Claims

1. Use of a population of pluripotent hematopoietic cells comprising a nucleic acid cassette that encodes an autoantigen-binding protein in the manufacture of a medicament for treating or preventing an autoimmune disease in a patient in need thereof, wherein the nucleic acid cassette is operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ regulatory T (Treg) cells.

2. Use of a population of pluripotent hematopoietic cells comprising a nucleic acid cassette that encodes an autoantigen-binding protein in the manufacture of a medicament for suppressing activity and/or proliferation of a population of autoreactive effector immune cells in a patient diagnosed as having an autoimmune disease, wherein the nucleic acid cassette is operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells.

3. Use of a population of pluripotent hematopoietic cells comprising a nucleic acid cassette that encodes an autoantigen-binding protein in the manufacture of a medicament for inducing apoptosis of an autoreactive effector immune cell in a patient diagnosed as having an autoimmune disease, wherein the nucleic acid cassette is operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells.

4. Use of a population of pluripotent hematopoietic cells comprising a nucleic acid cassette that encodes an autoantigen-binding protein in the manufacture of a medicament for protecting endogenous tissue from an autoimmune response in a patient diagnosed as having an autoimmune disease, wherein the nucleic acid cassette is operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells.

5. Use of a population of pluripotent hematopoietic cells comprising a nucleic acid cassette that encodes an autoantigen-binding protein in the manufacture of a medicament for reducing inflammation in a patient diagnosed as having an autoimmune disease, wherein the nucleic acid cassette is operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells.

6. The use of any one of claims 1-5, wherein:

(i) the pluripotent hematopoietic cells are hematopoietic stem cells (HSCs) or hematopoietic progenitor cells (HPCs);

(ii) the pluripotent hematopoietic cells are embryonic stem cells;

(iii) the pluripotent hematopoietic cells are induced pluripotent stem cells;

(iv) the pluripotent hematopoietic cells are lymphoid progenitor cells;

(v) the pluripotent hematopoietic cells are CD34+ cells;

(vi) the population of pluripotent hematopoietic cells is to be administered systemically to the patient;

(vii) the pluripotent hematopoietic cells are autologous with respect to the patient;

(viii) the pluripotent hematopoietic cells are allogeneic with respect to the patient;

(ix) the pluripotent hematopoietic cells are transduced ex vivo with a viral vector comprising the nucleic acid cassette that encodes the autoantigen-binding protein;

(x) the pluripotent hematopoietic cells are transfected ex vivo with a polynucleotide comprising the nucleic acid cassette that encodes the autoantigen-binding protein; or

(xi) the pluripotent hematopoietic cells are obtained by delivering to the cells a nuclease that catalyzes a single-strand break or a double-strand break at a target position within the genome of the cell.

7. The use of claim 6, wherein:

(i) the population of pluripotent hematopoietic cells is to be administered to the patient by way of intravenous injection;

(ii) the pluripotent hematopoietic cells are HLA-matched to the patient;

(iii) the nuclease is delivered to the cells in combination with a guide RNA (gRNA) that hybridizes to the target position within the genome of the cell;

(iv) the nuclease is a clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein; or

(v) while the cells are contacted with the nuclease, the cells are additionally contacted with a template polynucleotide comprising the nucleic acid cassette that encodes the autoantigen-binding protein.

8. The use of claim 7, wherein:

(i) the CRISPR-associated protein is CRISPR-associated protein 9 (Cas9) or CRISPR-associated protein 12a (Cas12a);

(ii) the template polynucleotide comprises a 5’ homology arm and a 3’ homology arm having nucleic acid sequences that are sufficiently similar to the nucleic acid sequences located 5’ to the target position and 3’ to the target position, respectively, to promote homologous recombination; or

(iii) the nuclease, gRNA, and/or template polynucleotide are delivered to the cells by contacting the cells with a viral vector that encodes the nuclease, gRNA, and/or template polynucleotide.

