

CLAIMS:

1. A chimeric costimulatory receptor (CoStAR) comprising an extracellular ligand binding fragment of a first costimulatory receptor and an intracellular signalling fragment of a second costimulatory receptor fused to a tumour-associated antigen specific binding domain linked to a signalling domain.
2. A CoStAR as claimed in claim 1, comprising a full-length costimulatory receptor fused to a tumour-associated antigen specific binding domain, wherein said costimulatory receptor full-length includes the entire mature protein lacking the leader sequence and which has up to 5 amino acid deletions or mutations at the very N-terminus of the mature protein.
3. A chimeric costimulatory receptor (CoStAR) comprising a full-length costimulatory receptor fused to a tumour-associated antigen specific binding domain, wherein said costimulatory receptor full-length includes the entire mature protein lacking the leader sequence and which has up to 5 amino acid deletions or mutations at the very N-terminus of the mature protein.
4. The CoStAR of any preceding claim wherein said costimulatory receptor is selected from the group of CD2, CD9, CD26, CD27, CD28, CD29, CD38, CD40, CD43, CD46, CD49d, CD55, CD73, CD81, CD82, CD99, CD100, CD134 (OX40), CD137 (41BB), CD150 (SLAM), CD270 (HVEM), CD278 (ICOS), CD357 (GITR), or EphB6.
5. A CoStAR as of any preceding claim wherein the costimulatory domain comprises or consists of two or more costimulatory receptor domains fused into a single receptor format, with the joining region being a direct fusion, or containing a linker region such as a linker up to 50 amino acids in length.
6. The CoStAR of any preceding claim wherein said antigen specific binding domain is selected from:
 - I. A single chain antibody fragment which binds a tumour associated antigen including, but not limited to, carcinoembryonic antigen (CEA), 5T4, mellanotransferrin (CD228), Her2, EGFR, GPC3, melanoma-associated chondroitin sulphate proteoglycan (MCSP/CSPG4), CD71, SM5-1, folate receptor or CA125; or
 - II. A single chain antibody fragment which binds a tumour specific peptide (p)-major histocompatibility (MHC) complex; or

- III. A tumour specific pMHC complex antigen specific single chain T-cell receptor (scTCR); or
 - IV. A natural antigen binding polypeptide such as, but not limited to, transferrin; or
 - V. A domain which bind an antibody (e.g. Fc binding domains such as, but not limited to, CD16, CD32 or CD64), or other indirect method of antigen recognition.
- 7. A fusion protein which comprises a polypeptide according to any preceding claim fused to a protein of interest (POI) such as a marker gene (e.g. DYKDDDDK (SEQ ID NO:14) epitope tag, CD34, CD19 etc), chimeric antigen receptor (CAR), a T cell receptor (TCR) or another receptor of immunotherapeutic use for adoptive cell therapy.
 - 8. A fusion protein according to claim 8, which comprises a self-cleaving peptide between the polypeptide and the protein of interest.
 - 9. A nucleic acid sequence, such as:
 - I. that shown in SEQ ID NO:1, capable of encoding a polypeptide, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level, according to claim 1 and 2; or
 - II. a fusion protein, such as that shown in SEQ ID NOs:3 to 12 or a variant thereof having at least 80% sequence identity at the protein level.
 - 10. A vector which comprises a nucleic acid sequence according to claim 10.
 - 11. A vector according to claim 10, which also comprises a transgene of interest.
 - 12. A vector according to claim 11, wherein the transgene of interest encodes a chimeric antigen receptor, a T-cell receptor or another receptor of immunotherapeutic use for adoptive cell therapy, such that when the vector is used to transduce a target cell, the target cell co-expresses a polypeptide according to any of claims 1 and a chimeric antigen receptor, T-cell receptor or another receptor of immunotherapeutic interest.
 - 13. A cell which expresses a polypeptide according to claim 1.
 - 14. A cell according to claim 13 which co-expresses the polypeptide of claim 1 and a POI at the cell surface.
 - 15. A cell which comprises a nucleic acid sequence according to claim 10.
 - 16. A cell according to any of claims 13-16, which is a T cell.

