

CLAIMS

1. A cell which co-expresses:
- (i) a first chimeric antigen receptor (CAR) at the cell surface, comprising an antigen-binding domain which binds to CD19;
- (ii) a second CAR at the cell surface, comprising an antigen-binding domain which binds to CD22;
- (iii) dominant negative SHP2 (dSHP2); and
- (iv) dominant negative TGF β receptor II (dnTGF β RII).
2. A cell according to claim 1, wherein each CAR comprises an intracellular signalling domain, wherein the intracellular signalling domain of the first CAR comprises a TNF receptor family endodomain; and the intracellular signalling domain of the second CAR comprises a co-stimulatory endodomain.
3. A cell according to claim 2, wherein the co-stimulatory domain is CD28 co-stimulatory endodomain.
4. A cell according to claim 2 or 3, wherein the TNF receptor family endodomain is OX-40 or 4-1BB endodomain.
5. A cell according to any of claims 2, 3 or 4, wherein the intracellular signalling domain of the first and the second CAR also comprises an ITAM-containing domain.
6. A cell according to any one of claims 1 to 5, wherein each CAR comprises:
- (i) an antigen-binding domain;
- (ii) a spacer; and
- (iii) a trans-membrane domain;
- wherein the spacer of the first CAR is different to the spacer of the second CAR.
7. A cell according to claim 6, wherein the spacer of the second CAR comprises cartilage oligomeric matrix protein (COMP) coiled coil domain.
8. A cell according to any of claims 1 to 7, wherein the first CAR comprises a CD19-binding domain which comprises
- a) a heavy chain variable region (VH) having complementarity determining regions (CDRs) with the following sequences:

CDR1 – SYWMN (SEQ ID No. 1);

CDR2 – QIWPGDGDNTYNGKFK (SEQ ID No. 2)

CDR3 – RETTTVGRYYYAMDY (SEQ ID No. 3); and

b) a light chain variable region (VL) having CDRs with the following sequences:

5 CDR1 – KASQSVDYDGDSYLN (SEQ ID No. 4);

CDR2 – DASNLVS (SEQ ID No. 5)

CDR3 – QQSTEDPWT (SEQ ID No. 6).

9. A cell according to claim 8, wherein the CD19 binding domain comprises a VH domain
10 having the sequence shown as SEQ ID No. 7, or SEQ ID NO 8; or a VL domain having the
sequence shown as SEQ ID No 9, SEQ ID No. 10 or SEQ ID No. 11 a variant thereof having
at least 90% sequence identity which retains the capacity to bind CD19.

10. A cell according to claim 9, wherein the CD19 binding domain comprises the sequence
15 shown as SEQ ID No 12, SEQ ID No. 13 or SEQ ID No. 14 or a variant thereof having at least
90% sequence identity which retains the capacity to bind CD19.

11. A cell according to any of claims 1 to 7, wherein the second CAR comprises a CD22-
binding domain which comprises

20 a) a heavy chain variable region (VH) having complementarity determining regions (CDRs)
with the following sequences:

CDR1 – NYWIN (SEQ ID No. 15);

CDR2 – NIYPSDSFTNYNQKFKD (SEQ ID No. 16)

CDR3 – DTQERSWYFDV (SEQ ID No. 17); and

25 b) a light chain variable region (VL) having CDRs with the following sequences:

CDR1 – RSSQSLVHSNQNTYLH (SEQ ID No. 18);

CDR2 – KVSNRFS (SEQ ID No. 19)

CDR3 – SQSTHVPWT (SEQ ID No. 20).

30 12. A cell according to claim 11, wherein the CD22 binding domain comprises a VH domain
having the sequence shown as SEQ ID No. 21, or SEQ ID NO 22; or a VL domain having the
sequence shown as SEQ ID No 23, or SEQ ID No. 24 or a variant thereof having at least 90%
sequence identity which retains the capacity to bind CD22.

35 13. A cell according to claim 11, wherein the CD22 binding domain comprises the
sequence shown as SEQ ID No 25 or SEQ ID No. 26 or a variant thereof having at least 90%
sequence identity which retains the capacity to bind CD22.

14. A cell according to any one of claims 1 to 13, wherein the first CAR has the structure:

AgB1-spacer1-TM1-TNF-ITAM

in which:

AgB1 is the antigen-binding domain of the first CAR;

spacer1 is the spacer of the first CAR;

TM1 is the transmembrane domain of the first CAR;

TNF is a TNF receptor endodomain; and

ITAM is an ITAM-containing endodomain;

and the second CAR has the structure:

AgB2-spacer2-TM2-costim-ITAM

in which:

AgB2 is the antigen-binding domain of the second CAR;

spacer2 is the spacer of the second CAR;

TM2 is the transmembrane domain of the second CAR;

costim is a co-stimulatory domain; and

ITAM is an ITAM-containing endodomain.

15. A nucleic acid sequence encoding both the first and second chimeric antigen receptors (CARs) as defined in any of claims 1 to 14, dSHP2, and dnTGFβRII.

