

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.

In another aspect, the present disclosure refers to an engineered cell comprising: (i.) a chimeric antigen receptor polypeptide comprising an antigen recognition domain selected from the group consisting of CD3, CD4, CD5, CD19, CD20, CD33, CD123, BCMA, GD2, and GD3; a signal peptide; a hinge region; a transmembrane domain; a co-stimulatory domain; and a signaling 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.

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.

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

lymphoblastic leukemia (B-ALL), blastic plasmacytoid dendritic cell neoplasm, lung cancer, liver cancer, brain cancer, osteosarcoma, breast cancer, prostate cancer and cell proliferative diseases by administering any of the engineered cells described above to a patient in need thereof.

In another example, the present disclosure provides a method of treating an autoimmune disease, said method including administering an engineered cell as described herein to a patient in need thereof; wherein said autoimmune disease comprises systemic lupus erythematosus (SLE), multiple sclerosis (MS), Inflammatory bowel disease (IBD), Rheumatoid arthritis, Sjögren syndrome, dermatomyositis, autoimmune hemolytic anemia, Neuromyelitis optica (NMO), NMO Spectrum Disorder (NMOSD), idiopathic thrombocytopenic purpura (ITP), antineutrophil cytoplasmic autoantibodies (ANCAs) associated with systemic autoimmune small vessel vasculitis syndromes or microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA, Wegener's granulomatosis), or eosinophilic granulomatosis with polyangiitis (EGPA, Churg-Strauss syndrome) and TTP (thrombotic thrombocytopenic purpura)

The present disclosure provides chimeric antigen receptors (CARs) targeting non-hematologic malignancies, compositions and methods of use thereof.

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 (s). In a further example, an enhancer can be selected from at least one of the group including, but not limited, IL-2, IL-7, IL-12, IL-15, IL-15/IL-15sush, IL-15/IL-15sushi anchor, IL-15/IL-15RA, IL-18, IL-21, IL-21 anchor, PD-1, PD-L1, CSF1R, CTAL-4, TIM-3, cytoplasmic domain of IL-15 receptor alpha, 4-1BBL, IL-21, IL-21 anchor and TGFR beta, receptors.

In some examples, CAR having an antigen recognition domain (s) is part of an expression cassette. In a preferred example, the expressing gene or the cassette may include an

accessory gene or a tag or a part thereof. The accessory gene may be an inducible suicide gene or a part thereof, including, but not limited to, caspase 9 gene. The “suicide gene” ablation approach improves safety of the gene therapy and kills cells only when activated by a specific compound or a molecule. In some examples, the epitope tag is a c-myc tag, CD52, streptavidin-binding peptide (SBP), truncated EGFR gene (EGFRt) or a part or a combination thereof.

In some examples, CAR cells can be ablated by administering an anti-CD52 monoclonal antibody (CAMPATH) to a subject.

In another example, the present disclosure provides methods for treating soft tissue tumors, carcinoma, sarcomas, leukemia, and cell proliferative diseases by administering any of the engineered cells described above to a patient in need thereof.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: CAR construction and expression

(A) Two discrete CAR units: an anti-BCMA CAR comprised of: a CD8-derived hinge (H) and transmembrane (TM) regions, and 4-1BB co-activation domains linked to the CD3 ζ signaling domain is fused to a complete anti-CS1 CAR by a self-cleaving P2A peptide. A strong spleen focus forming virus promoter (SFFV) and a CD8 leader sequence were used for efficient expression of the BC1cCAR (BCMA-CS1 cCAR) molecule on the T-cell surface. (B) Expression of BC1cCAR was measured by FACS against control T-cells. BCMA also called CD269.

Figure 2: In vitro evaluation of BC1cCAR T-cells against myeloma cell lines

(A) BC1cCAR and control T-cells cultured with MM1S and RPMI-8226 cells for 24 hours at E:T ratios of 2:1 and 5:1. Target cells were stained by Cytotracker dye (CMTMR) to distinguish them from effector T-cells, and are indicated in red. Populations were gated by BCMA, CS1, and CMTMR. (B) BC1cCAR and control T-cells were incubated with U266 (BCMA⁺CS1^{dim}) cells under similar conditions. (C) Graphical summary of BC1cCAR T-cell in vitro cytotoxicity against various myeloma cell lines.

Figure 3: Primary patient cell phenotypes

Primary cells were assayed by FACS for BCMA and CS1 expression. Density plots represent major antigen populations.

Figure 4: Characterization of BC1cCAR T-cell anti-tumor activity against primary myeloma tumor cells

(A) Co-cultures against BCMA⁺CS1⁺ primary myeloma cells (MM7-G) were carried out over 24 hours and target cells pre-stained with CMTMR. Populations were gated by BCMA and CS1, along with CMTMR, and flow cytometry plots show target tumor populations in red (left). Bar graph summarizing in vitro cytotoxicity (right). (B) Co-cultures with MM10-G primary cells were conducted under similar conditions. BCMA⁺CS1⁺ double positive populations (purple) and CS1⁺ only populations (dark blue) by FACS. Specific cytotoxicity summarized (below). (C) BCMA^{dim}CS1^{dim} primary cells (MM11-G) show BC1cCAR anti-tumor activity over a range of E:T dosages. (D) Summary panel graph showing results of BC1cCAR in vitro screening.