17. A method for making a cell according to any of claims 13-16 which comprises the step of transducing or transfecting a cell with a vector according to any of claims 10 to 12.
18. A method for selecting cells expressing a POI which comprises the following steps:
 - I. detecting expression of the POI epitope on the surface of cells transfected or transduced with a vector according to any of claims 10 to 12; and
 - II. selecting cells which are identified as expressing the POI epitope.
19. A method for preparing a purified population of cells enriched for cells expressing a POI which comprises the step of selecting cells expressing a POI from a population of cells using a method according to claim 19.
20. A method according to claim 19, which comprises the ex vivo transduction or transfection of a population of cells isolated from a tissue or cell donor with:
 - I. a vector according to any of claims 10 to 12; and
 - II. selecting cells expressing the POI from the transduced/transfected population of cells by a method according to claim 18.
21. A cell population which is enriched for cells expressing a polypeptide according to claim 1, and thus enriched for cells expressing a POI.
22. A method for tracking transduced cells in vivo which comprises the step of detection of expression of a polypeptide according to claim 1 at the cell surface.
23. A method for treating a disease in a subject, which comprises the step of administering a cell according to any of claims 13 to 16, or a cell population according to claim 21 to the subject.
24. A method according to claim 24 which comprises the following steps:
 - I. transducing or transfecting a sample of cells isolated from a subject with a vector according to any of claims 10 to 12, and
 - II. returning the transduced/transfected cells to a patient.
25. A method according to claim 24 for treating cancer.
26. A cell according to any of claims 13 to 16 or a cell population according to claim 21 for use in the treatment of disease and/or adoptive cell transfer.
27. A CoStAR receptor comprising a tumour associated antigen binding domain according to claim 6, fused to:
 - I. a fusion signalling domain comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and

- II. the intracellular domain from human CD137, such as that shown in SEQ ID NO:4 or a variant thereof having at least 80% or 90% sequence identity at the protein level.
28. A CoStAR receptor comprising a tumour associated antigen binding domain according to claim 6, fused to:
- I. a fusion signalling domain comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and
 - II. the intracellular domain from human CD134, such as that shown in SEQ ID NO:5 or a variant thereof having at least 80% or 90% sequence identity at the protein level.
29. A CoStAR receptor comprising a tumour associated antigen binding domain according to claim 6, fused to:
- I. a fusion signalling domain comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and
 - II. the intracellular domain from human CD2, such as that shown in SEQ ID NO:6 or a variant thereof having at least 80% or 90% sequence identity at the protein level.
30. A CoStAR receptor comprising a tumour associated antigen binding domain according to claim 6, fused to:
- I. a fusion signalling domain comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and
 - II. the intracellular domain from human CD29, such as that shown in SEQ ID NO:7 or a variant thereof having at least 80% or 90% sequence identity at the protein level.
31. A CoStAR receptor comprising a tumour associated antigen binding domain according to claim 6, fused to:
- I. a fusion signalling domain comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and

- II. the intracellular domain from human GITR, such as that shown in SEQ ID NO:8 or a variant thereof having at least 80% or 90% sequence identity at the protein level.
32. A CoStAR receptor comprising a tumour associated antigen binding domain according to claim 6, fused to:
- I. a fusion signalling domain comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and
 - II. the intracellular domain from human IL2R γ , such as that shown in SEQ ID NO:9 or a variant thereof having at least 80% or 90% sequence identity at the protein level.
33. A CoStAR receptor comprising a tumour associated antigen binding domain according to claim 6, fused to:
- I. a fusion signalling domain comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and
 - II. the intracellular domain from human CD40, such as that shown in SEQ ID NO:10 or a variant thereof having at least 80% or 90% sequence identity at the protein level.
34. A CoStAR receptor comprising a tumour associated antigen binding domain according to claim 6, fused to:
- I. a fusion signalling domain comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and
 - II. the intracellular domain from human CD150, such as that shown in SEQ ID NO:11 or a variant thereof having at least 80% or 90% sequence identity at the protein level.
35. A CoStAR receptor comprising a tumour associated antigen binding domain according to claim 6, fused to:
- I. a fusion signalling domain comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and

- II. the intracellular domain from human CD2 and human CD40, such as that shown in SEQ ID NO:12 or a variant thereof having at least 80% or 90% sequence identity at the protein level.

