4-1BB (CD137) have been added alone (second generation) or in combination (third generation) to enhance survival and increase proliferation of CAR modified T cells. CARs have successfully allowed T cells to be redirected against antigens expressed at the surface of tumor cells from various malignancies including lymphomas and solid tumors (Jena, Dotti et al. 2010, Blood 116(7):1035-44).

Adoptive immunotherapy, which involves the transfer of autologous or allogeneic antigen-specific T cells generated *ex vivo*, is a promising strategy to treat viral infections and cancer as confirmed by the increase in the number of CAR-T cells approved by the US Food and Drug Administration (FDA) (e.g. Novartis' anti-CD19 CAR-T tisagenlecleucel (Kymriah™) for the treatment of precursor B-cell acute lymphoblastic leukemia, Kite Pharma's anti-CD19 CAR-T axicabtagene ciloleucel (Yescarta™) for certain types of large B-cell lymphoma in adult patients).

Despite the progress in research and development of CAR-T cell therapy, there still remains a need for improved CAR-T cells which could target a wider range of cancers as well as recurrent cancers and/or cancers for which the expression of the cancer-associated antigens are very variable and evolve with the time or during or after the treatments.

Summary

In one aspect, provided herein is 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.

In another aspect, provided herein is a pharmaceutical composition comprising the engineered immune cells as described herein, and a pharmaceutically acceptable excipient.

In another aspect, provided herein is 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).

In another aspect, provided herein is a vector comprising the isolated polynucleotide as described herein.

In another aspect, provided herein is a method of preparing the engineered immune cell as described herein, comprising introducing into an immune cell the polynucleotide as described herein, or a vector as described herein.

In another aspect, provided herein is the engineered immune cell as described herein, for use as a medicament.

In another aspect, provided herein is use of the engineered immune cell as described herein, in the manufacture of a medicament for the treatment of a cancer associated with CD20 and/or CD22 expression.

The inventors have developed new CAR-T cells targeting CD20 and CD22 antigens, which can be activated by the tumor cells expressing CD20 and CD22 at variable levels, and constitute an improvement over CAR-T cells of the prior art.

A first aspect relates to 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 the Variable Heavy chain (VH) of SEQ ID NO: 11 and the Variable Light chain (VL) of SEQ ID NO: 12, 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: