

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 example, the present disclosure provides an engineered cell including a chimeric antigen receptor polypeptide comprising a signal peptide, antigen recognition domain, a hinge region, a transmembrane domain, a signaling domain, and a co-stimulatory domain; wherein said antigen recognition domain comprises one of FcER1A, FcER1, Ig E, CD19, BCMA, or CD45.

In another example, the present disclosure provides an engineered polypeptide including a chimeric antigen receptor polypeptide including a signal peptide, antigen recognition domain, a hinge region, a transmembrane domain, a signaling domain, and a co-stimulatory domain; wherein said antigen recognition domain includes one of FcER1A, CD19, BCMA, or CD45; and at least one enhancer; wherein a high efficiency cleavage site is disposed between the chimeric antigen receptor polypeptide and enhancer.

In another example, the present disclosure provides an engineered cell including a first chimeric antigen receptor polypeptide including a first signal peptide, first antigen recognition domain, a first hinge region, a first transmembrane domain, a first signaling domain, and a first co-stimulatory domain; and a second chimeric antigen receptor polypeptide including a second signal peptide, second antigen recognition domain, a second hinge region, a second transmembrane domain, a second signaling domain, and a second co-stimulatory domain; wherein the first antigen recognition domain and second antigen recognition domain are different; and the first antigen recognition domain and second antigen rejection domain are selected from the group consisting of CD4, CD19, CD33, CD123, CLL-1, BAFFR, BCMA, and CS-1.

In another example, the present disclosure provides an engineered polypeptide including a first chimeric antigen receptor polypeptide including a first signal peptide, first antigen recognition domain, a first hinge region, a first transmembrane domain, a first signaling domain, and a first co-stimulatory domain; and a second chimeric antigen receptor polypeptide including a second signal peptide, second antigen recognition domain, a second hinge region, a second transmembrane domain, a second signaling domain, and a second co-stimulatory domain; wherein the first antigen recognition domain and the second antigen recognition domain are

different; and the first antigen recognition domain and second antigen rejection domain are selected from the group consisting of CD4, CD19, CD33, CD123, BAFFR, CLL-1, BCMA, and CS-1.

In one aspect, the present disclosure provides the use of an engineered cell in the manufacture of a medicament for treating asthma; wherein the engineered cell comprises: a chimeric antigen receptor polypeptide comprising a signal peptide, antigen recognition domain, a hinge region, a transmembrane domain, a signaling domain, and a co-stimulatory domain; and wherein the chimeric antigen receptor comprises FcER1A.

In another aspect, the present disclosure provides the use of an engineered cell in the manufacture of a medicament for treating an autoimmune disease; 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 (ANCA)s 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); wherein the engineered cell comprises: a chimeric antigen receptor polypeptide comprising a signal peptide, antigen recognition domain, a hinge region, a transmembrane domain, a signaling domain, and a co-stimulatory domain; and wherein said antigen recognition domain comprises FcER1A.

In an example, the present disclosure provides a method of treating an autoimmune disease, said method including administering an engineered cell described above 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 (ANCA)s 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).

In another aspect, the present disclosure provides the use of an engineered polynucleotide in the manufacture of a medicament for treating an autoimmune disease; 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 (ANCA) 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); wherein the engineered polynucleotide comprises a chimeric antigen receptor polypeptide comprising a signal peptide, antigen recognition domain, a hinge region, a transmembrane domain, a signaling domain, and a co-stimulatory domain; and wherein said antigen recognition domain comprises FcER1A; and at least one enhancer; wherein a high efficiency cleavage site is disposed between the chimeric antigen receptor polypeptide and enhancer.

In yet another aspect, the present disclosure provides the use of an effective amount of engineered cells in the manufacture of a medicament for treating asthma in a patient; wherein the engineered cells comprise: a chimeric antigen receptor polypeptide comprising a signal peptide, antigen recognition domain, a hinge region, a transmembrane domain, a signaling domain, and a co-stimulatory domain; and wherein said antigen recognition domain comprises FcER1A. In yet another aspect, the present disclosure provides the use of engineered polynucleotides in the manufacture of a medicament for preventing or mediating organ rejection in a patient; wherein the engineered polynucleotide comprises a chimeric antigen receptor polypeptide comprising a signal peptide, antigen recognition domain, a hinge region, a transmembrane domain, a signaling domain, and a co-stimulatory domain; and wherein said antigen recognition domain comprises FcER1A; and at least one enhancer; wherein a high efficiency cleavage site is disposed between the chimeric antigen receptor polypeptide and enhancer. In another example, the present disclosure provides a method of treating asthma. The method includes administering an engineered cell described above.

