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

in which the spacers of the first and second CARs have sufficiently similar dimensions to cause the CARs to co-localise at the T cell membrane.

2. A T cell according to Claim 1, in which the spacers of the first and second CARs are sufficiently different as to prevent cross-pairing.

3. A T cell according to Claim 1 or 2, in which the spacers of the first and second CARs are orthologous.

4. A T cell according to Claim 1, 2 or 3, in which the ligation-on inhibitory endodomain comprises a phosphatase domain.

5. A T cell according to Claim 4, in which the ligation-on inhibitory endodomain comprises at least the phosphatase domain of PTPN6.

6. A T cell according to any of Claims 1, 2 or 3, in which the ligation-on inhibitory endodomain comprises at least one ITIM domain.

7. A T cell according to Claim 6, in which activity of the ligation-on inhibitory endodomain is enhanced by co-expression of a PTPN6-CD45 or –CD148 fusion protein.

8. A T cell according to any preceding claim, in which the CAR comprising the activating endodomain comprises an antigen-binding domain which binds CD33 and the CAR which comprises the ligation-on inhibitory endodomain comprises an antigen-binding domain which binds CD34.

9. 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.

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

11. A nucleic acid sequence according to Claim 10, 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.

12. A nucleic acid sequence according to Claim 11, in which coexpr encodes a sequence comprising a self-cleaving peptide.

13. A nucleic acid sequence according to Claim 11 or 12, in which alternative codons are used in regions of sequence encoding the same or similar amino acid sequences, in order to avoid homologous recombination.

14. 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 9, 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 9, 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.

15. A kit comprising: a first vector which comprises the first nucleic acid sequence as defined in Claim 14; and a second vector which comprises the second nucleic acid sequence as defined in Claim 14.

16. A kit according to Claim 15, in which the vectors are integrating viral vectors or transposons.

17. A vector comprising a nucleic acid sequence according to any of Claims 10 to 13.

18. A retroviral vector or a lentiviral vector or a transposon according to Claim 17.

19. A method for making a T cell according to any of Claims 1 to 9, which comprises the step of introducing: a nucleic acid sequence according to any of Claims 10 to 13; a first nucleic acid sequence and a second nucleic acid sequence as defined in Claim 14; and/or a first vector and a second vector as defined in Claim 15 or a vector according to Claim 17 or 18, into a T cell.

20. A method according to Claim 19, in which the T cell is from a sample isolated from a subject.

21. A pharmaceutical composition comprising a plurality of T cells according to any of Claims 1 to 9.

22. A pharmaceutical composition according to Claim 21 for use in treating and/or preventing a disease.

23. Use of a T cell according to any of Claims 1 to 9 in the manufacture of a medicament for treating and/or preventing a cancer.

24. Use of a T cell according to Claim 23, in which the pharmaceutical composition according to Claim 21 is to be administered to a subject.

25. Use according to Claim 24, 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 10 to 13; a first nucleic acid sequence and a second nucleic acid sequence as defined in Claim 14; a first vector and a second vector as defined in Claim 15 or 16 or a vector according to Claim 17 or 18.

26. Use of a T cell according to Claim 24 or 25, in which the disease to be treated is a cancer.

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

in which the spacers of the first and second CARs have sufficiently similar dimensions to cause the CARs to co-localise at the T cell membrane.

28. A cell composition comprising CAR expressing T cells according to Claim 1 and/or CAR expressing NK cells according to Claim 27 made by transducing a blood-sample *ex vivo* with a nucleic acid encoding the first and second CARs.