ALLOGENEIC CELL THERAPY OF B CELL MALIGNANCIES USING GENETICALLY ENGINEERED T CELLS TARGETING CD19

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing dates of U.S. Provisional Application No. 62/840,913, filed April 30, 2019, the entire contents of which are incorporated by reference herein.

BACKGROUND OF THE INVENTION

Chimeric antigen receptor (CAR) T cell therapies are adoptive T cell therapeutics used to treat human malignancies. Although CAR T cell therapy has led to tremendous clinical success, including durable remission in relapsed/refractory non-Hodgkin lymphoma (NHL) and pediatric acute lymphoblastic leukemia (ALL), the approved products are autologous and require patient-specific cell collection and manufacturing. Because of this, some patients have experienced disease progression or death while awaiting treatment. Accordingly, there remains a need for improved CAR T cell therapeutics.

SUMMARY

The present disclosure is based, at least in part, on the development of allogeneic cell therapy for B cell malignancies such as transformed FL or DLBCL using genetically engineered T cells (*e.g.*, CTX110 cells, *a.k.a.*, TC1 cells) expressing an anti-CD19 chimeric antigen receptor (CAR) and having disrupted *TRAC* gene and *B2M* gene. The allogeneic CAR-T cell therapy disclosed herein showed treatment efficacies in human patients having B cell malignancies disclosed herein, including complete responses in certain patients and long durability of responses. Further, the allogeneic CAR-T cell therapy disclosed herein exhibited desired pharmacokinetic features in the human patients, including prolonged CAR-T cell expansion and persistence after infusion.

In one aspect, the present disclosure provides a use of a population of genetically engineered T cells in the manufacture of a pharmaceutical composition for treating a B-cell malignancy in a human patient, wherein the pharmaceutical composition comprises T cells that comprise:

(a) a disrupted T cell receptor alpha constant (*TRAC*) gene,

(b) a nucleic acid coding for a chimeric antigen receptor (CAR) that binds CD19, wherein the CAR comprises an anti-CD19 single chain variable fragment (scFv) that comprises a heavy chain variable region set forth in SEQ ID NO: 51, and a light chain

variable region set forth in SEQ ID NO: 52, and wherein the nucleic acid is inserted in the disrupted *TRAC* gene, and

(c) a disrupted beta 2-microglobulin (*β2M*) gene;

wherein the pharmaceutical composition is to be administered to a human patient who has been subjected to lymphodepletion treatment; and

wherein the pharmaceutical composition is to be administered to the human patient at a dose of about $1x10^7$ to about $1x10^9$ CAR^+ T cells.

Accordingly, some examples of the present disclosure provides a method for treating a B-cell malignancy in a human patient, the method comprising: (i) subjecting a human patient having a B-cell malignancy to a lymphodepletion treatment; and (ii) administering to the human patient a population of genetically engineered T cells after step (i). In some examples, step (i) can be performed about 2-7 days prior to step (ii). In some examples, the population of genetically engineered T cells is allogeneic.

The population of the genetically engineered T cells may comprise T cells that comprise: (a) a disrupted T cell receptor alpha constant (*TRAC*) gene, (b) a nucleic acid coding for a chimeric antigen receptor (CAR) that binds CD19, wherein the CAR comprises an anti-CD19 single chain variable fragment (scFv) that comprises a heavy chain variable region set forth in SEQ ID NO: 51, and a light chain variable region set forth in SEQ ID NO: 52, and wherein the nucleic acid is inserted in the disrupted *TRAC* gene, and (c) a disrupted beta 2-microglobulin (*β2M*) gene. In some examples, the disrupted *TRAC* gene comprises a deletion of a fragment comprising the nucleotide sequence of SEQ ID NO: 26.

In some examples, the population of genetically engineered T cells is administered to the human patient at a dose of about $1x10^7$ to about $1x10^9$ CAR^+ T cells. In some examples, the population of genetically engineered T cells is administered to the human patient at a dose of about $1x10^7$ CAR^+ T cells. In some examples, the population of genetically engineered T cells is administered to the human patient at a dose of about $3x10^7$ CAR^+ T cells. In some examples, the population of genetically engineered T cells is administered to the human patient at a dose of about $1x10^8$ CAR^+ T cells. In some examples, the population of genetically engineered T cells is administered to the human patient at a dose of about $3x10^8CAR^+$ T cells. In some examples, the population of genetically engineered T cells is administered to the human patient at a dose of about $1x10^9$ CAR^+ T cells. In any event, the population of genetically engineered T cells administered to the human patient per dose contains no more than $7 x10^4$ TCR^+ T cells/kg.

In some examples, the lymphodepletion treatment in step (i) comprises co-administration to the human patient fludarabine at about 30 mg/m^2 and cyclophosphamide at about 500-750 mg/m^2 per day for three days. For example, the lymphodepletion treatment in step (i) comprises co-administration to the human patient fludarabine at about 30 mg/m^2 and cyclophosphamide at about 500 mg/m^2 per day for three days. In other examples, the lymphodepletion treatment in step (i) comprises co-administration to the human patient fludarabine at about 30 mg/m^2 and cyclophosphamide at about 750 mg/m^2 per day for three days.

In some examples, prior to step (i), the human patient does not show one or more of the following features: (a) significant worsening of clinical status, (b) requirement for supplemental oxygen to maintain a saturation level of greater than 91%, (c) uncontrolled cardiac arrhythmia, (d) hypotension requiring vasopressor support, (e) active infection, and (f) grade ≥2 acute neurological toxicity.

In some examples, after step (i) and prior to step (ii), the human patient does not show one or more of the following features: (a) active uncontrolled infection; (b) worsening of clinical status compared to the clinical status prior to step (i); and (c) grade ≥2 acute neurological toxicity.

Any of the methods disclosed herein may further comprise (iii) monitoring the human patient for development of acute toxicity after step (ii); and (iv) managing the acute toxicity if occurs. In some examples, step (iii) can be performed for at least 28 days after administration of the population of genetically engineered T cells. Exemplary acute toxicity may comprise tumor lysis syndrome (TLS), cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), B cell aplasia, hemophagocytic lymphohistiocytosis (HLH), cytopenia, graft-versus-host disease (GvHD), hypertension, renal insufficiency, or a combination thereof.

In some examples, the B cell malignancy is non-Hodgkin lymphoma. Examples include, but are not limited to, diffuse large B cell lymphoma (DLBCL), high grade B cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangement, transformed follicular lymphoma (FL), or grade 3b FL. In some instances, DLBCL is DLBCL not otherwise specified (NOS). In some examples, the B cell malignancy is refractory and/or relapsed.

In some examples, the human patient may have at least one measurable lesion that is fluorodeoxyglucose positron emission tomography (PET)-positive. In some examples, the human patient has undergone one or more lines of prior anti-cancer therapies. In some examples, the human patient has undergone two or more lines of prior anti-cancer therapies.

Exemplary prior anti-cancer therapies may comprise an anti-CD20 antibody, an anthracycline-containing regimen, or a combination thereof.

In some examples, the human patient has refractory or relapsed transformed FL and has undergone at least one line of chemotherapy for disease after transformation to DLBCL. In other examples, the B cell malignancy is refractory, and the human patient has progressive disease on last therapy, or has stable disease following at least two cycles of therapy with duration of stable disease of up to 6 months. In yet other examples, the human patient has failed prior autologous hematopoietic stem cell transplantation (HSCT) or ineligible for prior autologous HSCT. Alternatively or in addition, the human patient is subject to an additional anti-cancer therapy after treatment with the population of genetically engineered T cells.

In any of the methods disclosed herein, the human patient has one or more of the following features:

(a) has an Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1;

(b) adequate renal, liver, cardiac, and/or pulmonary function;

(c) free of prior gene therapy or modified cell therapy;

(d) free of prior treatment comprising an anti-CD19 antibody;

(e) free of prior allogeneic HSCT;

(f) free of detectable malignant cells from cerebrospinal fluid;

(g) free of brain metastases;

(h) free of prior central nervous system disorders;

(i) free of unstable angina, arrhythmia, and/or myocardial infarction;

(j) free of uncontrolled infection;

(k) free of immunodeficiency disorders or autoimmune disorders that require immunosuppressive therapy; and

(l) free of infection by human immunodeficiency virus, hepatitis B virus, or hepatitis C virus.

In any of the methods disclosed herein, the anti-CD19 CAR expressed by the genetically engineered T cells may comprise an extracellular antigen binding domain, which is an anti-CD19 scFv comprising the amino acid sequence of SEQ ID NO: 47. In some examples, the anti-CD19 CAR may comprise the amino acid sequence of SEQ ID NO: 40.

In some examples, the nucleic acid encoding the anti-CD19 CAR is inserted at the site of deletion in the disrupted *TRAC* gene. In some examples, the disrupted *TRAC* gene comprises the nucleotide sequence of SEQ ID NO: 54. Alternatively or in addition, the

disrupted *β2M* gene in the population of genetically engineered T cells comprises at least one of the nucleotide sequence set forth in SEQ ID NOs: 9-14.

In some examples, at least 90% of the T cells in the population of genetically engineered T cells do not express a detectable level of TCR surface protein. For example, at least 70% of the T cells in the population of genetically engineered T cells do not express a detectable level of TCR surface protein; at least 50% of the T cells in the population of genetically engineered T cells do not express a detectable level of B2M surface protein; and/or at least 30% of the T cells in the population of genetically engineered T cells express a detectable level of the CAR. In some examples, at least 99.5% of the T cells in the population of genetically engineered T cells do not express a detectable level of TCR surface protein. In some examples, at least 70% of the T cells in the population of genetically engineered T cells do not express a detectable level of B2M surface protein. In specific examples, at least 85% of the T cells in the population of the genetically engineered T cells do not express a detectable level of B2M surface protein. In some examples, at least 50% of the T cells in the population of genetically engineered T cells express a detectable level of the CAR. In specific examples, at least 70% of the T cells in the population of genetically engineered T cells express a detectable level of the CAR.

In a specific example, the population of genetically engineered T cells for use in any of the methods disclosed herein are CTX110 cells.

In any of the methods disclosed herein, the population of genetically engineered T cells are administered to the human patient *via* intravenous infusion. In some examples, the population of genetically engineered T cells may be suspended in a cryopreservation solution.

Also within the scope of the present disclosure are pharmaceutical compositions for use in treating a B-cell malignancy, the pharmaceutical composition comprising any of the population of genetically engineered T cells disclosed herein (*e.g.*, the CTX110 cells), as well as use of the genetically engineered T cells for manufacturing a medicament for use in treating a B-cell malignancy as disclosed herein.

The details of one or more examples of the disclosure are set forth in the description below. Other features or advantages of the present disclosure will be apparent from the following drawings and detailed description of several examples, and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a series of flow cytometry plots of human primary T-cells, TRAC-/B2M-CD19CAR+T cells (TC1), 8 days post-editing. The graphs show reduced surface expression of TRAC and B2M. TCR/MHC I double knockout cells express high levels of the CAR transgene (bottom panel). Negative selection of TC1 cells with purification beads leads to a reduction in TCR positive cells (right panel).

FIG. 2 is a graph depicting high editing rates achieved at the TRAC and B2M loci in TRAC-/B2M-CD19CAR+T cells (TC1). Surface expression of TCR and MHCI, which is the functional output of gene editing, was measured and plotted as editing percentage on the y-axis. High efficiency (*e.g.*, greater than 50%) site-specific integration and expression of the CAR from the TRAC locus were detected. These data demonstrate greater than 50% efficiency for the generation of TRAC-/B2M-/anti-CD19CAR+T cells.

FIG. 3 is a graph depicting a statistically significant decrease in tumor volume (mm^3) ($p = 0.007$) in NOG Raji mice following treatment with TRAC-/β2M-/CD19 CAR+ T cells (TC1).

