

antigen receptor(s); (2)CAR design; (3)tumor heterogeneity, particularly the variance in the surface expression of tumor antigens. Targeting single antigen carries the risk of immune escape and this could be overcome by targeting multiple desired antigens.

Most CAR chimeric antigen receptors are scFvs derived from monoclonal antibodies and some of these monoclonal antibodies have been used in the clinical trials or treatment for diseases. However, they have limited efficacy, which suggests that alternative and more potent targeting approaches, such as CARs are required. scFvs are the most commonly used chimeric antigen receptor for CARs. However, CAR affinity binding and locations of the recognized epitope on the antigen could affect the function. Additionally the level of the surface CAR expression on the T cells or NK cells is affected by an appropriate leader sequence and promoter. Furthermore, overexpressed CAR proteins can be toxic to cells.

Therefore, there remains a need for improved chimeric antigen receptor-based therapies that allow for more effective, safe, and efficient targeting of T-cell associated malignancies.

SUMMARY

In one aspect, the present disclosure provides 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 antibody binding 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 antibody binding domain, a second hinge region, a second transmembrane domain, a second CD28 or 4-1BBz co-stimulatory domain, and a second signaling domain;

and wherein the first antibody binding domain and the second antibody binding domain are different from each other, and each bind to a different target, wherein the targets of the first and second antibody binding domains irrespective of order are CD19 and CD20, or CD123 and CD33, or BMCA (CD269) and CD19, or and BMCA (CD269) and CS1, 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 aspect, 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 aspect, 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 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 aspect, 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.

In another example, the present disclosure provides an engineered polypeptide including a chimeric antigen receptor and an enhancer.

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5 In another example, the present disclosure provides an engineered chimeric antigen receptor polypeptide, the polypeptide including: a signal peptide, a CD45 antigen recognition domain, a hinge region, a transmembrane domain, at least one co-stimulatory domain, and a signaling domain. In another example, the present disclosure provides a polynucleotide encoding for the aforementioned polypeptide.

10 In another example, the present disclosure provides an engineered cell having the engineered polypeptide or polynucleotide described above.

 In another example, the present disclosure provides a method of reducing the number of target cells including the steps of (i.) contacting said target cells with an effective amount of an engineered cell having at least one chimeric antigen receptor polypeptide, for engineered cells
15 having multiple chimeric antigen receptor polypeptides, each chimeric antigen receptor polypeptides are independent; and (ii.) optionally, assaying for the reduction in the number of said cells. The target cells include at least one cell surface antigen selected from the group consisting of interleukin 6 receptor, NY-ESO-1, alpha fetoprotein (AFP), glypican-3 (GPC3), BAFF-R, BCMA, TACI, LeY, CD5, CD13, CD14, CD15 CD19, CD20, CD22, CD33, CD41,
20 CD45, CD61, CD64, CD68, CD117, CD123, CD138, CD267, CD269, CD38, Flt3 receptor, and CS1.

In another example, the present disclosure provides methods for treating B-cell lymphoma, T-cell lymphoma, multiple myeloma, chronic myeloid leukemia, B-cell acute lymphoblastic leukemia (B-ALL), and cell proliferative diseases by administering any of the engineered cells described above to a patient in need thereof.

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BRIEF DESCRIPTION OF DRAWINGS

Figure 1. A schematic representation of cCAR construct (hereinafter, "multiple CAR or compound CAR"). Multiple or compound CAR targets multiple antigens (e.g. cell type 1 or cell type 2 or the same cell type). Multiple or cCAR T cell immunotherapies comprises individual component CAR comprising a different or same antigen recognition domain, a hinge region, a transmembrane domain, various co-stimulatory domain(s) and an intracellular signaling domain.

Figure 2A. A schematic representation of cCAR-T construct. The construct comprises a SFFV promoter driving the expression of-multiple modular units of CARs linked by a P2A peptide. Upon cleavage of the linker, the cCARs split and engage upon targets expressing CD33 and/or CD123. As a novel cCAR construct, the activation domains of the construct may include, but is not limited to, 4-1BB on the CD33 CAR segment and a CD28 region on the CD123 CAR.

Figure 2B. A Western blot depicting the expression of transduced CD33CD123 cCAR-T cells. The figure depicts expression of two different CAR proteins, i.e., CD33 CAR and CD123 CARs. The cCAR-T cells expressing both CD33 and CD123 CARs upon cleavage of the linker generate two distinct and consistently intense protein bands. Green Fluroscent Protein (GFP) is included as negative control.

Figure 2C. Flow cytometry representing the efficiency of transduction. Upper panel shows the lentiviral titer for CD33CD123 cCARs (also referred to as CD33CD123-2G-CAR)

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 antibody binding domain, a first hinge region, a first transmembrane domain, a first CD28 or 4-1BB co-stimulatory domain, and a first signaling domain; and
 - 10 (ii) the second CAR comprises a second signal peptide, a second antibody binding domain, a second hinge region, a second transmembrane domain, a second CD28 or 4-1BBz co-stimulatory domain, and a second signaling domain;and wherein the first antibody binding domain and the second antibody binding domain are different from each other, and each bind to a different target, wherein the targets of the first and second antibody binding domains irrespective of order are CD19 and CD20, or CD123 and CD33, or BMCA (CD269) and CD19, or and BMCA (CD269) and CS1, wherein the first and second co-stimulatory domains are intracellular.
- 15 2. The engineered cell according to claim 1, wherein the targets of the first and second antigen recognition domains are, irrespective of order, CD19 and CD20.
- 20 3. 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.
4. The engineered cell according to claim 1, wherein the targets of the first and second antigen recognition domains are, irrespective of order, CD19 and BCMA.
5. 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.
- 25 6. The engineered cell according to claim 5, wherein the high efficiency cleavage site is selected from the group consisting of P2A, T2A, E2A, and F2A.

7. The engineered cell according to any one of claims claim 1-6, wherein the first co-stimulatory domain and the second co-stimulatory domain are different.
8. The engineered cell according to any one of claims 1-7, wherein the first co-stimulatory domain comprises CD28, and the second co-stimulatory domain comprises 4-1BB.
- 5 9. The engineered cell according to claim 9, wherein the T-cell is a CD4 T-cell or CD8 T-cell.
10. The engineered cell according to claim 9, wherein the Natural Killer cell is a NKT cell or NK-92 cell.
11. Use of an engineered cell according to claim 3 in the manufacture of a medicament for
10 treating myeloid leukemia.
12. Use of an engineered cell according to claim 5 in the manufacture of a medicament for treating multiple myeloma.
13. An *in vitro* method of reducing the number of target cells, the method comprising the steps of:
- 15 i. contacting said target cells with an effective amount of an engineered cell according to any one of claims 1-11; 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.
- 20 14. The engineered cell according to any one of claims 1, 4 or 5, wherein the BCMA antigen recognition domain comprises APRIL ligand or BAFF ligand or a fragment thereof.
15. The engineered cell according to any one of claims 1-10, wherein the first co-stimulatory domain and the second co-stimulatory domain are same.
16. The engineered cell according to any one of claims 1-10, wherein the first co-stimulatory
25 domain and the second co-stimulatory domain comprise 4-1BB co-stimulatory domain.
17. Use of an engineered cell according to claim 4, in the manufacture of a medicament for depleting B cells and/or plasma cells in a patient with an autoimmune disease.

18. Use of an engineered cell according to claim 4, in the manufacture of a medicament for treating multiple myeloma.
19. Use of an engineered cell according to claim 2, in the manufacture of a medicament for treating B cell lymphoma or B cell leukemia.