In another example, the present disclosure provides a method of treating organ rejection. The method includes administering an engineered cell described above.

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.

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

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 embodiment, the present disclosure provides a polynucleotide encoding for the aforementioned polypeptide.

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 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 interleukin 6 receptor, ROR1, PSMA, PSCA, MAGE A3, Glycolipid, glypican 3, F77, GD-2, WT1, CEA, HER-2/neu, IL13R α 2, Met, mesothelin, EGFR, EGFRvIII, MUC16, NKG2D ligands, thyroglobulin, 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, CD45, BCMA, CS1, BAFF receptor, TACI, CD3, CD4, CD8, CD5, CD7, CD2, CD45, CD70 and CD138.

In another example, the present disclosure provides methods for treating B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma, multiple myeloma, acute myeloid leukemia, chronic myeloid leukemia, chronic myeloproliferative neoplasms, myelodysplastic syndromes, granulocytic sarcoma, histiocytic sarcoma, B-cell acute lymphoblastic leukemia (B-ALL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), chronic myelomonocytic leukemia, and cell proliferative diseases by administering any of the engineered cells described above to a patient in need thereof.

In some examples, the disclosed disclosure comprises methods and compositions of controlling the proliferation of T cells, for instance, CAR T cells or therapeutic T cells using CAMPATH. The methods further relate to compositions and methods for ablating CAR T cells using CAMPATH after tumor depletion or in emergency cases, for example, unexpected side effects caused by CAR Therapy. In further examples, CD52 is incorporated into CD5 CAR engineered cell or any CAR engineered cell and can be used as a "safety switch" for CAR therapy.

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

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 Fluorescent Protein (GFP) is included as negative control.

5 **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) tested on 293FT HEK (human embryonic kidney) cells to gauge maximum transduction efficiency before usage on UCB (umbilical cord blood) and PB (peripheral blood) T-cells. Lower panel shows CD33CD123 cCAR (also referred to as CD33CD123-2G-CAR) T-cells

CLAIMS

1. Use of an engineered cell in the manufacture of a medicament for treating asthma;

wherein the engineered cell comprises: a chimeric antigen receptor polypeptide comprising a signal peptide, antigen recognition domain, a hinge region, a transmembrane domain, a signaling domain, and a co-stimulatory domain; and

wherein said antigen recognition domain comprises FcER1A.

2. Use of an engineered cell in the manufacture of a medicament for treating an autoimmune disease; 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);

wherein the engineered cell comprises: a chimeric antigen receptor polypeptide comprising a signal peptide, antigen recognition domain, a hinge region, a transmembrane domain, a signaling domain, and a co-stimulatory domain; and

wherein said antigen recognition domain comprises FcER1A.

3. Use of an engineered polynucleotide in the manufacture of a medicament for treating an autoimmune disease; 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 (ANCA) 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);

wherein the engineered polynucleotide comprises a chimeric antigen receptor polypeptide comprising a signal peptide, antigen recognition domain, a hinge region, a transmembrane domain, a signaling domain, and a co-stimulatory domain; and

wherein said antigen recognition domain comprises FcER1A; and at least one enhancer; wherein a high efficiency cleavage site is disposed between the chimeric antigen receptor polypeptide and enhancer.

4. Use of an effective amount of engineered cells in the manufacture of a medicament for treating asthma in a patient;

wherein the engineered cells comprise: a chimeric antigen receptor polypeptide comprising a signal peptide, antigen recognition domain, a hinge region, a transmembrane domain, a signaling domain, and a co-stimulatory domain; and

wherein said antigen recognition domain comprises FcER1A.

5. Use of an effective amount of engineered polynucleotides in the manufacture of a medicament for preventing or mediating organ rejection in a patient;

wherein the engineered polynucleotide comprises a chimeric antigen receptor polypeptide comprising a signal peptide, antigen recognition domain, a hinge region, a transmembrane domain, a signaling domain, and a co-stimulatory domain; and

wherein said antigen recognition domain comprises FcER1A; and at least one enhancer;

- 5 wherein a high efficiency cleavage site is disposed between the chimeric antigen receptor polypeptide and enhancer.