outcomes. The ideal target(s) in neuroblastoma or other soft tissue tumors (such as sarcomas), diseases of great antigenic diversity, has not been established. The identification of appropriate target (s) is an important step for the CAR design and the CAR design is required to address tumor heterogeneity, CAR persistency and tumor microenvironment suppression. There is no general rule that CAR design is efficacious and safe.

Therefore, there remains a need for improved chimeric antigen receptor-based therapies that allow for more effective, safe, and efficient targeting of soft tissue tumors.

SUMMARY

In one aspect, the present disclosure refers to an ex vivo engineered T cell or NK cellco-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 first polynucleotide encoding a first chimeric antigen receptor polypeptide (first CAR), a second polynucleotide encoding a second chimeric antigen receptor polypeptide (second CAR), a nucleotide encoding a first viral self-cleavage peptide disposed between the first CAR and second CAR, under the transcriptional control of a single promoter, wherein: (i.) the first CAR polypeptide comprises a first antigen recognition domain selected from the group consisting of CS-1, CD5, CD19, CD20, CD123, BCMA, CD38, CLL-1, and CD33; a first signal peptide; a first hinge region; a first transmembrane domain; a first co-stimulatory domain; and a first signaling domain; and (ii.) the second CAR comprises a second antigen recognition domain selected from the group consisting of CS-1, CD5, CD19, CD20, CD123, BCMA, CD38, CLL-1, and CD33; 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 and the second antigen recognition domain are different; wherein the engineered T cell or NK cell comprises an enhancer selected from the group consisting of IL-15/IL-15sushi, IL-15/IL-15 sushi anchor, 4-1BBL, and IL-15, wherein the engineered T cell or NK cell comprises SEQ ID NO. 42 (a BCMA-CS 1 compound chimeric antigen receptor (cCAR) polypeptide and IL-15/IL- 15 sushi); SEQ ID NO. 34 (a CD123- CD33 cCAR polypeptide and IL-15/IL-15 sushi); SEQ ID NO. 60 (a CLL1-CD33 cCAR polypeptide and IL-15/IL-15sushi); SEQ ID NO.40 (a BCMA-CD38 cCAR polypeptide, 4- 1BBL and IL-15/IL-15sushi; SEQ ID NO. 36 (CD123- CLL1 cCAR polypeptide, and IL-15/IL-15sushi); SEQ ID NO. 28 (a CD20-CD19 cCAR

polypeptide, and IL-15/IL-15sushi; or SEQ ID NO. 50 (CD269-A7D- L-15/IL-15sushi anchor-2G), and wherein said enhancer is separated from the first CAR and second CAR by a nucleotide encoding a second viral self-cleavage peptide that flanks either end of the two distinct CAR units.

In yet another aspect, the present disclosure refers to the use of an engineered cell as described herein in the manufacture of a medicament for treating a cell proliferative disease, wherein

CLAIMS

1. An ex vivo 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 first polynucleotide encoding a first chimeric antigen receptor polypeptide (first CAR), a second polynucleotide encoding a second chimeric antigen receptor polypeptide (second CAR), a nucleotide encoding a first viral self-cleavage peptide disposed between the first CAR and second CAR, under the transcriptional control of a single promoter, wherein:

(i.) the first CAR polypeptide comprises a first antigen recognition domain selected from the group consisting of CS-1, CD5, CD19, CD20, CD123, BCMA, CD38, CLL-1, and CD33; a first signal peptide; a first hinge region; a first transmembrane domain; a first co-stimulatory domain; and a first signaling domain; and

(ii.) the second CAR comprises a second antigen recognition domain selected from the group consisting of CS-1, CD5, CD19, CD20, CD123, BCMA, CD38, CLL-1, and CD33; 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 and the second antigen recognition domain are different;

wherein the engineered T cell or NK cell comprises an enhancer selected from the group consisting of IL-15/IL-15sushi, IL-15/IL-15 sushi anchor, 4-1BBL, and IL-15; and

wherein the engineered T cell or NK cell comprises SEQ ID NO. 42 (a BCMA-CS 1 compound chimeric antigen receptor (cCAR) polypeptide and IL-15/IL- 15 sushi); SEQ ID NO. 34 (a CD123- CD33 cCAR polypeptide and IL-15/IL- 15 sushi); SEQ ID NO. 60 (a CLL1-CD33 cCAR polypeptide and IL-15/IL-15sushi); SEQ ID NO.40 (a BCMA-CD38 cCAR polypeptide, 4- 1BBL and IL-15/IL-15sushi; SEQ ID NO. 36 (CD123- CLL1 cCAR polypeptide, and IL-15/IL-15sushi); SEQ ID NO. 28 (a CD20-CD19 cCAR polypeptide, and IL-15/IL-15sushi; or SEQ ID NO. 50 (CD269-A7D- IL-15/IL-15sushi-2G), and wherein said enhancer is separated from the first CAR and second CAR by a nucleotide encoding a second viral self-cleavage peptide that flanks either end of the two distinct CAR units.

2. The engineered cell according to claim 1, wherein the enhancer is secreted by the engineered cell.

3. The engineered cell according to claim 1 or 2, wherein the engineered cell is an NK T cell, T cell, or NK cell.

4. The engineered cell according to any one of claims 1-3, wherein the engineered cell comprises at least two enhancers.

5. Use of an engineered cell according to claim 3 in the manufacture of a medicament for treating a cell proliferative disease, wherein the medicament is to be administered to a patient in need thereof, and wherein the cell proliferative disease comprises a T-cell malignancy, leukemia, or a lymphoma.

6. The use according to claim 5, wherein the cell proliferative disease comprises a soft tissue tumor.

7. The use according to claim 5 or 6, wherein the soft tissue tumor is selected from the group consisting of brain tumor, lung cancer, or osteosarcoma.