

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.

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 claim 1 or 2, wherein none of the extracellular domain of said CAR20 and CAR22 comprises a safety switch comprising a rituximab-specific mimotope such as SEQ ID NO: 22.~~

~~4. The genetically engineered immune cell according to any one of claims 1 to 3, wherein said CARs 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. The genetically engineered immune cell according to claim 4, wherein:~~

~~—said nucleic acid of (ii) comprises the signal peptide of SEQ ID NO: 1, the scFv of SEQ ID NO: 17, the CD8alpha hinge of SEQ ID NO: 4, the CD8alpha transmembrane domain of SEQ ID NO: 6, the 4-1BB co-stimulation domain of SEQ ID NO: 8, and the CD3 zeta signalisation domain of SEQ ID NO: 9); and~~

~~—said nucleic acid of (iv) comprises the signal peptide of SEQ ID NO: 1, the scFv of SEQ ID NO: 13, the CD8alpha hinge of SEQ ID NO: 4, the CD8alpha transmembrane domain of SEQ ID NO: 6, the 4-1BB co-stimulation domain of SEQ ID NO: 8, and the CD3 zeta signalisation domain of SEQ ID NO: 9.~~

~~6.3.~~ The genetically engineered immune cell according to any one of claims ~~4~~1 and ~~5~~2, wherein said exogenous nucleic acid comprises the nucleic acid sequence of SEQ ID NO: 32.

~~7.4.~~ The genetically engineered immune cell according to any one of claims 1 to ~~6~~3, wherein said engineered immune cell is selected from the group consisting of a T-cell, a NK-cell, and a macrophage.

~~8. The genetically engineered immune cell according to any one of claims 1 to 7, wherein said engineered immune cell is a T-cell, for instance a cytotoxic T cell.~~

~~9. The genetically engineered immune cell according to any one of claims 1 to 8, wherein said engineered immune cell is comprised in a population of cells, such as a population~~

~~of immune cells, in particular a population of T-cells, a population of NK-cells, and/or a population of macrophages.~~

~~10.5.~~_____ The genetically engineered immune cell according to any one of claims 1 to ~~94~~, wherein said engineered immune cell is a T-cell that is TCR negative.

~~11. The genetically engineered immune cell according to claim 10, wherein said engineered T-cell expresses a short hairpin RNA (shRNA) or small interfering (siRNA) directed against a polynucleotide sequence encoding a component of TCR.~~

~~12. The genetically engineered immune cell according to claim 10, wherein said engineered T-cell is mutated in its TCR alpha and/or TCR beta alleles.~~

~~13.6.~~_____ The genetically engineered immune cell according to ~~any one of claims 10-5 or 12~~, wherein said engineered T-cell has at least one allele encoding TCR alpha, TCR beta, and/or CD3 that has been inactivated by mutation.

~~14.7.~~_____ The genetically engineered immune cell according to any one of claims ~~8-5~~ to ~~136~~, wherein said engineered T-cell has at least one allele selected from $\beta 2m$, PD1, CTLA4, dCK, CD52 and/or GR that has been inactivated.

~~15. The genetically engineered immune cell according to any one of claims 9 to 14, wherein said engineered T-cell has a CD52 allele that has been inactivated.~~

~~16. The genetically engineered immune cell according to any one of claims 1 to 15, wherein said engineered cell, preferably a T-cell, is a primary cell.~~

~~17. The genetically engineered immune cell according to any one of claims 1 to 16, wherein said engineered cell, preferably a T-cell, is a mammalian cell, preferably a human cell.~~

~~18. The genetically engineered immune cell according to any one of claims 1 to 17, wherein said engineered cell, preferably a T-cell, expresses no further CAR than said CAR22 and CAR20.~~

~~19. A population of immune cells comprising the engineered immune cells according to any one of claims 1 to 18.~~

~~20. A population of T-cells comprising the engineered T-cells according to any one of claims 9 to 18.~~

~~21.8.~~_____ A pharmaceutical composition comprising the engineered immune cells according to any one of claims 1 to ~~187~~, and a pharmaceutically acceptable excipient.

~~22. A pharmaceutical composition comprising a population of immune cells according to any one of claims 19 to 20, and a pharmaceutically acceptable excipient.~~

~~23. A pharmaceutical composition comprising the engineered T-cells according to any one of claims 10 to 18, and a pharmaceutically acceptable excipient.~~

~~24. A pharmaceutical composition comprising a population of T-cells according to claim 20, and a pharmaceutically acceptable excipient.~~

~~25.9.~~_____ An isolated polynucleotide encoding a CAR20 and CAR22 comprising:

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 (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 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

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 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 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 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).

a) a nucleic acid encoding a CAR22 comprising:

- 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) ~~a nucleic acid encoding a CAR20 comprising:~~
- 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.~~
26. ~~The polynucleotide of claim 25, 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).~~
27. ~~The polynucleotide of claim 25 or 26, wherein said polynucleotide does not comprise a nucleic acid encoding a further CAR than said CAR22 and CAR20.~~
28. ~~The polynucleotide of any one of claims 25 to 27, wherein said polynucleotide does not comprise a nucleic acid encoding rituximab-specific mimotope of SEQ ID NO: 22.~~
29. ~~The polynucleotide of any one of claims 25 to 28:~~