9. The use of any one of claims 1-8, wherein:

(i) the one or more lineage-specific transcription regulatory elements comprise a Foxp3 promoter;

(ii) the one or more lineage-specific transcription regulatory elements comprise a CNS1 enhancer;

(iii) the one or more lineage-specific transcription regulatory elements comprise a CNS2 enhancer;

(iv) the one or more lineage-specific transcription regulatory elements comprise a CNS3 enhancer;

(v) the one or more lineage-specific transcription regulatory elements comprise a CNS0 enhancer;

(vi) the autoantigen-binding protein is a single-chain polypeptide;

(vii) the autoantigen-binding protein is a chimeric antigen receptor (CAR);

(viii) the autoantigen-binding protein is a multi-chain protein;

(ix) upon administration of the population of pluripotent hematopoietic cells to the patient, the administered cells, or progeny thereof, differentiate into CD4+CD25+ Treg cells; or

(x) the patient is a mammal and the cells are mammalian cells.

10. The use of claim 9, wherein:

(i) the Foxp3 promoter has the nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4, or a nucleic acid sequence that is at least 85% identical thereto;

(ii) the Foxp3 promoter specifically binds transcription factor Nr4a and/or Foxo;

(iii) the CNS1 enhancer has the nucleic acid sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8, or a nucleic acid sequence that is at least 85% identical thereto;

(iv) the CNS1 enhancer specifically binds transcription factor AP-1, NFAT, Smad3, and/or Foxo;

(v) the CNS2 enhancer has the nucleic acid sequence of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12, or a nucleic acid sequence that is at least 85% identical thereto;

(vi) the CNS2 enhancer specifically binds transcription factor Runx, Foxp3, Ets-1, CREB, Stat5, NFAT, and/or c-Rel;

(vii) the CNS3 enhancer has the nucleic acid sequence of SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, or SEQ ID NO: 16, or a nucleic acid sequence that is at least 85% identical thereto;

(viii) the CNS3 enhancer specifically binds transcription factor Foxo and/or c-Rel;

(ix) the CNS0 enhancer has the nucleic acid sequence of SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, or a nucleic acid sequence that is at least 85% identical thereto;

(x) the CNS0 enhancer specifically binds transcription factor Satb1 and/or Stat5;

(xi) the autoantigen-binding protein is a full-length antibody, a dual-variable immunoglobulin domain, a diabody, a triabody, an antibody-like protein scaffold, a Fab fragment, or a $F(ab')_2$ molecule; or

(xii) the mammal is a human and the cells are human cells.

11. The use of any one of claims 1-10, wherein the autoimmune disease is type 1 diabetes, Alopecia Areata, Ankylosing Spondylitis, Antiphospholipid Syndrome, Autoimmune Addison's Disease, Autoimmune Hemolytic Anemia, Autoimmune Hepatitis, Behcet's Disease, Bullous Pemphigoid, Cardiomyopathy, Celiac Sprue-Dermatitis, Chronic Fatigue Immune Dysfunction Syndrome (CFIDS), Chronic Inflammatory Demyelinating Polyneuropathy, Churg-Strauss Syndrome, Cicatricial Pemphigoid, CREST Syndrome, Cold Agglutinin Disease, Crohn's Disease, Essential Mixed Cryoglobulinemia, Fibromyalgia-Fibromyositis, Graves' Disease, Guillain-Barré, Hashimoto's Thyroiditis, Hypothyroidism, Idiopathic Pulmonary Fibrosis, Idiopathic Thrombocytopenia Purpura (ITP), IgA Nephropathy, Juvenile Arthritis, Lichen Planus, Lupus, Ménière's Disease, Mixed Connective Tissue Disease, Multiple Sclerosis, Myasthenia Gravis,

Neuromyelitis Optica, Pemphigus Vulgaris, Pernicious Anemia, Polyarteritis Nodosa, Polychondritis, Polyglandular Syndromes, Polymyalgia Rheumatica, Polymyositis and Dermatomyositis, Primary Agammaglobulinemia, Primary Biliary Cirrhosis, Psoriasis, Raynaud's Phenomenon, Reiter's Syndrome, Rheumatic Fever, Rheumatoid Arthritis, Sarcoidosis, Scleroderma, Sjögren's Syndrome, Stiff-Man Syndrome, Takayasu Arteritis, Temporal Arteritis/Giant Cell Arteritis, Ulcerative Colitis, Uveitis, Vasculitis, Vitiligo, or Wegener's Granulomatosis.

