INSULIN TREATMENT TO IMPROVE T CELL ENGINEERING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is an International Application which claims priority to and benefit of U.S. Provisional Application No. 63/387,068 filed December 12, 2022 and U.S. Provisional Application No. 63/608,697 filed December 11, 2023, the contents of each of which are incorporated herein by reference in their entreties and for all purposes.

BACKGROUND

[0002] Genetic engineering has nearly limitless potential as a tool to improve human health. Indeed, genetic engineering has introduced and transformed therapies in every aspect of medical practice today. Of particular interest is the genetic engineering of T cells. T cell based therapies have become powerful new medicines, especially in the realms of cancer and immune system regulation. However, significant hurdles remain with respect to improving the viability and expansion capacity of genetically engineered T cells.

[0003] Disclosed herein, *inter alia*, are solutions to these and other problems in the art.

BRIEF SUMMARY

[0004] In an example, provided herein, is a method of editing an endogenous gene in a population of T cells, the method including: contacting the population of T cells with a gene editing reagent or a polynucleotide encoding a gene editing reagent under conditions to allow the polynucleotide or gene editing reagent to enter the cell, and culturing the population of T cells in the presence of one or more of; insulin, an insulin analog, an insulin agonist and/or an insulin partial agonist before and/or during, and/or after the contacting step to obtain an engineered population of T cells. In one aspect, provided herein, is a method of editing an endogenous gene in a population of T cells *in vitro*, the method comprising: contacting the population of T cells with a gene editing reagent or a polynucleotide encoding a gene editing reagent under conditions to allow the polynucleotide or gene editing reagent to enter the cell, and culturing the population of T cells in the presence of one or more of; insulin, an insulin analog, an insulin agonist and/or an insulin partial agonist before and/or during, and/or after the contacting step to obtain an engineered population of T cells.

[0005] In another aspect, provided herein, is a method of monitoring of cell viability for an engineered population of T cells, the method comprising measuring mitochondrial function and cell metabolism over time.

[0006] In another aspect, provided herein, is a method of monitoring cell viability for an engineered population of T cells, the method comprising measuring a cell metabolism marker over time.

[0007] In another example, provided herein, is a method of increasing cell viability of a population of engineered T cells, including contacting a population of T cells in the presence of insulin, an insulin analog, an insulin agonist, an insulin partial agonist, a gene editing reagent, or a polynucleotide encoding a gene editing reagent thereby forming the population of engineered T cells, wherein the population of engineered T cells has increased cell viability, growth, and/or gene editing efficiencies relative to a population of engineered T cells that are not contacted with insulin, insulin analog, insulin agonist, or insulin partial agonist, wherein the population of engineered T cells are administered to a subject in need thereof.

[0008] In another example, provided herein, is a method of increasing gene editing efficiency in a population of engineered T cells, including contacting a population of T cells with insulin, an insulin analog, an insulin agonist, an insulin partial agonist, a gene editing reagent, and a polynucleotide, thereby forming the population of engineered T cells, wherein the population of engineered T cells has increased gene editing efficiency relative to a population of engineered T cells that are not contacted with insulin, insulin analog, insulin agonist, or insulin partial agonist.

[0009] In another example, provided herein, is a method for increasing expansion of a population of engineered T cells, including: (i) contacting a population of T cells with insulin, an insulin analog, an insulin agonist, and/or an insulin partial agonist and a polynucleotide, thereby forming the population of engineered T cells, and (ii) expanding the population of engineered T cells, thereby forming a population of expanded engineered T cells, wherein the insulin, insulin analog, insulin agonist, and/or insulin partial agonist increases the population of expanded engineered T cells relative to a population of engineered T cells that are not contacted with insulin, insulin analog, insulin agonist, and/or insulin partial agonist.

[0010] In another example, provided herein, is an engineered population of T cells, made by the method provided herein including examples thereof. In another aspect, provided herein, is an engineered population of T cells, made by the method as disclosed herein.

[0011] In another example, provided herein, is a pharmaceutical composition comprising the engineered T cell provided herein including examples thereof. In another aspect, provided herein, is a pharmaceutical composition comprising the engineered T cell as disclosed herein.

[0012] In another example, provided herein, is a method of treating a disease in a subject in need thereof, comprising administering a therapeutically effective amount of the engineered T cell

provided herein including examples thereof or the pharmaceutical composition provided herein including examples thereof. In another aspect, provided herein, is use of the engineered T cell as disclosed herein or the pharmaceutical composition as disclosed herein, in the manufacture of a medicament for treating a disease in a subject in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. 1. Insulin treatment stimulates Akt phosphorylation in T cells. Western blot analysis of Akt, Erk and STAT5 and their respective phosphorylated forms in activated human CD8+ T cells with (+Insulin) or without (Control) insulin pre-treatment. Samples were obtained 15, 30, and 180 minutes (min) post insulin treatment in media without glucose. All measurements were performed using Image Lab software. C-Cas3: cleaved Caspase 3.

[0014] FIGs. 2A-2F. Insulin pre-treatment (24 h) of cultured T cells prior to electroporation improves culture viability, expansion, and total edited cells (TECs) levels. FIGs 2A-2C show T cell final drug product (FDP) expansion folds after a 15-day T cell engineering process with different pre-transfection (TFX) insulin treatment concentrations, calculated based on seeding density on day 2. Data obtained from 3 individual donors pre-treated with different concentrations of insulin. FIGs 2D-2F show total edited cells (TEC) number in FDP was calculated for the T cells engineered as indicated in FIGs. 2A-2C. Numbers in each column are rounded up to whole integers. TEC was calculated by multiplying the expansion fold with the knock-in percentage and was based on 1 million cell seeding density post-transfection per group. Insulin concentrations used: Low: 1 µg/ml, Mid: 5 µg/ml, High: 25 µg/ml. CTR: control.

[0015] FIGs. 3A-3E. Insulin pre-treatment enhances expression/activation of Akt and Erk signaling pathways in T cells. Western blot of Akt and Erk expression and phosphorylation with (Insulin Pre-24 h) or without (CTR) insulin pre-treatment (FIG. 3A). FIGs. 3B-3E show quantification of Western blot data for Akt and Erk expression and phosphorylation. Total Akt (FIG. 3B) and Erk (FIG. 3D) expression was calculated from the ratio of the protein band intensity to the internal control (actin) band intensity. Protein phosphorylation levels were calculated from the ratio of Akt (FIG. 3C) or Erk (FIG. 3E) phosphorylation band intensity to the total Akt of Erk protein band intensity. 0N, 2N represent day 0 and day 2 (prior to transfection), respectively; 2, 3, 4, 6, 8, 11, 15 (or 2T, 3T, 4T, 6T, 8T, 11T, 15T) represent days post 0N (TFX