The large majority of patients having B cell malignancies, including non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM), are significant contributors to cancer mortality. The response of B cell malignancies to various forms of treatment is mixed. Traditional methods of treating B cell malignancies, including chemotherapy and radiotherapy, have limited utility due to toxic side effects. Immunotherapy with anti-CD19, anti-CD20, anti-CD22, anti-CD23, anti-CD52,anti-CD80, and anti-HLA-DR therapeutic antibodies have provided limited success, due in part to poor pharmacokinetic profiles, rapid elimination of antibodies by serum proteases and filtration at the glomerulus, and limited penetration into the tumor site and expression levels of the target antigen on cancer cells. Attempts to use genetically modified cells expressing chimeric antigen receptors (CARs) have also met with limited success. In addition, the therapeutic efficacy of a given antigen binding domain used in a CAR is unpredictable: if the antigen binding domain binds too strongly, the CAR T cells induce massive cytokine release resulting in a potentially fatal immune reaction deemed a "cytokine storm," and if the antigen binding domain binds too weakly, the CAR T cells do not display sufficient therapeutic efficacy in clearing cancer cells.

BRIEF SUMMARY

The disclosure generally provides improved vectors for generating T cell therapies and methods of using the same.

In various embodiments, a chimeric antigen receptor (CAR) is provided comprising: an extracellular domain that comprises a murine anti-BCMA (B cell maturation antigen) antibody or antigen binding fragment thereof that binds one or more epitopes of a human BCMA polypeptide; a transmembrane domain, one or more intracellular co-stimulatory signaling domains, and a primary signaling domain.

In particular embodiments, the murine anti-BCMA antibody or antigen binding fragment that binds the human BCMA polypeptide is selected from the group consisting of: a Camel Ig, Ig NAR, Fab fragments, Fab' fragments, F(ab)'2 fragments, F(ab)'3 fragments, Fv, single chain Fv antibody ("scFv"), bis-scFv, (scFv)2, minibody, diabody, triabody, tetrabody, disulfide stabilized Fv protein ("dsFv"), and single-domain antibody (sdAb, Nanobody).

In particular embodiments, the one or more co-stimulatory signaling domains are from a co-stimulatory molecule selected from the group consisting of: CD28, CD134, and CD137.

In additional embodiments, the one or more co-stimulatory signaling domains are from a co-stimulatory molecule selected from the group consisting of: CD28, CD134, and CD137.

In additional embodiments, the one or more co-stimulatory signaling domains is from CD28.

In particular embodiments, the one or more co-stimulatory signaling domains is from CD134.

In other embodiments, the one or more co-stimulatory signaling domains is from CD137.

In certain embodiments, a CAR comprises a hinge region polypeptide.

In further embodiments, the hinge region polypeptide comprises a hinge region of CD8α.

In some embodiments, a CAR comprises a spacer region.

In additional embodiments, the spacer region polypeptide comprises CH2 and CH3 regions of IgG1 or IgG4.

In particular embodiments, a CAR comprises a signal peptide.

In further embodiments, the signal peptide comprises an IgG1 heavy chain signal polypeptide, granulocyte-macrophage colony stimulating factor receptor 2 (GM-CSFR2) signal peptide, or a CD8α signal polypeptide.

In one embodiment, a CAR comprises an amino acid sequence as set forth in SEQ ID NO: 9.

In various embodiments, a polynucleotide encoding a CAR contemplated herein, is provided.

In one aspect, a polynucleotide encoding a CAR is provided, wherein the polynucleotide sequence is set forth in SEQ ID NO: 10.

In various certain embodiments, a vector comprising a polynucleotide encoding a CAR contemplated herein or as set forth in SEQ ID NO: 10 is provided.

In one aspect, there is provided a vector comprising the polynucleotide as contemplated herein.

In certain embodiments, the vector is an expression vector.

In some embodiments, the polyadenylation sequence is a bovine growth hormone polyadenylation or signal rabbit β-globin polyadenylation sequence.

In additional embodiments, a polynucleotide encoding a CAR contemplated herein comprises an optimized Kozak sequence.

In further embodiments, the promoter operably linked to the polynucleotide encoding a CAR contemplated herein is selected from the group consisting of: a cytomegalovirus immediate early gene promoter (CMV), an elongation factor 1 alpha promoter (EF1-α), a phosphoglycerate kinase-1 promoter (PGK), a ubiquitin-C promoter (UBQ-C), a cytomegalovirus enhancer/chicken beta-actin promoter (CAG), polyoma enhancer/herpes simplex thymidine kinase promoter (MC1), a beta actin promoter (β-ACT), a simian virus 40 promoter (SV40), and a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) promoter.

In various embodiments, an immune effector cell is provided comprising a vector contemplated herein. In various embodiments, the immune effector cell is transduced with a vector contemplated herein.

In one aspect, there is provided an *ex vivo* or *in vitro* immune effector cell comprising the vector as contemplated herein.

In further embodiments, the immune effector cell is selected from the group consisting of: a T lymphocyte and a natural killer (NK) cell.

In some embodiments, the immune effector cell is transduced with the vector of any one of claims 28 to 46 and is activated and stimulated in the presence of an inhibitor of the PI3K pathway, thereby maintaining proliferation of the transduced immune effector cells compared to the proliferation of transduced immune effector cells that were activated and stimulated in the absence of the inhibitor of the PI3K pathway.