Figure 5: Functional validation of BC1cCAR antigen specificity

(A) A CML cell line (K562) was transduced to stably express either BCMA or CS1. Histogram population shifts in their respective antigen expression ranges show expression. (B) Short term (4 hour – 8 hour) cultures of BC1cCAR T-cells against either BCMA-K562 or CS1-K562 show antigen specific cytotoxicity correlating with E:T dosage increase. Wild-type K562 cells were

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.
2. The engineered cell according to claim 1, wherein the engineered cell includes SEQ ID NO. 42 (a BCMA-CS1 cCAR polypeptide and IL-15/IL-15sushi); SEQ ID NO. 34 (a CD123-CD33 cCAR polypeptide and IL-15/IL-15sushi); 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. 42 (a BCMA-CS1 cCAR polypeptide and IL-15/IL-15sushi); SEQ ID NO. 34 (a CD123-CD33 cCAR polypeptide, 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); SEQ ID NO. 52 (a CD20-CD19 cCAR polypeptide); SEQ ID NO. 1 (IL-21 anchor polypeptide); or SEQ ID NO. 50 (super2 polypeptide).
3. The engineered cell according to any one of claims 1-2, wherein the enhancer is secreted by the engineered cell.
4. The engineered cell according to any one of claims 1-3, wherein the engineered cell is an NK T cell, T cell, or NK cell.

5. The engineered cell according to any one of claims 1-4, wherein the engineered cell comprises at least two enhancers.
6. An engineered cell comprising:
 - (i.) a chimeric antigen receptor polypeptide comprising an antigen recognition domain selected from the group consisting of CD3, CD4, CD5, CD19, CD20, CD33, CD123, BCMA, GD2, and GD3; a signal peptide; a hinge region; a transmembrane domain; a co-stimulatory domain; and a signaling 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.
7. The engineered cell according to claim 6, wherein the engineered cell includes SEQ ID NO. 56 (a GD2 chimeric antigen receptor polypeptide); SEQ ID NO. 58 (a GD2 chimeric antigen receptor polypeptide, 4-1BBL ligand and IL-15/IL-15sushi); SEQ ID NO. 49 (a CD5 chimeric antigen receptor polypeptide and IL-15/IL-15sushi); SEQ ID NO. 22 (a CD4 chimeric antigen receptor polypeptide and IL-15/IL-15sushi); SEQ ID NO. 20 (a CD4 chimeric antigen receptor polypeptide, 4-1BBL and IL-15/IL-15sushi); SEQ ID NO. 18 (a CD3 chimeric antigen receptor polypeptide, 4-1BBL and IL-15/IL-15sushi); SEQ ID NO. 24 (a CD19 chimeric antigen receptor polypeptide and IL-15/IL-15sushi); SEQ ID NO. 26 (a CD19 chimeric antigen receptor polypeptide, 4-1BBL and IL-15/IL-15sushi); SEQ ID NO. 30 (a CD33 chimeric antigen receptor polypeptide, 4-1BBL and IL-15/IL-15sushi); SEQ ID NO. 32 (a CD123 chimeric antigen receptor polypeptide, 4-1BBL and IL-15/IL-15sushi); SEQ ID NO. 38 (a BCMA chimeric antigen receptor polypeptide, 4-1BBL and IL-15/IL-15sushi); SEQ ID NO. 46 (a GD2 chimeric antigen receptor polypeptide, 4-1BBL and IL-15/IL-15sushi); SEQ ID NO. 56 (a GD2 chimeric antigen receptor polypeptide); SEQ ID NO. 32 (a CD123b chimeric antigen receptor polypeptide and 4-1BBL ligand, and IL-15/IL-15sushi); SEQ ID NO. 54 (a CD45 chimeric antigen receptor polypeptide and IL-15/IL-15sushi); SEQ ID NO. 44 (a CLL-1 CAR polypeptide, 4-1BBL and IL-15/IL-15sushi); SEQ ID NO. 40 (a BCMA-CD38a chimeric antigen receptor polypeptides and 4-1BBL ligand, and IL-15/IL-15sushi); SEQ ID NO. 21 (a CD33 CAR polypeptide, 4-1BBL and IL-15/IL-15sushi; or SEQ ID NO. 50 (super2 polypeptide).
8. The engineered cell according to any one of claims 6-7, wherein the enhancer is secreted by the engineered cell.

9. The engineered cell according to any one of claims 6-8, wherein the engineered cell is an NK T cell, T-cell, or NK cell.
10. The engineered cell according to any one of claims 6-9, wherein the engineered cell comprises at least two enhancers.
11. Use of an engineered cell according to any one of claims 1-5 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.
12. The use according to claim 11, wherein the cell proliferative disease comprises a t-cell malignancy, leukemia, or a lymphoma.
13. The use according to any one of claims 11-12, wherein the engineered cell comprises NK T cell, T-cell, or NK cell.
14. Use of an engineered cell according to any one of claims 6-10 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.
15. The use according to claim 14, wherein the cell proliferative disease comprises a t-cell malignancy, leukemia, or a lymphoma.
16. The use according to any one of claims 14-15, wherein the engineered cell comprises NK T cell, T-cell, or NK cell.
17. The use according to any one of claims 14-16, wherein the engineered cell comprises SEQ ID NO. 56 (a GD2 chimeric antigen receptor polypeptide); SEQ ID NO. 58 (a GD2 chimeric antigen receptor polypeptide, 4-1BBL ligand and IL-15/IL-15sushi); or SEQ ID NO. 46 (a GD2 chimeric antigen receptor polypeptide, 4-1BBL and IL-15/IL-15sushi).
18. The use according to any one of claims 14-17, wherein the cell proliferative disease comprises a soft tissue tumor.
19. The use according to any one of claims 14-18, wherein the soft tissue tumor is selected from the group consisting of brain tumor, lung cancer, or osteosarcoma.
20. The use according to any one of claims 14-19, wherein the engineered cell comprises NK T cell, T-cell, or NK cell.