CLAIMS

1. A chimeric costimulatory receptor (CoStAR) comprising a tumour-associated antigen specific binding fragment, a first costimulatory receptor fragment comprising the stimulatory domain of CD28, and a second costimulatory receptor fragment comprising the stimulatory domain of CD40.
2. A CoStAR according to Claim 1, comprising a full-length CD28 and/or CD40 costimulatory receptor fragment, in which the costimulatory receptor fragment includes the entire mature costimulatory receptor protein, lacking the leader sequence and up to 5 amino acids at the N- terminus of the mature costimulatory receptor protein.
3. A chimeric costimulatory receptor (CoStAR) comprising a full-length costimulatory receptor fused to a tumour-associated antigen specific binding fragment, in which the costimulatory receptor full-length includes the entire mature protein lacking the leader sequence and up to 5 amino acids at the N- terminus of the mature protein.
4. A CoStAR according to Claim 1, 2 or 3, in which the costimulatory fragment comprises or consists of two or more costimulatory receptor fragments fused into a single receptor format, with the joining region being a direct fusion, or containing a linker region such as a linker up to 50 amino acids in length.
5. A CoStAR according to any preceding claim, in which the antigen specific binding fragment is selected from:
 - (I) a single chain antibody fragment which binds a tumour associated antigen including, but not limited to, carcinoembryonic antigen (CEA), 5T4, melanotransferrin (CD228), Her2, EGFR, GPC3, melanoma-associated chondroitin sulphate proteoglycan (MCSP/CSPG4), CD71, SM5-1, folate receptor or CA125; or
 - (II) a single chain antibody fragment which binds a tumour specific peptide (p)- major histocompatibility (MHC) complex; or III. A tumour specific pMHC complex antigen specific single chain T-cell receptor (scTCR); or

- (III) a tumour specific pMHC complex antigen specific single chain T-cell receptor (seTCR); or
 - (IV) a natural antigen binding polypeptide such as, but not limited to, transferrin; or
 - (V) a fragment which bind an antibody (e.g. Fc binding domains such as, but not limited to, CD16, CD32 or CD64), or other indirect method of antigen recognition.
6. A fusion protein which comprises a CoStAR according to any preceding claim fused to a protein of interest (POI) such as a marker gene (e.g. DYKDDDDK (SEQ ID NO: 14) epitope tag, CD34, CD19 etc), chimeric antigen receptor (CAR), a T cell receptor (TCR) or another receptor of immunotherapeutic use for adoptive cell therapy.
 7. A fusion protein according to Claim 6, which comprises a self-cleaving peptide between the CoStAR and the protein of interest.
 8. A nucleic acid sequence of SEQ ID NO: 1, capable of encoding:
 - (I) a protein comprising a sequence of SEQ ID NO: 2 or a variant thereof having at least 80% sequence identity at the protein level; or
 - (II) a protein comprising a sequence of any one of SEQ ID NOs: 3 to 12 or a variant thereof having at least 80% sequence identity at the protein level;
 in which the protein does not comprise a CD3-zeta domain.
 9. A cell which expresses a CoStAR according to Claim 1.
 10. A cell according to Claim 9 which co-expresses the CoStAR according to Claim 1 and a POI at the cell surface.
 11. A cell population which is enriched for cells expressing a CoStAR according to Claim 1.
 12. A method for tracking transduced cells *in vivo* which comprises the step of detection of expression of a CoStAR according to Claim 1 at the cell surface.
 13. A CoStAR receptor comprising a tumour associated antigen binding fragment according to Claim 5, fused to:

(I) a fusion signalling fragment comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and II. the intracellular fragment from human CD 137, such as that shown in SEQ ID NO: 4 or a variant thereof having at least 80% or 90% sequence identity at the protein level.

14. A CoStAR receptor comprising a tumour associated antigen binding fragment according to Claim 5, fused to:

(I) a fusion signalling fragment comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and

(II) the intracellular fragment from human CD 134, such as that shown in SEQ ID NO: 5 or a variant thereof having at least 80% or 90% sequence identity at the protein level.

15. A CoStAR receptor comprising a tumour associated antigen binding fragment according to Claim 5, fused to:

(I) a fusion signalling fragment comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and

(II) the intracellular fragment from human CD2, such as that shown in SEQ ID NO: 6 or a variant thereof having at least 80% or 90% sequence identity at the protein level.