What Is Claimed Is:

1. Use of a population of genetically engineered T cells in the manufacture of a pharmaceutical composition for treating a B-cell malignancy in a human patient, wherein the pharmaceutical composition comprises T cells that comprise:

(a) a disrupted T cell receptor alpha constant (*TRAC*) gene,

(b) a nucleic acid coding for a chimeric antigen receptor (CAR) that binds CD19, wherein the CAR comprises an anti-CD19 single chain variable fragment (scFv) that comprises a heavy chain variable region set forth in SEQ ID NO: 51, and a light chain variable region set forth in SEQ ID NO: 52, and wherein the nucleic acid is inserted in the disrupted *TRAC* gene, and

(c) a disrupted beta 2-microglobulin (*β2M*) gene;

wherein the pharmaceutical composition is to be administered to a human patient who has been subjected to lymphodepletion treatment; and

wherein the pharmaceutical composition is to be administered to the human patient at a dose of about $1x10^7$ to about $1x10^9$ CAR^+ T cells.

2. The use of claim 1, wherein the disrupted *TRAC* gene comprises a deletion of a fragment comprising the nucleotide sequence of SEQ ID NO: 26,

3. The use of claim 1 or claim 2, wherein the pharmaceutical composition to be administered to the human patient per dose contains no more than $7 x10^4$ TCR^+ T cells/kg.

4. The use of any one of claims 1-3, wherein fludarabine was to be co-administered at about 30 mg/m^2 with cyclophosphamide at about 500-750 mg/m^2 per day for three days to the human patient during the lymphodepletion treatment.

5. The use of claim 4, wherein fludarabine was to be co-administered at about 30 mg/m^2 with cyclophosphamide at about 500 mg/m^2 per day for three days, or fludarabine was to be co-administered at about 30 mg/m^2 with cyclophosphamide at about 750 mg/m^2 per day for three days.

6. The use of any one of claims 1-5, wherein the population of genetically engineered T cells is to be administered to the human patient at a dose of about $1x10^7$, about $3x10^7$, about $1x10^8$, about $3x10^8$, or about $1x10^9$ CAR^+ T cells.

7. The use of any one of claims 1-6, wherein prior to lymphodepletion treatment , the human patient does not show one or more of the following features:

(a) significant worsening of clinical status,

(b) requirement for supplemental oxygen to maintain a saturation level of greater than 91%,

(c) uncontrolled cardiac arrhythmia,

(d) hypotension requiring vasopressor support,

(e) active infection, and

(f) grade ≥2 acute neurological toxicity.

8. The use of any one of claims 1-7, wherein the lymphodepletion treatment was performed about 2-7 days prior to administration of the pharmaceutical composition.

9. The use of any one of claims 1-8, wherein after lymphodepletion treatment and prior to administration of the pharmaceutical composition, the human patient does not show one or more of the following features:

(a) active uncontrolled infection;

(b) worsening of clinical status compared to the clinical status; and

(c) grade ≥2 acute neurological toxicity.

10. The use of any one of claims 1-9, further comprising monitoring the human patient for development of acute toxicity after administration of the pharmaceutical composition ; and managing the acute toxicity if occurs.

11. The use of claim 10, wherein monitoring is performed for at least 28 days after administration of the pharmaceutical composition.

12. The use of claim 10 or claim 11, wherein the acute toxicity comprises tumor lysis syndrome (TLS), cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), B cell aplasia, hemophagocytic lymphohistiocytosis (HLH), cytopenia, graft-versus-host disease (GvHD), hypertension, renal insufficiency, or a combination thereof.

13. The use of any one of claims 1-12, wherein the B cell malignancy is non-Hodgkin lymphoma, which optionally is selected from the group consisting of diffuse large B cell lymphoma (DLBCL), high grade B cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangement, transformed follicular lymphoma (FL), and grade 3b FL.

14. The use of claim 13, wherein DLBCL is DLBCL not otherwise specified (NOS).

15. The use of any one of claims 1-14, wherein the human patient has at least one measurable lesion that is fluorodeoxyglucose positron emission tomography (PET)-positive.

16. The use of any one of claims 1-15, wherein the B cell malignancy is refractory and/or relapsed.

17. The use of any one of claims 1-16, wherein the human patient has undergone one or more lines of prior anti-cancer therapies.