16. A nucleic acid sequence according to claim 15, which has the following structure:

module1-coexpr-AgB1-spacer1-TM1-coexpr-AgB2-spacer2-TM2-coexpr-module2

in which

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

spacer1 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;

coexpr is a nucleic acid sequence enabling co-expression

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

spacer2 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; *module1* and *module2* are nucleic acid sequences encoding either dominant negative SHP2 (dSHP2) or dominant negative TGF β RII (dnTGF β RII), wherein when *module1* encodes dSHP2 *module2* encodes dnTGF β RII and when *module2* encodes dnTGF β RII *module1* encodes dSHP2;

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.

17. A nucleic acid sequence according to claim 16, wherein *coexpr* encodes a sequence comprising a self-cleaving peptide.

18. A nucleic acid sequence according to claim 16 or 17, wherein alternative codons are used in regions of sequence encoding the same or similar amino acid sequences, in order to avoid homologous recombination.

19. A vector comprising a nucleic acid sequence according to any of claims 15 to 18.

20. A retroviral vector or a lentiviral vector or a transposon according to claim 19.

21. A method for making a cell according to any of claim 1 to 14, which comprises the step of introducing: a nucleic acid sequence according to any of claims 15 to 18; or a vector according to claim 19 or 20, into a cell.

22. A method according to claim 21, wherein the cell is from a sample isolated from a subject.

23. A pharmaceutical composition comprising a plurality of cells according to any of claims 1 to 14.

24. A method for treating and/or preventing a disease, which comprises the step of administering a pharmaceutical composition according to claim 23 to a subject.

25. A method according to claim 24, which comprises the following steps:

- (i) isolation of a cell-containing sample from a subject;
- (ii) transduction or transfection of the cells with: a nucleic acid sequence according to any of claims 15 to 18; or a vector according to claim 19 or 20; and

(iii) administering the cells from (ii) to the subject.

26. A method according to claim 24 or 25, wherein the disease is a cancer.

5 27. A method according to claim 26, wherein the cancer is a B cell malignancy.

28. A pharmaceutical composition according to claim 23 for use in treating and/or preventing a disease.

10 29. The use of a cell according to any of claims 1 to 14 in the manufacture of a medicament for treating and/or preventing a disease.

30. A kit which comprises

15 (i) a first nucleic acid sequence encoding the first chimeric antigen receptor (CAR), which nucleic acid sequence has the following structure:

AgB1-spacer1-TM1

in which

20 AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the first CAR which binds to CD19;

spacer1 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;

(ii) a second nucleic acid sequence encoding the second chimeric antigen receptor, which nucleic acid sequence has the following structure:

25

AgB2-spacer2-TM2

in which

AgB2 is a nucleic acid sequence encoding the antigen-binding domain of the second CAR which binds to CD22;

30 spacer2 is a nucleic acid sequence encoding the spacer of the second CAR; and

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

(iii) a third nucleic acid sequence encoding dSHP2 and dnTGFβRII as described herein.

35 31. A kit comprising: a first vector which comprises the first nucleic acid sequence as defined in claim 30; a second vector which comprises the second nucleic acid sequence as

defined in claim 30; and a third vector which comprises the third nucleic acid sequence as defined in claim 30.

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1. A cell which co-expresses:
 - (i) a first chimeric antigen receptor (CAR) at the cell surface, comprising an antigen-binding domain which binds to CD19;
 - 5 (ii) a second CAR at the cell surface, comprising an antigen-binding domain which binds to CD22;
 - (iii) dominant negative SHP2 (dSHP2); and
 - (iv) dominant negative TGF β receptor II (dnTGF β RII).
2. A cell according to Claim 1, in which each CAR comprises an intracellular signalling domain, in which the intracellular signalling domain of the first CAR comprises a TNF receptor family endodomain; and the intracellular signalling domain of the second CAR comprises a co-stimulatory endodomain.
- 10 3. A cell according to Claim 2, in which the co-stimulatory domain is CD28 co-stimulatory endodomain.
4. A cell according to Claim 2 or 3, in which the TNF receptor family endodomain is OX-40 or 4-1BB endodomain.
- 15 5. A cell according to Claim 2, 3 or 4, in which the intracellular signalling domain of the first and the second CAR also comprises an ITAM-containing domain.
6. A cell according to any preceding claim, in which each CAR comprises:
 - 20 (i) an antigen-binding domain;
 - (ii) a spacer; and
 - (iii) a trans-membrane domain;

in which the spacer of the first CAR is different to the spacer of the second CAR, optionally in which the spacer of the second CAR comprises cartilage oligomeric matrix protein (COMP)

- 25 coiled coil domain.
- 7. A cell according to any preceding claim, in which the first CAR comprises a CD19-binding domain which comprises

- (a) a heavy chain variable region (VH) having complementarity determining regions (CDRs) with the following sequences:

CDR1 – SYWMN (SEQ ID No. 1);

CDR2 – QIWPGDGDNTNYNGKFK (SEQ ID No. 2)

