

(i) the first CAR comprises a first signal peptide, a first antigen recognition domain, a first hinge region, a first transmembrane domain, a first CD28 or 4-1BB co-stimulatory domain, and a first signaling domain; and

(ii) the second CAR comprises a second signal peptide, a second antigen recognition domain, a second hinge region, a second transmembrane domain, a second CD28 or 4-1BB co-stimulatory domain, and a second signaling domain;

and wherein the first antigen recognition domain and the second antigen recognition domain are different from each other, and each bind to a different target, wherein the targets of the first and second antigen recognition domains irrespective of order are CD123 and CD33, or BCMA (CD269) and CS1, and wherein the first and second co-stimulatory domains are intracellular.

In another aspect, the present disclosure provides a use of an engineered cell as described herein in the manufacture of a medicament for treating myeloid leukemia. In another aspect, the present disclosure provides a use of an engineered cell as described herein in the manufacture of a medicament for treating multiple myeloma.

In another example, the present disclosure provides an *in vitro* method of reducing the number of target cells, the method comprising the steps of:

i. contacting said target cells with an effective amount of an engineered cell as described herein; and

ii. assaying for the reduction in the number of said cells;

wherein said target cells comprise at least one cell surface antigen selected from the group consisting of BCMA, CD19, CD33, CD123, CD20 and CS1.

In another example, the present disclosure provides a use of an engineered cell as described herein, in the manufacture of a medicament for depleting B cells and/or plasma cells in a patient with an autoimmune disease. In another example, the present disclosure provides a use of an engineered cell as described herein, in the manufacture of a medicament for treating multiple myeloma. In another example, the present disclosure provides a use of an engineered cell as described herein, in the manufacture of a medicament for treating B cell lymphoma or B cell leukemia.

In one example, the present disclosure provides an engineered cell having a first chimeric antigen receptor polypeptide including a first antigen recognition domain, a first signal peptide, a first hinge region, a first transmembrane domain, a first co-stimulatory domain, and a first signaling domain; and a second chimeric antigen receptor polypeptide including a second antigen recognition domain, a second signal peptide, a second hinge region, a second transmembrane domain, a second co-stimulatory domain, and a second signaling domain; wherein the first antigen recognition domain is different than the second antigen recognition domain.

CLAIMS

1. An engineered T cell or NK cell co-expressing two distinct chimeric antigen receptor (CAR) units at the cell surface, wherein the engineered T cell or NK cell comprises a nucleotide sequence comprising from 5' to 3' a promoter, a first polynucleotide encoding a first chimeric antigen receptor polypeptide (CAR), a nucleotide encoding a first self-cleavage peptide, and a second polynucleotide encoding a second chimeric antigen receptor polypeptide (CAR) wherein:
 - (i) the first CAR comprises a first signal peptide, a first antigen recognition domain, a first hinge region, a first transmembrane domain, a first CD28 or 4-1BB co-stimulatory domain, and a first signaling domain; and
 - (ii) the second CAR comprises a second signal peptide, a second antigen recognition domain, a second hinge region, a second transmembrane domain, a second CD28 or 4-1BB co-stimulatory domain, and a second signaling domain;and wherein the first antigen recognition domain and the second antigen recognition domain are different from each other, and each bind to a different target, wherein the targets of the first and second antigen recognition domains irrespective of order are CD123 and CD33, or BCMA (CD269) and CS1, and wherein the first and second co-stimulatory domains are intracellular.
2. The engineered cell according to claim 1, wherein the targets of the first and second antigen recognition domains are, irrespective of order, CD33 and CD123.
3. The engineered cell according to claim 1, wherein the targets of the first and second antigen recognition domains are, irrespective of order, BCMA and CS1.
4. The engineered cell according to claim 3, wherein the high efficiency cleavage site is selected from the group consisting of P2A, T2A, E2A, and F2A.
5. The engineered cell according to any one of claims 1-4, wherein the first co-stimulatory domain and the second co-stimulatory domain are different.
6. The engineered cell according to any one of claims 1-5, wherein the first co-stimulatory domain comprises CD28, and the second co-stimulatory domain comprises 4-1BB.
7. The engineered cell according to claim 1, wherein the T-cell is a CD4 T-cell or CD8 T-cell.

8. The engineered cell according to claim 1, wherein the Natural Killer cell is a NKT cell or NK-92 cell.
9. Use of an engineered cell according to claim 2 in the manufacture of a medicament for treating myeloid leukemia.
- 5 10. Use of an engineered cell according to claim 3 in the manufacture of a medicament for treating multiple myeloma.