iPSC-DERIVED EFFECTOR CELLS FOR AUTOIMMUNE DISEASE TREATMENT

RELATED APPLICATIONS

[0001] The application claims priority to U.S. Provisional Application Serial No. 63/581,612, filed September 8, 2023, to U.S. Provisional Application Serial No. 63/557,427, filed February 23, 2024, and to U.S. Provisional Application Serial No. 63/644,427, filed May 8, 2024, the disclosures of which are hereby incorporated by reference in their entireties.

INCORPORATED BY REFERENCE OF SEQUENCE LISTING

[0002] The Sequence Listing titled 184143-654601_SL.xml, which was created on September 5, 2024 and is 20,563 bytes in size, is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present disclosure is broadly concerned with the field of off-the-shelf immunocellular products. More particularly, the present disclosure is concerned with strategies for developing multifunctional effector cells capable of delivering therapeutically relevant properties *in vivo*. The cell products developed under the present disclosure address critical limitations of patient-sourced cell therapies.

BACKGROUND OF THE INVENTION

[0004] The field of adoptive cell therapy is currently focused on using patient- and donor-sourced cells, which makes it particularly difficult to achieve consistent manufacturing of cancer immunotherapies and to deliver therapies to all patients who may benefit. There is also the need to improve the efficacy and persistence of adoptively transferred lymphocytes to promote favorable patient outcomes. Lymphocytes such as T cells and natural killer (NK) cells are potent anti-tumor effectors that play an important role in innate and adaptive immunity. However, the use of these immune cells for adoptive cell therapies remains challenging and has unmet needs for improvement. Therefore, significant opportunities remain to harness the full potential of T and NK cells, or other lymphocytes in adoptive immunotherapy.

SUMMARY

[0005] There is a need for functionally improved effector cells that address issues ranging from response rate, cell exhaustion, loss of transfused cells (survival and/or persistence), off-target toxicity, off-target effect, to efficacy against various diseases.

[0006] It is an object of examples of the present disclosure to provide methods and compositions for adoptive cell therapy, wherein the adoptive cell therapy includes administering an adoptive cell therapy product generated from derivative non-pluripotent cells differentiated from a single cell derived iPSC (induced pluripotent stem cell) clonal line, which iPSC line comprises one or several genetic modifications in its genome. Said one or several genetic modifications include, in some examples, one or more of DNA insertion, deletion, and substitution, and which modifications are retained and remain functional in subsequently derived cells after differentiation, expansion, passaging and/or transplantation.

[0006a] In one aspect, the present disclosure relates to use of an adoptive cell therapy product in the manufacture of a medicament for use in combinatorial therapy for treating lupus or a symptom thereof in a subject, wherein said medicament is to be administered to the subject in combination with one or more doses of a chemotherapy, and wherein the subject is to be administered with one or more doses of the chemotherapy followed by one or more doses of the medicament in a first effective amount; wherein the adoptive cell therapy product comprises an engineered T lineage effector cell comprising (i) expression of a CD19-CAR (chimeric antigen receptor) at a T-cell receptor α constant (TRAC) locus, and (ii) a T cell receptor (TCR) knockout.

[0006b] In another aspect, the present disclosure relates to use of an adoptive cell therapy product in the manufacture of a medicament for use in combinatorial therapy for treating lupus or a symptom thereof in a subject, wherein said medicament is to be administered to the subject in combination with one or more doses of a chemotherapy, and wherein the subject is to be administered with one or more doses of chemotherapy followed by one or more doses of the medicament in a first effective amount; wherein the adoptive cell therapy product comprises an engineered T lineage effector cell comprising (i) expression of a CD19-CAR (chimeric antigen receptor) at a T-cell receptor α constant (TRAC) locus, and (ii) a T cell receptor (TCR) knockout; and wherein the subject previously received and failed to respond to one or more prior treatments comprising glucocorticoids, CY, mycophenolic acid or its derivatives, belimumab, methotrexate, azathioprine, anifrolumab, rituximab, obinutuzumab, cyclosporin, tacrolimus, or voclosporin.

[0006c] In another aspect, the present disclosure relates to use of an adoptive cell therapy product in the manufacture of a medicament for treating lupus or a symptom thereof in a subject, wherein the subject is to be administered one or more doses of the medicament in a first effective amount; wherein the adoptive cell therapy product comprises an engineered NK lineage effector cell comprising a CD38 knockout and expression of (i) a CD19-CAR (chimeric antigen receptor); (ii) an alloimmune defense receptor (ADR) targeting 4-1BB; (iii) a high-affinity, non-cleavable CD16 (hnCD16); and (iv) an interleukin-15 (IL15)/IL15 receptor fusion protein (IL15RF).