12. The use of any one of claims 1-11, wherein the autoantigen is myelin oligodendrocyte glycoprotein, aquaporin 4, actin, tubulin, myosin, tropomyosin, vimentin, fibronectin, collagen I, collagen II, collagen III, collagen IV, collagen V, heparin, laminin, collagenase, cardiolipin, glucocerebroside, phosphatidylethanolamine, cholesterol, enolase, aldolase, acid phosphatase, annexin 33 kDa, annexin 67 kDa, cytochrome P450C, catalase, peroxidase, tyrosinase, ribonuclease, histone II A, double-stranded DNA, single-stranded DNA, transferrin, fetuin, factor II, factor VII, fibrin, fibrinogen, C1, C1q, interleukin 2, interleukin 10, interleukin 4, interferon-γ, TNFαR, HSP60, HSP65, GAD, insulin, IA-2, ZnT8, MBP, AchR, myoglobulin, thyroglobulin, hemoglobin A, spectrin, TB PPD, LPS, MuSK, LRP4, the Fc portion of immunoglobin, citrullinated peptides, carbamylated peptides, the thyrotrophin receptor, or a protein expressed in the thyroid gland.

13. The use of claim 11, wherein:

(i) the autoimmune disease is multiple sclerosis and the autoantigen is myelin oligodendrocyte glycoprotein;

(ii) the autoimmune disease is type 1 diabetes and the autoantigen is insulin, GAD-65, IA-2, or ZnT8;

(iii) the autoimmune disease is rheumatoid arthritis and the autoantigen is collagen II, the Fc portion of immunoglobin, citrullinated peptides, carbamylated peptides, or HSP65;

(iv) the autoimmune disease is myasthenia gravis and the autoantigen is AChR, MuSK, or LRP4;

(v) the autoimmune disease is lupus and the autoantigen is histone II A;

(vi) the autoimmune disease is hypothyroidism and the autoantigen is a protein expressed in the thyroid gland;

(vii) the autoimmune disease is Graves’ disease and the autoantigen is the thyrotrophin receptor;

(viii) the autoimmune disease is pemphigus vulgaris and the autoantigen is double-stranded DNA;

(ix) the autoimmune disease is psoriasis and the autoantigen is double-stranded DNA; or

(x) the autoimmune disease is neuromyelitis optica and the autoantigen is aquaporin 4.

14. A pharmaceutical composition comprising (i) a population of pluripotent hematopoietic cells comprising a nucleic acid cassette that encodes an autoantigen-binding protein, wherein the nucleic acid cassette is operably linked to one or more lineage-specific transcription regulatory elements that are

active in CD4+CD25+ Treg cells, and (ii) one or more pharmaceutically acceptable excipients, carriers, or diluents.

15. The pharmaceutical composition of claim 14, wherein:

(i) the pluripotent hematopoietic cells are HSCs or HPCs;

(ii) the pluripotent hematopoietic cells are embryonic stem cells;

(iii) the pluripotent hematopoietic cells are induced pluripotent stem cells;

(iv) the pluripotent hematopoietic cells are lymphoid progenitor cells;

(v) the pluripotent hematopoietic cells are CD34+ cells;

(vi) the pluripotent hematopoietic cells are transduced ex vivo with a viral vector comprising the nucleic acid cassette that encodes the autoantigen-binding protein;

(vii) the pluripotent hematopoietic cells are transfected ex vivo with a polynucleotide comprising the nucleic acid cassette that encodes the autoantigen-binding protein;

(viii) the pluripotent hematopoietic cells are obtained by delivering to the cells a nuclease that catalyzes a single-strand break or a double-strand break at a target position within the genome of the cell;