~~—wherein said nucleic acid of a) comprises a promoter (such as the EF1alpha promoter) that controls the expression of said CAR22 composed of the signal peptide of SEQ ID NO: 1, the scFv of SEQ ID NO: 13, the CD8alpha hinge of SEQ ID NO: 4, the CD8alpha transmembrane domain of SEQ ID NO: 6, the 4-1BB co-stimulation domain of SEQ ID NO: 8, and the CD3-zeta signalisation domain of SEQ ID NO: 9;~~

~~—wherein said nucleic acid of b) encodes said CAR20 composed of the signal peptide of SEQ ID NO: 1, the scFv of SEQ ID NO: 17, the CD8alpha hinge of SEQ ID NO: 4, the CD8alpha transmembrane domain of SEQ ID NO: 6, the 4-1BB co-stimulation domain of SEQ ID NO: 8, and the CD3-zeta signalisation domain of SEQ ID NO: 9; and~~

~~—wherein a nucleic acid encoding for a self-cleaving peptide (such as P2A of SEQ ID NO: 19) is present between said nucleic acid of a) and said nucleic acid of b), allowing the simultaneous expression of said CAR20 and CAR22.~~

30. The polynucleotide of any one of claims 25 to 28,

~~—wherein said nucleic acid of b) comprises a promoter (such as the EF1alpha promoter) that controls the expression of said CAR20 composed of the signal peptide of SEQ ID NO: 1, the scFv of SEQ ID NO: 17, the CD8alpha hinge of SEQ ID NO: 4, the CD8alpha transmembrane domain of SEQ ID NO: 6, the 4-1BB co-stimulation domain of SEQ ID NO: 8, and the CD3-zeta signalisation domain of SEQ ID NO: 9;~~

~~—wherein said nucleic acid of a) encodes said CAR22 composed of the signal peptide of SEQ ID NO: 1, the scFv of SEQ ID NO: 13, the CD8alpha hinge of SEQ ID NO: 4, the CD8alpha transmembrane domain of SEQ ID NO: 6, the 4-1BB co-stimulation domain of SEQ ID NO: 8, and the CD3-zeta signalisation domain of SEQ ID NO: 9; and~~

~~—wherein a nucleic acid encoding for a self-cleaving peptide (such as P2A of SEQ ID NO: 19) is present between said nucleic acid of b) and said nucleic acid of a), allowing the simultaneous expression of said CAR22 and CAR20.~~

31. The isolated polynucleotide of claim 29 comprising the nucleic acid sequence of SEQ ID NO: 31 (CAR22xCAR20 construct).

32.10. The isolated polynucleotide of claim ~~30~~9 comprising the nucleic acid sequence of SEQ ID NO: 32 (CAR20xCAR22 construct).

33.11. A vector comprising the isolated polynucleotide of any one of claims ~~25~~9 to ~~32~~10.

~~34. A host cell comprising the vector of claim 33.~~

35.12. A method of preparing the engineered immune cell according to any one of claims 1 to ~~18~~7, comprising introducing into an immune cell the polynucleotide according to any one of claims ~~25~~9 to ~~32~~10, or a vector according to claim ~~33~~11.

36.13. The engineered immune cell according to any one of claims 1 to ~~18~~7, for use as a medicament.

~~37.14.~~ Use of The engineered immune cell according to any one of claims 1 to ~~18~~7,
~~for use in the manufacture of a medicament for the~~ treatment of a cancer associated
with CD20 and/or CD22 expression.

~~38. The engineered immune cell according to any one of claims 1 to 18, for use in the
treatment of a hematological cancer, in particular a CD22 and/or CD20-associated
hematological cancer, more particularly a relapse refractory CD22 and/or CD20-
associated hematological cancer, even more particularly an aggressive form of said
CD22 and/or CD20-associated hematological cancer.~~

~~39.15.~~ The engineered immune cell for use according to claim ~~37 or 38~~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).

~~40. The engineered immune cell for use according to claim 39, wherein said cancer is non-
Hodgkin lymphoma or acute lymphocytic leukemia.~~

~~41. The engineered immune cell for use according to any one of claims 37 to 40, wherein
said cancer is associated with a low expression of CD20 and/or CD22.~~

~~42. The engineered immune cell for use according to any one of claims 37 to 41, wherein
said cancer is a relapsing non-Hodgkin lymphoma.~~

~~43. A method of treating a patient suffering from a cancer associated with CD20 and/or
CD22 expression, comprising administering to said patient an effective amount of an
engineered immune cell according to any one of claims 1 to 18, or a population of
immune cells according to any one of claims 19 to 20.~~

~~44. The method of treatment according to claim 43, 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).~~

~~45. The method of treatment according to any one of claims 43 to 44, wherein said cancer
is non-Hodgkin lymphoma or acute lymphocytic leukemia.~~

~~46. The method of treatment according to any one of claims 43 to 45, wherein said cancer
is associated with a low expression of CD20 and/or CD22.~~

~~47. The method of treatment according to any one of claims 43 to 46, wherein said
cancer is a relapsing non-Hodgkin lymphoma.~~