

CLAIMS

1. A genetically engineered immune cell expressing a Chimeric Antigen Receptor (CAR) specific for CD22 (CAR22) and a Chimeric Antigen Receptor specific for CD20 (CAR20) at its cell surface,

a) wherein said CAR22 comprises:

i) at least one extracellular domain comprising:

- an antigen binding domain specific for CD22 comprising a Variable Heavy chain (VH) comprising an amino acid sequence having at least 80% identity with SEQ ID NO: 11 and comprising the H-CDRs of amino acid sequences SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO: 43, and a Variable Light chain (VL) comprising an amino acid sequence having at least 80% identity with SEQ ID NO: 12 and comprising the L-CDRs of amino acid sequences SEQ ID NO: 44, SEQ ID NO: 45, and SEQ ID NO: 46, optionally a leader sequence,

- a hinge domain from CD8alpha,

ii) a transmembrane domain from CD8alpha, and

iii) an intracellular domain comprising a 4-1BB stimulatory domain and a CD3zeta signalling domain; and

b) wherein said CAR20 comprises:

i) at least one extracellular domain comprising:

- an antigen binding domain specific for CD20 comprising a Variable Heavy chain (VH) comprising an amino acid sequence having at least 80% identity with SEQ ID NO: 15 and comprising the H-CDRs of amino acid sequences SEQ ID NO: 47, SEQ ID NO: 48, and SEQ ID NO: 49, and a Variable Light chain (VL) comprising an amino acid sequence having at least 80% identity with SEQ ID NO: 16 and comprising the L-CDRs of amino acid sequences SEQ ID NO: 50, SEQ ID NO: 51, and SEQ ID NO: 52, optionally a leader sequence,

- a hinge domain from CD8alpha,

ii) a transmembrane domain from CD8alpha, and

iii) an intracellular domain comprising a 4-1BB stimulatory domain and a CD3zeta signalling domain;

wherein said CAR22 and said CAR20 are encoded by an exogenous nucleic acid incorporated in said immune cell's genome; and wherein said exogenous nucleic acid comprises, from 5' to 3':

(i) a promoter (such as the EF1alpha promoter)

(ii) a nucleic acid encoding said CAR20,

(iii) a nucleic acid encoding a self-cleaving peptide (such as P2A of SEQ ID NO: 19);

(iv) a nucleic acid encoding said CAR22,

whereby the same promoter controls the expression of said CAR20 and CAR22.

- 5 2. The genetically engineered immune cell according to claim 1, wherein said CAR22 comprises the amino acid sequence of SEQ ID NO: 14, and wherein said CAR20 comprises the amino acid sequence of SEQ ID NO: 18.
3. The genetically engineered immune cell according to any one of claims 1 and 2, wherein said exogenous nucleic acid comprises the nucleic acid sequence of SEQ ID
10 NO: 32.
4. The genetically engineered immune cell according to any one of claims 1 to 3, wherein said engineered immune cell is selected from the group consisting of a T-cell, a NK-cell, and a macrophage.
5. The genetically engineered immune cell according to any one of claims 1 to 4, wherein
15 said engineered immune cell is a T-cell that is TCR negative.
6. The genetically engineered immune cell according to claim 5, wherein said engineered T-cell has at least one allele encoding TCR alpha, TCR beta, and/or CD3 that has been inactivated by mutation.
7. The genetically engineered immune cell according to any one of claims 5 to 6, wherein
20 said engineered T-cell has at least one allele selected from β 2m, PD1, CTLA4, dCK, CD52 and/or GR that has been inactivated.
8. A pharmaceutical composition comprising the engineered immune cells according to any one of claims 1 to 7, and a pharmaceutically acceptable excipient.
9. An isolated polynucleotide encoding a CAR20 and CAR22 comprising:
25 a) a promoter (such as the EF1alpha promoter) that controls the expression of a CAR20, followed by a nucleic acid encoding said CAR20, wherein said CAR20 comprises:
i) at least one extracellular domain comprising:
- an antigen binding domain specific for CD20 comprising a Variable Heavy chain
30 (VH) comprising an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identity with SEQ ID NO: 15 and comprising the H-CDRs of amino acid sequences SEQ ID NO: 47, SEQ ID NO: 48, and SEQ ID NO: 49, and a Variable Light chain (VL) comprising an amino acid
35 sequence having at least 80%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identity with SEQ ID NO: 16 and comprising the L-CDRs of amino acid sequences SEQ ID NO: 50, SEQ ID NO: 51, and SEQ ID NO: 52, optionally a leader sequence,

- a hinge domain from CD8alpha,
 - ii) a transmembrane domain from CD8alpha, and
 - iii) an intracellular domain comprising a 4-1BB stimulatory domain and a CD3zeta signalling domain; and
- 5 b) a nucleic acid encoding a CAR22, wherein said CAR22 comprises:
- i) at least one extracellular domain comprising:
 - an antigen binding domain specific for CD22 comprising a Variable Heavy chain (VH) comprising an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identity with SEQ ID NO: 11 and
 - 10 comprising the H-CDRs of amino acid sequences SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO: 43, and a Variable Light chain (VL) comprising an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identity with SEQ ID NO: 12 and comprising the L-CDRs of amino acid sequences SEQ ID NO: 44, SEQ ID NO: 45, and SEQ ID NO: 46, optionally a
 - 15 leader sequence,
 - a hinge domain from CD8alpha,
 - ii) a transmembrane domain from CD8alpha, and
 - iii) an intracellular domain comprising a 4-1BB stimulatory domain and a CD3zeta signalling domain;
- 20 wherein said nucleic acids of a) and b) are on a single nucleic acid molecule, and wherein a nucleic acid sequence encoding a self-cleaving peptide (such as P2A, T2A, E2A, or F2A) is located between said nucleic acids of a) and b).
10. The isolated polynucleotide of claim 9 comprising the nucleic acid sequence of SEQ ID NO: 32 (CAR20xCAR22 construct).
- 25 11. A vector comprising the isolated polynucleotide of any one of claims 9 to 10.
12. A method of preparing the engineered immune cell according to any one of claims 1 to 7, comprising introducing into an immune cell the polynucleotide according to any one of claims 9 to 10, or a vector according to claim 11.
- 30 13. The engineered immune cell according to any one of claims 1 to 7, for use as a medicament.
14. Use of the engineered immune cell according to any one of claims 1 to 7, in the manufacture of a medicament for the treatment of a cancer associated with CD20 and/or CD22 expression.
- 35 15. The use according to claim 14, wherein said cancer is selected from the group consisting of lymphoma, Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), leukemia, multiple myeloma (MM), B-chronic lymphocytic leukemia (B-CLL), hairy cell

leukemia (HCL), acute lymphocytic leukemia (ALL), acute lymphocytic cancer, acute myeloid leukemia (AML).