(ix) the one or more lineage-specific transcription regulatory elements comprise a Foxp3 promoter;

(x) the one or more lineage-specific transcription regulatory elements comprise a CNS1 enhancer;

(xi) the one or more lineage-specific transcription regulatory elements comprise a CNS2 enhancer;

(xii) the one or more lineage-specific transcription regulatory elements comprise a CNS3 enhancer;

(xiii) the one or more lineage-specific transcription regulatory elements comprise a CNS0 enhancer;

(xiv) the autoantigen-binding protein is a single-chain polypeptide;

(xv) the autoantigen-binding protein is a CAR;

(xvi) the autoantigen-binding protein is a multi-chain protein; or

(xvii) the autoantigen is myelin oligodendrocyte glycoprotein, actin, tubulin, myosin, tropomyosin, vimentin, fibronectin, collagen I, collagen II, collagen III, collagen IV, collagen V, heparin, laminin, collagenase, cardiolipin, glucocerebroside, phosphatidylethanolamine, cholesterol, enolase, aldolase, acid phosphatase, annexin 33 kDa, annexin 67 kDa, cytochrome P450C, catalase, peroxidase, tyrosinase, ribonuclease, histone II A, double stranded DNA, single stranded DNA, transferrin, fetuin, factor II, factor VII, fibrin, fibrinogen, C1, C1q, interleukin 2, interleukin 10, interleukin 4, interferon-γ, TNFαR, HSP60, HSP65, GAD, insulin, IA-2, ZnT8, MBP, AchR, myoglobulin, thyroglobulin, hemoglobin A, spectrin, TB PPD, LPS, MuSK, LRP4, the Fc portion of immunoglobin, citrullinated peptides, carbamylated peptides, the thyrotrophin receptor, or a protein expressed in the thyroid gland.

16. The pharmaceutical composition of claim 15, wherein:

(i) the nuclease is delivered to the cells in combination with a gRNA that hybridizes to the target position within the genome of the cell;

(ii) the nuclease is a CRISPR-associated protein;

(iii) while the cells are contacted with the nuclease, the cells are additionally contacted with a template polynucleotide comprising the nucleic acid cassette that encodes the autoantigen-binding protein;

(iv) the Foxp3 promoter has the nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4, or a nucleic acid sequence that is at least 85% identical thereto;

(v) the Foxp3 promoter specifically binds transcription factor Nr4a and/or Foxo;

(vi) the CNS1 enhancer has the nucleic acid sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8, or a nucleic acid sequence that is at least 85% identical thereto;

(vii) the CNS1 enhancer specifically binds transcription factor AP-1, NFAT, Smad3, and/or Foxo;

(viii) the CNS2 enhancer has the nucleic acid sequence of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12, or a nucleic acid sequence that is at least 85% identical thereto;

(ix) the CNS2 enhancer specifically binds transcription factor Runx, Foxp3, Ets-1, CREB, Stat5, NFAT, and/or c-Rel;

(x) the CNS3 enhancer has the nucleic acid sequence of SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, or SEQ ID NO: 16, or a nucleic acid sequence that is at least 85% identical thereto;

(xi) the CNS3 enhancer specifically binds transcription factor Foxo and/or c-Rel;

(xii) the CNS0 enhancer has the nucleic acid sequence of SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, or a nucleic acid sequence that is at least 85% identical thereto;

(xiii) the CNS0 enhancer specifically binds transcription factor Satb1 and/or Stat5; or

(xiv) the autoantigen-binding protein is a full-length antibody, a dual-variable immunoglobulin domain, a diabody, a triabody, an antibody-like protein scaffold, a Fab fragment, or a $F(ab')_2$ molecule.

17. The pharmaceutical composition of claim 16, wherein:

(i) the CRISPR-associated protein is Cas9 or Cas12a;

(ii) the template polynucleotide comprises a 5' homology arm and a 3' homology arm having nucleic acid sequences that are sufficiently similar to the nucleic acid sequences located 5' to the target position and 3' to the target position, respectively, to promote homologous recombination; or

(iii) the nuclease, gRNA, and/or template polynucleotide are delivered to the cells by contacting the cells with a viral vector that encodes the nuclease, gRNA, and/or template polynucleotide.

