

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 receptor (LNGFR), wherein said at least part of the LNGFR is suitable for facilitating immunoselection and identification of cells transduced with said CAR, and wherein the extracellular spacer comprises the first three TNFR-Cys domains of LNGFR. In another aspect, the present invention refers to 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. 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 a 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: NHVDPCLPCTVCE~~TER~~QLRECTRW. Preferably the NHVDPCLPCTVCE~~TER~~QLRECTRW 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.

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 receptor (LNGFR), wherein said at least part of the LNGFR is suitable for facilitating immunoselection and identification of cells transduced with said CAR, and wherein the extracellular spacer comprises the first three TNFR-Cys domains of LNGFR.
2. The CAR according to claim 1 wherein the spacer lacks the intracellular domain of LNGFR.
3. The CAR according to claim 1 or 2 wherein the spacer comprises all four TNFR-Cys domains of LNGFR.
4. 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.
5. The CAR according to any preceding claim wherein the spacer comprises the serine/threonine-rich stalk of LNGFR.
6. The CAR according to claims 1 to 4 wherein the spacer lacks the serine/threonine-rich stalk of LNGFR.
7. The CAR according to claim 1 or 2 wherein said spacer comprises the entire extracellular domain of LNGFR.
8. 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.
9. 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.
10. The CAR according to claim 9 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.
11. 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 10;
 - (iii) a transmembrane domain;
 - (iv) optionally at least one costimulatory domain; and
 - (v) an intracellular signaling domain.
12. The CAR according to claim 11 wherein the antigen-specific targeting domain comprises an antibody.
 13. The CAR according to claim 12 wherein the antigen-specific targeting domain is a single chain variable fragment.
 14. The CAR according to any one of claims 11 to 13 wherein the antigen-specific targeting domain targets a tumour antigen.
 15. The CAR according to claim 14 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.
 16. The CAR according to claim 14 wherein the tumour antigen is isoform 6 of CD44 (CD44v6).
 17. The CAR according to any one of claims 11 to 16 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.
 18. The CAR according to claim any one of claims 11 to 17 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.
 19. The CAR according to any one of claims 11 to 18 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.
 20. The CAR according to any one of claims 11 to 19 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.

21. A polynucleotide encoding a CAR of any one of claims 1 to 20.
22. A vector comprising the polynucleotide of claim 21.
23. The vector according to claim 22 wherein the vector is a viral vector.
24. A cell comprising a CAR according to any one of claims 1 to 20, a polynucleotide according to claim 22 or a vector according to claim 22 or 23.
25. The cell according to claim 24 wherein the cell is a T-cell.
26. A pharmaceutical composition comprising the cell of claim 24 or 25.
27. The CAR according to any one of claims 11 to 20, or a polynucleotide according to claim 21, a vector according to claim 22 or 23 or a cell according to claim 24 or 25, for use in treating cancer.
28. The CAR according to claim 20, 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.