[0007] In one example, of the present disclosure provides a method of treating lupus or a symptom thereof in a subject. In some examples, the method comprises (a) administering to the subject one or more doses of chemotherapy; and (b) after step (a), administering to the subject one or more doses of an adoptive cell therapy product in a first effective amount; wherein the adoptive cell therapy product comprises an engineered T lineage effector cell comprising (i) expression of a CD19-CAR (chimeric antigen receptor) at a T-cell receptor α constant (TRAC) locus, and (ii) a T cell receptor (TCR) knockout.

[0008] In some examples, the method further comprises (c) after step (b), administering to the subject one or more doses of an additional chemotherapy, wherein the additional chemotherapy is the same or different as the chemotherapy of step (a); and (d) after step (c), administering to the subject one or more doses of an adoptive cell therapy product in a second effective amount that is the same or different as the first effective amount.

[0009] In some examples, the lupus or the symptom thereof comprises: (i) cutaneous lupus; (ii) lupus nephritis; (iii) neuropsychiatric lupus; or (iv) pericarditis, atherosclerosis, angina, Raynaud's syndrome, renal disease, metabolic syndrome, thyroid disorder, fibromyalgia, respiratory tract infection, skin infection, or urinary system infection.

[00010] In some examples, the chemotherapy comprises (a) one or more of cyclophosphamide (CY) or fludarabine (FLU); or (b) bendamustine. In some examples, the chemotherapy is administered one or more days prior to administering the adoptive cell therapy product; optionally wherein the chemotherapy is administered on one or more of (i) three days before the adoptive cell therapy product, (ii) four days before the adoptive cell therapy product, or (iii) five days before the adoptive cell therapy product. In some examples, the chemotherapy comprises cyclophosphamide administered at a daily dose of about 250 mg/m^2 to about 600 mg/m^2 and fludarabine administered at a daily dose of about 20 mg/m^2 to about 40 mg/m^2 for 3 consecutive days starting from about 4-6 days prior to day 1 of administering the adoptive cell therapy product. In some examples, the chemotherapy comprises bendamustine and is administered at a daily dose of about 30 mg/m^2 to about 150 mg/m^2 for 2

consecutive days starting from about 4-6 days prior to day 1 of administering the adoptive cell therapy product.

[00011] In some examples, the engineered T lineage effector cell is derived from an engineered induced pluripotent stem cell (iPSC) comprising a TCR knockout, and a polynucleotide encoding the CD19-CAR. In some examples, the first effective amount of the adoptive cell therapy product is about 1.8×10^8 cells to about 9×10^9 cells and is optionally escalated. In some examples, the first effective amount of the adoptive cell therapy product is about 3.6×10^8 cells, about 1×10^9 cells, about 3×10^9 cells, or about 9×10^9. In some examples, the adoptive cell therapy product is cryopreserved, and then thawed prior to administering. In some examples, the adoptive cell therapy product is FT819.

[00012] In some examples, the subject has had a lupus diagnosis. In some examples, the subject is positive in at least one of (a) an anti-nuclear antibody; (b) an anti-dsDNA antibody; or (c) an anti-Smith antibody. In some examples, treating lupus comprises obtaining lupus remission or low activity in a subject. In some examples, remission or reduced disease activity is defined by DORIS (Definition of remission in systemic lupus erythematosus) complete remission, DORIS clinical remission on treatment, and/or LLDAS (lupus low disease activity state). In some examples, treating lupus comprises controlling lupus flare. In some examples, the treatment comprises longer disease-free intervals off immunosuppressive medications, decreases autoantibody production in the subject; and/or reconstitutes healthy B cell compartment in the subject as compared to B cells under active lupus. In some examples, the subject previously received one or more prior treatments comprising glucocorticoids, CY, mycophenolic acid or its derivatives, belimumab, methotrexate, azathioprine, anifrolumab, rituximab, obinutuzumab, cyclosporin, tacrolimus, or voclosporin. In some examples, the subject did not respond to the one or more prior treatments.

[00013] In one example, the present disclosure provides a method of treating lupus or a symptom thereof in a subject. In some examples, the method comprises (a) administering to the subject one or more doses of chemotherapy; and (b) after step (a), administering to the subject one or more doses of an adoptive cell therapy product in a first effective amount; wherein the adoptive cell therapy product comprises an engineered T lineage effector cell comprising (i) expression of a CD19-CAR (chimeric antigen receptor) at a T-cell receptor α constant (TRAC) locus, and (ii) a T cell receptor (TCR) knockout; and wherein the subject previously received and failed to respond to one or more prior treatments comprising glucocorticoids, CY, mycophenolic acid or its derivatives, belimumab, methotrexate, azathioprine, anifrolumab, rituximab, obinutuzumab, cyclosporin, tacrolimus, or voclosporin.

