

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 engineered cell comprising: (i.) a first chimeric antigen receptor polypeptide comprising 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.) a second chimeric antigen receptor polypeptide comprising 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 first antigen recognition domain and the second antigen recognition domain each have a single antigen recognition domain; and wherein the engineered 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 cell includes SEQ ID NO. 42 (a BCMA-CS 1 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. 18 (a CD5-CD38 chimeric antigen receptor polypeptide); 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); SEQ ID NO. 52 (a CD20-CD19 cCAR polypeptide); SEQ ID NO. 1 (IL-21 anchor polypeptide); or SEQ ID NO. 50 (super2 polypeptide).

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

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.

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, and the first antigen recognition domain and second antigen rejection domain are selected from the group consisting of interleukin 6 receptor, NY-ESO-1, alpha fetoprotein (AFP), glypican-3 (GPC3), BAFF-R, BAFF, APRIL, BCMA, TACI, LeY, CD5, CD4, CD3, CD2, CD52, GD2, CD13, CD14, CD15 CD19, CD20, CD22, CD33, CD30, CD41, CD45, CD61, CD64, CD68, CD117, CD123, CD138, CD267, CD269, CD38, Flt3 receptor, CD4, CLL-1 and CS1(SLAMF7).

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

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 having multiple chimeric antigen receptor polypeptides, each chimeric antigen receptor polypeptide is 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 GD2, GD3, , ROR1, PSMA, PSCA (prostate stem cell antigen), MAGE A3, Glycolipid, glypican 3, F77, GD-2, WT1, CEA, HER-2/neu, MAGE-3, MAGE-4, MAGE-5, MAGE- 6, alpha-fetoprotein, CA 19-9, CA 72-4, NY-ESO, FAP, ErbB, c-Met, MART-1, MUC1, MUC2, MUC3, MUC4, MUC5, CD30, EGFRvIII, CD33, CD123, CLL-1, immunoglobulin kappa and lambda, CD38, CD52, CD19, CD20, CD22, CD38, BCMA, CS1, NKG2D receptor, April receptor, BAFF receptor, TACI, CD3, CD4, CD8, CD5, CD7, CD2, and CD138. The target antigens can also include viral or fungal antigens, such as E6 and E7 from the human papillomavirus (HPV) or EBV (Epstein Barr virus) antigens.

In another example, the present disclosure provides methods for treating B-cell lymphoma, T-cell lymphoma, multiple myeloma, chronic myeloid leukemia, acute myeloma leukemia, myelodysplastic syndromes, chronic myeloproliferative neoplasms, B-cell acute

CLAIMS

1. An engineered cell comprising:

(i.) a first chimeric antigen receptor polypeptide comprising 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.) a second chimeric antigen receptor polypeptide comprising 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 first antigen recognition domain and the second antigen recognition domain each have a single antigen recognition domain; and

wherein the engineered 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 cell includes SEQ ID NO. 42 (a BCMA-CS 1 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. 18 (a CD5-CD38 chimeric antigen receptor polypeptide); 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); SEQ ID NO. 52 (a CD20-CD19 cCAR polypeptide); SEQ ID NO. 1 (IL-21 anchor polypeptide); or SEQ ID NO. 50 (super2 polypeptide).

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.