18. A kit comprising the pharmaceutical composition of any one of claims 14-17, wherein the kit further comprises a package insert instructing a user of the kit to administer the pharmaceutical composition to a human patient having an autoimmune disease.

19. A nucleic acid cassette encoding an autoantigen-binding protein, wherein the nucleic acid cassette is operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells.

20. The nucleic acid cassette of claim 19, wherein:

(i) the one or more lineage-specific transcription regulatory elements comprise a Foxp3 promoter;

(ii) the one or more lineage-specific transcription regulatory elements comprise a CNS1 enhancer;

(iii) the one or more lineage-specific transcription regulatory elements comprise a CNS2 enhancer;

(iv) the one or more lineage-specific transcription regulatory elements comprise a CNS3 enhancer;

(v) the one or more lineage-specific transcription regulatory elements comprise a CNS0 enhancer;

(vi) the autoantigen-binding protein is a single-chain polypeptide;

(vii) the autoantigen-binding protein is a CAR;

(viii) the autoantigen-binding protein is a multi-chain protein; or

(ix) the autoantigen is myelin oligodendrocyte glycoprotein, actin, tubulin, myosin, tropomyosin, vimentin, fibronectin, collagen I, collagen II, collagen III, collagen IV, collagen V, heparin, laminin, collagenase, cardiolipin, glucocerebroside, phosphatidylethanolamine, cholesterol, enolase, aldolase, acid phosphatase, annexin 33 kDa, annexin 67 kDa, cytochrome P450C, catalase, peroxidase, tyrosinase, ribonuclease, histone II A, double stranded DNA, single stranded DNA, transferrin, fetuin, factor II, factor VII, fibrin, fibrinogen, C1, C1q, interleukin 2, interleukin 10, interleukin 4, interferon-γ, TNFαR, HSP60, HSP65, GAD, insulin, IA-2, ZnT8, MBP, AchR, myoglobulin, thyroglobulin, hemoglobin A, spectrin, TB PPD, LPS, MuSK, LRP4, the Fc portion of immunoglobin, citrullinated peptides, carbamylated peptides, the thyrotrophin receptor, or a protein expressed in the thyroid gland.

21. The nucleic acid cassette of claim 20, wherein:

(i) the Foxp3 promoter has the nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4, or a nucleic acid sequence that is at least 85% identical thereto;

(ii) the Foxp3 promoter specifically binds transcription factor Nr4a and/or Foxo;

(iii) the CNS1 enhancer has the nucleic acid sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8, or a nucleic acid sequence that is at least 85% identical thereto;

(iv) the CNS1 enhancer specifically binds transcription factor AP-1, NFAT, Smad3, and/or Foxo;

(v) the CNS2 enhancer has the nucleic acid sequence of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12, or a nucleic acid sequence that is at least 85% identical thereto;

(vi) the CNS2 enhancer specifically binds transcription factor Runx, Foxp3, Ets-1, CREB, Stat5, NFAT, and/or c-Rel;

(vii) the CNS3 enhancer has the nucleic acid sequence of SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, or SEQ ID NO: 16, or a nucleic acid sequence that is at least 85% identical thereto;

(viii) the CNS3 enhancer specifically binds transcription factor Foxo and/or c-Rel;

(ix) the CNS0 enhancer has the nucleic acid sequence of SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, or a nucleic acid sequence that is at least 85% identical thereto;

(x) the CNS0 enhancer specifically binds transcription factor Satb1 and/or Stat5; or

(xi) the autoantigen-binding protein is a full-length antibody, a dual-variable immunoglobulin domain, a diabody, a triabody, an antibody-like protein scaffold, a Fab fragment, or a $F(ab')_2$ molecule.

22. A viral vector comprising the nucleic acid cassette of any one of claims 19-21.