[00014] In another example, the present disclosure provides a method of treating lupus or a symptom thereof in a subject, the method comprising administering to the subject one or more doses of an adoptive cell therapy product in a first effective amount; wherein the adoptive cell therapy product comprises an engineered NK lineage effector cell comprising a CD38 knockout and expression of (i) a CD19-CAR (chimeric antigen receptor); (ii) an alloimmune defense receptor (ADR) targeting 4-1BB; (iii) a high-affinity, non-cleavable CD16 (hnCD16); and (iv) an interleukin-15 (IL15)/IL15 receptor fusion protein (IL15RF). In one example, the method does not comprise administration of chemotherapy for lympho-conditioning to the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

[00015] **FIG. 1A and FIG. 1B** show exemplary treatment schemas to evaluate FT819 as an allogeneic cell therapy in combination with a chemotherapy. Abbreviations are as follows: CY (cyclophosphamide), DLT (dose-limiting toxicity), FLU (fludarabine), LTFU (long-term follow-up), PTFU (post-treatment follow-up). According to the illustrated schema, subjects are followed in post-treatment follow-up for up to 2 years after the cycle of FT819 treatment, and followed in long-term follow-up for up to 15 years after the FT819 treatment.

[00016] **FIG. 2** shows an exemplary dose-escalation and dose expansion schema for the use of FT819 in lupus treatment. Illustrated Dose Level 1 (DL1) is set at 3.6×10^8, and additional dose levels > DL1 (≤ 3 x the prior cleared dose level) may be explored. DL0 = 1.8×10^8 cells/dose (to be assessed if DL1 exceeds MTD).

[00017] **FIG. 3** shows CD19+ B cell depletion observed in a 72-hour killing assay against PBMCS (E:T 1:1) derived from a blood sample of a patient with SLE.

[00018] **FIG. 4** shows that FT819 displays better targeting of CD19+ B cells from blood samples of SLE patients in comparison to autologous-manufactured CAR T cells. Curves from left to right in each graph correspond to the legend entries from top to bottom, respectively.

[00019] **FIG. 5** shows FT819 PK (mean ± SEM) at 90 and 360 million cells in a Phase 1 clinical trial.

[00020] **FIG. 6** shows B-cell depletion in peripheral blood with sustained suppression of B cells during FT819 treatment cycle in patients.

[00021] **FIG. 7** shows B-cell depletion observed following a single dose of FT819 to two patients who are resistant to Cy/Flu conditioning chemotherapy.

[00022] **FIG. 8** shows CD19+ B cell depletion following a single-dose FT819 with bendamustine as an alternative to Cy/Flu conditioning chemotherapy.

CLAIMS

What is claimed is:

1. Use of an adoptive cell therapy product in the manufacture of a medicament for use in combinatorial therapy for treating lupus or a symptom thereof in a subject, wherein said medicament is to be administered to the subject in combination with one or more doses of a chemotherapy, and wherein the subject is to be administered with one or more doses of the chemotherapy followed by one or more doses of the medicament in a first effective amount;

wherein the adoptive cell therapy product comprises an engineered T lineage effector cell comprising (i) expression of a CD19-CAR (chimeric antigen receptor) at a T-cell receptor α constant (TRAC) locus, and (ii) a T cell receptor (TCR) knockout.

2. The use of claim 1, wherein
the subject is to be further administered with one or more doses of an additional chemotherapy, followed by one or more doses of the medicament in a second effective amount, wherein the additional chemotherapy is the same or different as the chemotherapy defined in claim 1; and

wherein the second effective amount that is the same or different as the first effective amount.

3. The use of claim 1, wherein the lupus or the symptom thereof comprises: (i) cutaneous lupus; (ii) lupus nephritis; (iii) neuropsychiatric lupus; or (iv) pericarditis, atherosclerosis, angina, Raynaud's syndrome, renal disease, metabolic syndrome, thyroid disorder, fibromyalgia, respiratory tract infection, skin infection, or urinary system infection.

4. The use of claim 1, wherein the chemotherapy comprises (a) one or more of cyclophosphamide (CY) or fludarabine (FLU); or (b) bendamustine.

