of claims 12 to 21, or a polynucleotide according to claim 22, a vector according to claim 23 or 24 or a cell according to claim 25 or 26, for use in treating cancer.
29. The CAR according to claim 21, a polynucleotide encoding said CAR, a vector comprising said polynucleotide or a host cell comprising said CAR, polynucleotide or vector, for use in treating tumours that express CD44.