16. A CoStAR receptor comprising a tumour associated antigen binding fragment according to Claim 5, fused to:

(I) a fusion signalling fragment comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and

(II) the intracellular fragment from human CD29, such as that shown in SEQ ID NO: 7 or a variant thereof having at least 80% or 90% sequence identity at the protein level.

17. A CoStAR receptor comprising a tumour associated antigen binding fragment according to Claim 5, fused to:

(I) a fusion signalling fragment comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and II. the intracellular fragment from human GITR, such as that shown in SEQ ID NO: 8 or a variant thereof having at least 80% or 90% sequence identity at the protein level.

18. A CoStAR receptor comprising a tumour associated antigen binding fragment according to Claim 5, fused to:

(I) a fusion signalling fragment comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and

(II) the intracellular fragment from human IL2Ry. such as that shown in SEQ ID NO:9 or a variant thereof having at least 80% or 90% sequence identity at the protein level.

19. A CoStAR receptor comprising a tumour associated antigen binding fragment according to Claim 5, fused to:

(I) a fusion signalling fragment comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and

(II) the intracellular fragment from human CD40, such as that shown in SEQ ID NO: 10 or a variant thereof having at least 80% or 90% sequence identity at the protein level.

20. A CoStAR receptor comprising a tumour associated antigen binding fragment according to Claim 5, fused to:

(I) a fusion signalling fragment comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and

- (II) the intracellular fragment from human CD 150, such as that shown in SEQ ID NO: 11 or a variant thereof having at least 80% or 90% sequence identity at the protein level.
21. A CoStAR receptor comprising a tumour associated antigen binding fragment according to Claim 5, fused to:
- (I) a fusion signalling fragment comprising or consisting of full length human CD28, such as that shown in SEQ ID NO:2 or a variant thereof having at least 80% sequence identity at the protein level; and
- (II) the intracellular fragment from human CD2 and human CD40, such as that shown in SEQ ID NO: 12 or a variant thereof having at least 80% or 90% sequence identity at the protein level.
22. A CoStar according to any of Claims 1 to 7, in which the stimulatory domain of CD28 comprises a sequence with at least 80% identity to the stimulatory domain of SEQ ID NO: 2.
23. A CoStar according to any of Claims 1 to 7, in which the stimulatory domain of CD40 comprises a sequence with at least 80% identity to the stimulatory domain of any one of SEQ ID NOs: 10 and/or 12.
24. A nucleic acid sequence encoding a CoStar according to any of Claims 1 to 7.
25. A vector which comprises a nucleic acid sequence according to Claim 24.
26. A vector according to Claim 25, which also comprises a transgene of interest.
27. A vector according to Claim 26, in which the transgene of interest encodes a chimeric antigen receptor, a T-cell receptor or another receptor of immunotherapeutic use for adoptive cell therapy, such that when the vector is used to transduce a target cell, the target cell co-expresses a CoStAR according to any of Claims 1 to 8 and a chimeric antigen receptor, T-cell receptor or another receptor of immunotherapeutic interest.
28. A cell which comprises a nucleic acid sequence according to Claim 24 and/or a vector according to any of Claims 25 to 27.
29. A cell according to Claim 28, which is a T cell.

30. A method for making a cell according to Claim 28 or 29, which comprises a step of transducing or transfecting a cell with a vector according to any of Claims 25 to 27.
31. A method for selecting cells expressing a POI which comprises the following steps:
- (I) detecting expression of the POI epitope on the surface of cells transfected or transduced with a vector according to any of Claims 25 to 27; and
 - (II) selecting cells which are identified as expressing the POI epitope.
32. A method for preparing a purified population of cells enriched for cells expressing a POI which comprises the step of selecting cells expressing a POI from a population of cells using a method according to Claim 31.
33. A method according to Claim 32, which comprises the *ex vivo* transduction or transfection of a population of cells isolated from a tissue or cell donor with:
- (I) a vector according to any of Claims 25 to 27; and
 - (II) selecting cells expressing the POI from the transduced/transfected population of cells by a method according to Claim 31.
34. Use of a cell according to Claim 28 or 29 or a cell population according to Claim 11 in the preparation of a medicament for the treatment of a disease and/or adoptive cell transfer.
35. Use according to Claim 34, which comprises transducing or transfecting a sample of cells isolated from a subject with a vector according to any of Claims 25 to 27, in which the transduced/transfected cells are to be returned to a patient.
36. Use according to Claim 34, in which the disease is cancer.