5. The use of claim 1, wherein the chemotherapy is to be administered one or more days prior to the administration of the medicament; optionally wherein the chemotherapy is to be administered on one or more of (i) three days before the adoptive cell therapy product, (ii) four days before the medicament, or (iii) five days before the medicament.

6. The use of claim 1, wherein the chemotherapy comprises cyclophosphamide to be administered at a daily dose of 250 mg/m^2 to 600 mg/m^2 and fludarabine to be administered at a daily dose of 20 mg/m^2 to 40 mg/m^2 for 3 consecutive days starting from 4-6 days prior to day 1 of the administration of the medicament.

7. The use of claim 1, wherein the chemotherapy comprises bendamustine and is to be administered at a daily dose of 30 mg/m^2 to 150 mg/m^2 for 2 consecutive days starting from 4-6 days prior to day 1 of the administration of the medicament.

8. The use of claim 1, wherein the engineered T lineage effector cell is derived from an engineered induced pluripotent stem cell (iPSC) comprising a TCR knockout, and a polynucleotide encoding the CD19-CAR.

9. The use of claim 1, wherein the first effective amount of the adoptive cell therapy product in the medicament is 1.8×10^8 cells to 9×10^9 cells, and is optionally escalated.

10. The use of claim 9, wherein the first effective amount of the adoptive cell therapy product in the medicament is about 3.6×10^8 cells, about 1×10^9 cells, about 3×10^9 cells, or about 9×10^9.

11. The use of claim 1, wherein the medicament is cryopreserved, and then thawed prior to its administration.

12. The use of claim 1, wherein the adoptive cell therapy product is FT819.

13. The use of claim 1, wherein the subject has had a lupus diagnosis.

14. The use of claim 1, wherein the subject is positive in at least one of (a) an anti-nuclear antibody; (b) an anti-dsDNA antibody; or (c) an anti-Smith antibody.

15. The use of any one of claims 1-14, wherein lupus remission or low activity is obtained in the subject following treatment with the combinatorial therapy.

16. The use of claim 15, wherein the lupus remission or low activity is defined by DORIS (Definition of remission in systemic lupus erythematosus) complete remission, DORIS clinical remission on treatment, and/or LLDAS (lupus low disease activity state).

17. The use of any one of claims 1-14, wherein lupus flare is controlled following treatment with the combinatorial therapy.

18. The use according to any one of claims 1-14, wherein the treating comprises longer disease-free intervals off immunosuppressive medications, decreases autoantibody production in the subject; and/or reconstitutes healthy B cell compartment in the subject as compared to B cells under active lupus.

19. The use according to any one of claims 1-14, wherein the subject previously received one or more prior treatments comprising glucocorticoids, CY, mycophenolic acid or its derivatives, belimumab, methotrexate, azathioprine, anifrolumab, rituximab, obinutuzumab, cyclosporin, tacrolimus, or voclosporin.

20. The use of claim 19, wherein the subject did not respond to the one or more prior treatments.

21. Use of an adoptive cell therapy product in the manufacture of a medicament for use in combinatorial therapy for treating lupus or a symptom thereof in a subject, wherein said medicament is to be administered to the subject in combination with one or more doses of a chemotherapy, and wherein

the subject is to be administered with one or more doses of chemotherapy followed by one or more doses of the medicament in a first effective amount;

wherein the adoptive cell therapy product comprises an engineered T lineage effector cell comprising (i) expression of a CD19-CAR (chimeric antigen receptor) at a T-cell receptor α constant (TRAC) locus, and (ii) a T cell receptor (TCR) knockout; and

wherein the subject previously received and failed to respond to one or more prior treatments comprising glucocorticoids, CY, mycophenolic acid or its derivatives, belimumab, methotrexate, azathioprine, anifrolumab, rituximab, obinutuzumab, cyclosporin, tacrolimus, or voclosporin.

22. Use of an adoptive cell therapy product in the manufacture of a medicament for treating lupus or a symptom thereof in a subject, wherein the subject is to be administered one or more doses of the medicament in a first effective amount;

wherein the adoptive cell therapy product comprises an engineered NK lineage effector cell comprising a CD38 knockout and expression of (i) a CD19-CAR (chimeric antigen receptor); (ii) an alloimmune defense receptor (ADR) targeting 4-1BB; (iii) a high-affinity, non-cleavable CD16 (hnCD16); and (iv) an interleukin-15 (IL15)/IL15 receptor fusion protein (IL15RF).

23. The use of claim 22, wherein the medicament is not used with the administration of chemotherapy for lympho-conditioning.