CLAIMS

1. A T cell which co-expresses a first chimeric antigen receptor (CAR) and second CAR at the cell surface, each CAR comprising:

(i) an antigen-binding domain;

(ii) a spacer;

(iii) a trans-membrane domain; and

(iv) an endodomain

in which the antigen binding domains of the first and second CARs bind to different antigens, in which the spacer of the first CAR has different dimensions to the spacer of the second CAR, such that when the first CAR and the second CAR bind their respective target antigens, the first CAR and second CAR become spatially separated on the T cell membrane, and in which one of the first or second CARs is an activating CAR comprising an activating endodomain and the other CAR is an inhibitory CAR comprising a ligation-off inhibitory endodomain, which inhibits T-cell activation by the activating CAR in the absence of inhibitory CAR ligation, but does not significantly inhibit T-cell activation by the activating CAR when the inhibitory CAR is ligated.

2. A T cell according to Claim 1, in which either the first spacer or the second spacer comprises a CD8 stalk and the other spacer comprises the hinge, CH2 and CH3 domain of IgG1.

3. A T cell according to Claim 1, in which the inhibitory endodomain comprises all or part of the endodomain from CD148 or CD45.

4. A T cell according to Claim 1 or 3, in which the antigen-binding domain of the first CAR binds CD5 and the antigen-binding domain of the second CAR binds CD19.

5. A T cell which comprises more than two CARs as defined in any preceding claim such that it is specifically stimulated by a cell, such as a T cell, bearing a distinct pattern of more than two antigens.

6. A nucleic acid sequence encoding both the first and second chimeric antigen receptors (CARs) as defined in any preceding claim.

7. A nucleic acid sequence according to Claim 6, which has the following structure:

AgB1-spacer1-TM1-endo1-coexpr-AbB2-spacer2-TM2-endo2

in which

AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the first CAR;

spacer 1 is a nucleic acid sequence encoding the spacer of the first CAR;

TM1 is a nucleic acid sequence encoding the transmembrane domain of the first CAR;

endo 1 is a nucleic acid sequence encoding the endodomain of the first CAR;

coexpr is a nucleic acid sequence enabling co-expression of both CARs

AgB2 is a nucleic acid sequence encoding the antigen-binding domain of the second CAR;

spacer 2 is a nucleic acid sequence encoding the spacer of the second CAR;

TM2 is a nucleic acid sequence encoding the transmembrane domain of the second CAR;

endo 2 is a nucleic acid sequence encoding the endodomain of the second CAR;

which nucleic acid sequence, when expressed in a T cell, encodes a polypeptide which is cleaved at the cleavage site such that the first and second CARs are co-expressed at the T cell surface.

8. A nucleic acid sequence according to Claim 7, in which coexpr encodes a sequence comprising a self-cleaving peptide.

9. A nucleic acid sequence according to Claim 7 or 8, in which alternative codons are used in regions of sequence encoding the same or similar amino acid sequences, in order to avoid homologous recombination.

10. A kit which comprises

(i) a first nucleic acid sequence encoding the first chimeric antigen receptor (CAR) as defined in any of Claims 1 to 5, which nucleic acid sequence has the following structure:

AgB1-spacer1-TM1-endo1

in which

AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the first CAR;

spacer 1 is a nucleic acid sequence encoding the spacer of the first CAR;

TM1 is a nucleic acid sequence encoding the transmembrane domain of the first CAR;

endo 1 is a nucleic acid sequence encoding the endodomain of the first CAR; and

(ii) a second nucleic acid sequence encoding the second chimeric antigen receptor (CAR) as defined in any of Claims 1 to 5, which nucleic acid sequence has the following structure:

AgB2-spacer2-TM2-endo2

AgB2 is a nucleic acid sequence encoding the antigen-binding domain of the second CAR;

spacer 2 is a nucleic acid sequence encoding the spacer of the second CAR;

TM2 is a nucleic acid sequence encoding the transmembrane domain of the second CAR;

endo 2 is a nucleic acid sequence encoding the endodomain of the second CAR.

11. A kit comprising: a first vector which comprises the first nucleic acid sequence as defined in Claim 10; and a second vector which comprises the second nucleic acid sequence as defined in Claim 10.

12. A kit according to Claim 11, in which the vectors are integrating viral vectors or transposons.

13. A vector comprising a nucleic acid sequence according to any of Claims 6 to 9.

14. A retroviral vector or a lentiviral vector or a transposon according to Claim 13.

15. A method for making a T cell according to any of Claims 1 to 5, which comprises the step of introducing: a nucleic acid sequence according to any of Claims 6 to 9; a first nucleic acid sequence and a second nucleic acid sequence as defined in Claim 10; and/or a first vector and a second vector as defined in Claim 11 or a vector according to Claim 13 or 14, into a T cell.

16. A method according to Claim 15, in which the T cell is from a sample isolated from a subject.

17. A pharmaceutical composition comprising a plurality of T cells according to any of Claims 1 to 6.

18. A pharmaceutical composition according to Claim 17 for use in treating and/or preventing a disease.

19. Use of a T cell according to any of Claims 1 to 5 in the manufacture of a medicament for treating and/or preventing cancer.

20. Use of a T cell according to Claim 19, in which a medicament comprising a plurality of the T cells is to be administered to a subject.

21. Use of a T cell according to Claim 20, in which the plurality of T cells is prepared by:

(i) isolating a T cell-containing sample from a subject; and

(ii) transducing or transfecting the T cells with: a nucleic acid sequence according to any of Claims 6 to 9; a first nucleic acid sequence and a second nucleic acid sequence as defined in Claim 10; a first vector and a second vector as defined in Claim 11 or 12 or a vector according to Claim 13 or 14.

22. Use of a T cell according to Claim 20 or 21, in which the disease to be treated is a cancer.

23. A natural killer (NK) cell which co-expresses a first chimeric antigen receptor (CAR) and second CAR at the cell surface, each CAR comprising:

(i) an antigen-binding domain;

(ii) a spacer

(iii) a trans-membrane domain; and

(iv) an endodomain

in which the antigen binding domains of the first and second CARs bind to different antigens, in which the spacer of the first CAR has different dimensions to the spacer of the second CAR, such that when the first CAR and the second CAR bind their respective target antigens, the first CAR and second CAR become spatially separated on the NK cell membrane and in which one of the first or second CARs is an activating CAR comprising an activating endodomain and the other CAR is an inhibitory CAR comprising a ligation-off inhibitory

endodomain which inhibits NK-cell activation by the activating CAR in the absence of inhibitory CAR ligation, but does not significantly inhibit NK-cell activation by the activating CAR when the inhibitory CAR is ligated.

24. A cell composition comprising CAR expressing T cells according to Claim 1 and/or CAR expressing NK cells according to Claim 23 made by transducing a blood-sample ex vivo with a nucleic acid encoding the first and second CARs.