

CLAIMS

1. A cell which co-expresses a first chimeric antigen receptor (CAR) and second CAR as separate molecules at the cell surface, each CAR comprising an antigen-binding domain, wherein the antigen-binding domain of the first CAR binds to CD19 and the antigen-binding domain of the second CAR binds to CD22.
2. A cell according to Claim 1, in which each CAR comprises:
 - (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.
3. A cell according to Claim 2, in which the antigen-binding domain of the second CAR binds to an epitope on Ig domain 1, 2, 3 or 4 of CD22.
4. A nucleic acid encoding both the first and second chimeric antigen receptors (CARs) as defined in any of Claims 1, 2 or 3.
5. A nucleic acid according to Claim 4, which has the following structure:

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

in which

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

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

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

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

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

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

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

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

6. A nucleic acid according to Claim 5, in which coexpr encodes a sequence comprising a self-cleaving peptide.

7. A nucleic acid according to Claim 5 or 6, in which alternative codons are used in regions of sequence encoding the same or similar amino acid sequences, in order to avoid homologous recombination.

8. A kit which comprises

(i) a first nucleic acid encoding the first chimeric antigen receptor (CAR) as defined in any of Claims 1 to 3, which nucleic acid has the following structure:

AgB1-spacer1-TM1

in which

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

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

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

and

(ii) a second nucleic acid encoding the second chimeric antigen receptor (CAR) as defined in any of Claims 1 to 3, which nucleic acid has the following structure:

AgB2-spacer2-TM2

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

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

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

9. A kit comprising: a first vector which comprises the first nucleic acid as defined in Claim 8; and a second vector which comprises the second nucleic acid as defined in Claim 8.

10. A kit according to Claim 9, in which the vectors are integrating viral vectors or transposons.

11. A vector comprising a nucleic acid according to any of Claims 4 to 7.
12. A retroviral vector or a lentiviral vector or a transposon according to Claim 11.
13. A method for making a cell according to any of Claims 1 to 3, which comprises the step of introducing: a nucleic acid according to any of Claims 4 to 7; a first nucleic acid and a
5 second nucleic acid as defined in Claim 8; and/or a first vector and a second vector as defined in Claim 9 or a vector according to Claim 11 or 12, into a cell.
14. A method according to Claim 13, in which the cell is from a sample isolated from a subject.
15. A pharmaceutical composition comprising a plurality of cells according to any of
10 Claims 1 to 3.
16. A pharmaceutical composition according to Claim 15 for use in treating a disease.
17. A pharmaceutical composition for use according to Claim 16, in which the plurality of cells is prepared by:
 - (i) isolating a cell-containing sample from a subject; and
 - 15 (ii) transducing or transfecting the cells with: a nucleic acid sequence according to any of Claims 4 to 7; a first nucleic acid sequence and a second nucleic acid sequence as defined in Claim 8; a first vector and a second vector as defined in Claim 9 or 10 or a vector according to Claim 11 or 12.
18. A pharmaceutical composition for use according to Claim 16 or 17, in which the disease
20 is a cancer.
19. A pharmaceutical composition for use according to Claim 18, in which the cancer is a B cell malignancy.
20. Use of a cell according to any of Claims 1 to 3 in the manufacture of a medicament for treating a B-cell malignancy.