In particular embodiments, the immune effector cell activated and stimulated in the presence of the inhibitor of PI3K pathway has increased expression of i) one or more markers selected from the group consisting of: CD62L, CD127, CD197, and CD38 or ii) all of the markers CD62L, CD127, CD197, and CD38 compared to an immune effector cell activated and stimulated in the absence of the inhibitor of PI3K pathway.

In one embodiment, the PI3K inhibitor is ZSTK474.

In various embodiments, a composition is provided comprising an immune effector cell contemplated herein and a physiologically acceptable excipient.

In one aspect, there is provided a composition comprising the *ex vivo* or *in vitro* immune effector cell as contemplated herein and a physiologically acceptable excipient.

In various embodiments, a method of generating an immune effector cell comprising a CAR contemplated herein is provided, comprising introducing into an immune effector cell a vector comprising a polynucleotide encoding the CAR.

In additional embodiments, the method further comprises stimulating the immune effector cell and inducing the cell to proliferate by contacting the cell with antibodies that bind CD3 and antibodies that bind to CD28; thereby generating a population of immune effector cells.

In particular embodiments, the immune effector cell is stimulated and induced to proliferate before introducing the vector.

In certain embodiments, the immune effector cells comprise T lymphocytes.

In particular embodiments, the immune effector cells comprise NK cells.

In particular embodiments, the cells are the activated and stimulated in the presence of an inhibitor of the PI3K pathway, thereby maintaining proliferation of the transduced immune effector cells compared to the proliferation of immune effector cells that are activated and stimulated in the absence of the inhibitor of the PI3K pathway

In some embodiments, the immune effector cells activated and stimulated in the presence of the inhibitor of PI3K pathway have increased expression of i) one or more markers selected from the group consisting of: CD62L, CD127, CD197, and CD38 or ii) all of the markers CD62L, CD127, CD197, and CD38 compared to immune effector cells activated and stimulated in the absence of the inhibitor of PI3K pathway.

In one embodiment, the PI3K inhibitor is ZSTK474.

In various embodiments, a method of treating a B cell related condition in a subject in need thereof is provided, comprising administering to the subject a therapeutically effect amount of a composition comprising BCMA CAR T cells contemplated herein and optionally, a pharmaceutically acceptable excipient.

In other embodiments, the B cell related condition is multiple myeloma, non-Hodgkin's lymphoma, B cell proliferations of uncertain malignant potential, lymphomatoid granulomatosis, post-transplant lymphoproliferative disorder, an immunoregulatory disorder, rheumatoid arthritis, myasthenia gravis, idiopathic

Claims:

1. A polynucleotide encoding a CAR, wherein the polynucleotide sequence is set forth in SEQ ID NO: 10.

2. A vector comprising the polynucleotide of claim 1.

3. The vector of claim 2, wherein the vector is an expression vector, an episomal vector, a viral vector, a retroviral vector, or a lentiviral vector.

4. The vector of claim 2, wherein the lentiviral vector is selected from the group consisting essentially of: human immunodeficiency virus 1 (HIV-1); human immunodeficiency virus 2 (HIV-2), visna-maedi virus (VMV) virus; caprine arthritis-encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV).

5. The vector according to any one of claims 2 to 4, comprising a left (5') retroviral LTR, a Psi (Ψ) packaging signal, a central polypurine tract/DNA flap (cPPT/FLAP), a retroviral export element; a promoter operably linked to the polynucleotide of claim 26 or claim 27; and a right (3') retroviral LTR.

6. The vector of claim 5, further comprising a heterologous polyadenylation sequence.

7. The vector of claim 5 or claim 6, wherein the promoter of the 5' LTR is replaced with a heterologous promoter.

8. The vector of claim 7, wherein the heterologous promoter is a cytomegalovirus (CMV) promoter, a Rous Sarcoma Virus (RSV)promoter, or an Simian Virus 40 (SV40) promoter.

9. The vector of any one of claims 5 to 8, wherein the 5' LTR or 3' LTR is a lentivirus LTR.

10. The vector of any one of claims 5 to 8, wherein the 3' LTR is a self-inactivating (SIN) LTR.

11. The vector of any one of claims 5 to 10, wherein the polyadenylation sequence is a bovine growth hormone polyadenylation or signal rabbit β-globin polyadenylation sequence.

12. The vector of any one of claims 5 to 11, wherein the promoter operably linked to the polynucleotide of claim 1 is selected from the group consisting of: a cytomegalovirus immediate early gene promoter (CMV), an elongation factor 1 alpha promoter (EF1-α), a phosphoglycerate kinase-1 promoter (PGK), a ubiquitin-C promoter (UBQ-C), a cytomegalovirus enhancer/chicken beta-actin promoter (CAG), polyoma enhancer/herpes simplex thymidine kinase promoter (MC1), a beta actin promoter (β-ACT), a simian virus 40 promoter (SV40), and a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) promoter.

13. An *ex vivo* or *in vitro* immune effector cell comprising the vector of any one of claims 2 to 12.

14. The *ex vivo* or *in vitro* immune effector cell of claim 13, wherein the immune effector cell is selected from the group consisting of: a T lymphocyte and a natural killer (NK) cell.

15. A composition comprising the *ex vivo* or *in vitro* immune effector cell of claim 13 or claim 14 and a physiologically acceptable excipient.