18. The use of claim 17, wherein the human patient has undergone two or more lines of prior anti-cancer therapies.

19. The use of claim 17 or claim 18, wherein the prior anti-cancer therapies comprise an anti-CD20 antibody, an anthracycline-containing regimen, or a combination thereof.

20. The use of claim 17, wherein the human patient has refractory or relapsed transformed FL and has undergone at least one line of chemotherapy for disease after transformation to DLBCL.

21. The use of any one of claims 16-19, wherein the B cell malignancy is refractory, and the human patient has progressive disease on last therapy, or has stable disease following at least two cycles of therapy with duration of stable disease of up to 6 months.

22. The use of any one of claims 1-21, wherein the human patient has failed prior autologous hematopoietic stem cell transplantation (HSCT) or ineligible for prior autologous HSCT.

23. The use of any one of claims 1-22, wherein the human patient is to be subjected to an additional anti-cancer therapy after administration of the pharmaceutical composition.

24. The use of any one of claims 1-23, wherein the human patient has one or more of the following features:

(a) has an Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1;

(b) adequate renal, liver, cardiac, and/or pulmonary function;

(c) free of prior gene therapy or modified cell therapy;

(d) free of prior treatment comprising an anti-CD19 antibody;

(e) free of prior allogeneic HSCT;

(f) free of detectable malignant cells from cerebrospinal fluid;

(g) free of brain metastases;

(h) free of prior central nervous system disorders;

(i) free of unstable angina, arrhythmia, and/or myocardial infarction;

(j) free of uncontrolled infection;

(k) free of immunodeficiency disorders or autoimmune disorders that require immunosuppressive therapy; and

(l) free of infection by human immunodeficiency virus, hepatitis B virus, or hepatitis C virus.

25. The use of any one of claims 1-24, wherein the anti-CD19 scFv comprises the amino acid sequence of SEQ ID NO: 47.

26. The use of claim 25, wherein the CAR that binds CD19 comprises the amino acid sequence of SEQ ID NO: 40.

27. The use of any one of claims 1-26, wherein the nucleic acid encoding the anti-CD19 CAR is inserted at the site of deletion in the disrupted *TRAC* gene.

28. The use of any one of claims 1-27, wherein the disrupted *TRAC* gene comprises the nucleotide sequence of SEQ ID NO: 54.

29. The use of any one of claims 1-28, wherein the disrupted *β2M* gene in the population of genetically engineered T cells comprises at least one of the nucleotide sequence set forth in SEQ ID NOs: 9-14.

30. The use of any one of claims 1-29, wherein the population of genetically engineered T cells is allogeneic.

31. The use of any one of claims 1-30, wherein at least 90% of the T cells in the population of genetically engineered T cells do not express a detectable level of TCR surface protein.

32. The use of any one of claims 1-31, wherein at least 70% of the T cells in the population of genetically engineered T cells do not express a detectable level of TCR surface protein, wherein at least 50% of the T cells in the population of genetically engineered T cells do not express a detectable level of B2M surface protein; and/or wherein at least 30% of the T cells in the population of genetically engineered T cells express a detectable level of the CAR.

33. The use of claim 32, wherein at least 99.5% of the T cells in the population of genetically engineered T cells do not express a detectable level of TCR surface protein.

34. The use of any one of claims 1-33, wherein at least 70% of the T cells in the population of genetically engineered T cells do not express a detectable level of B2M surface protein.

35. The use of claim 34, wherein at least 85% of the T cells in the population of the genetically engineered T cells do not express a detectable level of B2M surface protein.

36. The use of any one of claims 1-35, wherein at least 50% of the T cells in the population of genetically engineered T cells express a detectable level of the CAR.

37. The use of claim 36, wherein at least 70% of the T cells in the population of genetically engineered T cells express a detectable level of the CAR.

38. The use of any one of claims 1-37, wherein the population of genetically engineered T cells are to be administered to the human patient *via* intravenous infusion.

39. The use of any one of claims 1-38, wherein the population of genetically engineered T cells are suspended in a cryopreservation solution.