5 CDR3 – RETTTVGRYYYAMDY (SEQ ID No. 3); and

- (b) a light chain variable region (VL) having CDRs with the following sequences:

CDR1 – KASQSVDDYDGDSYLN (SEQ ID No. 4);

CDR2 – DASNLVS (SEQ ID No. 5)

10 CDR3 – QQSTEDPWT (SEQ ID No. 6), preferably in which the CD19 binding domain comprises a VH domain having the sequence shown as SEQ ID No. 7, or SEQ ID NO 8; or a VL domain having the sequence shown as SEQ ID No 9, SEQ ID No. 10 or SEQ ID No. 11 a variant thereof having at least 90% sequence identity which retains the capacity to bind CD19, more preferably in which the CD19 binding domain comprises the sequence shown as SEQ ID No 12, SEQ ID
15 No. 13 or SEQ ID No. 14 or a variant thereof having at least 90% sequence identity which retains the capacity to bind CD19.

8. A cell according to any of Claims 1 to 6, in which the second CAR comprises a CD22-binding domain which comprises

- 20 (a) a heavy chain variable region (VH) having complementarity determining regions (CDRs) with the following sequences:

CDR1 – NYWIN (SEQ ID No. 15);

CDR2 – NIYPSDSFTNYNQKFKD (SEQ ID No. 16)

CDR3 – DTQERSWYFDV (SEQ ID No. 17); and

- (b) a light chain variable region (VL) having CDRs with the following sequences:

25 CDR1 – RSSQSLVHSNGNTYLH (SEQ ID No. 18);

CDR2 – KVSNRFS (SEQ ID No. 19)

CDR3 – SQSTHVPWT (SEQ ID No. 20), preferably in which the CD22 binding domain comprises a VH domain having the sequence shown as SEQ ID No. 21, or

SEQ ID NO 22; or a VL domain having the sequence shown as SEQ ID No 23, or SEQ ID No. 24 or a variant thereof having at least 90% sequence identity which retains the capacity to bind CD22, more preferably in which the CD22 binding domain comprises the sequence shown as SEQ ID No 25 or SEQ ID No. 26 or a variant thereof having at least 90% sequence identity which retains the capacity to bind CD22.

9. A cell according to any preceding claim, in which the first CAR has the structure:

AgB1-spacer1-TM1-TNF-ITAM

in which:

AgB1 is the antigen-binding domain of the first CAR;

spacer1 is the spacer of the first CAR;

TM1 is the transmembrane domain of the first CAR;

TNF is a TNF receptor endodomain; and

ITAM is an ITAM-containing endodomain;

and the second CAR has the structure:

AgB2-spacer2-TM2-costim-ITAM

in which:

AgB2 is the antigen-binding domain of the second CAR;

spacer2 is the spacer of the second CAR;

TM2 is the transmembrane domain of the second CAR;

costim is a co-stimulatory domain; and

ITAM is an ITAM-containing endodomain.

10. A nucleic acid sequence encoding both the first and second chimeric antigen receptors (CARs) as defined in any preceding claim, dSHP2, and dnTGFβRII, preferably in which the

nucleic acid sequence has the following structure:

module1-coexpr-AgB1-spacer1-TM1-coexpr-AgB2-spacer2-TM2-coexpr-module2

in which

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

spacer1 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;

coexpr is a nucleic acid sequence enabling co-expression

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

spacer2 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;

10 *module1* and *module2* are nucleic acid sequences encoding either dominant negative SHP2 (dSHP2) or dominant negative TGF β RII (dnTGF β RII), in which when *module1* encodes dSHP2 *module2* encodes dnTGF β RII and when *module2* encodes dnTGF β RII *module1* encodes dSHP2;

15 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, more preferably in which *coexpr* encodes a sequence comprising a self-cleaving peptide.

11. A vector comprising a nucleic acid sequence according to Claim 10, preferably in which the vector is a retroviral vector or a lentiviral vector or a transposon.

20 12. A method for making a cell according to any preceding claim, which comprises the step of introducing: a nucleic acid sequence according to Claim 10; or a vector according to Claim 11, into a cell, preferably in which the cell is from a sample isolated from a subject.

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

25 14. A pharmaceutical composition according to Claim 13 for use in treating and/or preventing a disease.

15. A kit which comprises

(i) a first nucleic acid sequence encoding the first chimeric antigen receptor (CAR), which nucleic acid sequence has the following structure:

AgB1-spacer1-TM1

in which

5 *AgB1* is a nucleic acid sequence encoding the antigen-binding domain of the first CAR which binds to CD19;

spacer1 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;

10 (ii) a second nucleic acid sequence encoding the second chimeric antigen receptor, which nucleic acid sequence has the following structure:

AgB2-spacer2-TM2

in which

AgB2 is a nucleic acid sequence encoding the antigen-binding domain of the second CAR which binds to CD22;

15 *spacer2* is a nucleic acid sequence encoding the spacer of the second CAR; and

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

(iii) a third nucleic acid sequence encoding dSHP2 and dnTGFβRII as described herein.