testing provides the necessary information for regulators to be comfortable with dosing patients but does little to predict clinical efficacy.

[0005] In automated processes for producing cell-based therapies, there is often a need to optimize and modify various parameters of the process based on individual patient needs, and real-time changes during the methods. The present invention fulfills these needs by providing methods of assessing and optimizing cellular quality of a cell-based therapy, suitably in automated systems.

Summary

[0006] In some examples provided herein is a method for assessing and optimizing cellular quality of a cell-based therapy, comprising: determining one or more molecular characteristics of a pre-modified cell culture; genetically modifying the cell culture via an automated cell engineering system; determining the one or more molecular characteristics of the modified cell culture during and after the genetically modifying; and optimizing one or more parameters of the automated cell engineering system to alter the one or more molecular characteristics of the modified cell culture.

[0006a] In one aspect, the present disclosure refers to a method for assessing and optimizing cellular quality of a cell-based therapy, comprising:

(a) determining one or more molecular characteristics of a pre-modified cell culture;
(b) genetically modifying the cell culture via an automated cell engineering system;
(c) determining the one or more molecular characteristics of the modified cell culture during and after the genetically modifying; and
(d) optimizing one or more parameters of the automated cell engineering system to alter the one or more molecular characteristics of the modified cell culture before, during, and/or after the genetically modifying.

[0007] In further examples, provided herein is a method for assessing and optimizing cellular quality of a cell-based therapy, comprising: determining one or more molecular characteristics of a pre-modified cell culture; optimizing one or more parameters of an automated cell engineering systems to alter one the one or more molecular characteristics of the pre-modified cell culture; activating the pre-modified cell culture with an activation reagent to produce an activated cell culture; transducing the activated immune cell culture with a vector, to produce a transduced cell culture; expanding the transduced cell culture; concentrating the expanded cell culture of (e); harvesting the concentrated cell culture of (f) to produce a genetically modified cell culture; determining the one or more molecular characteristics of the cell culture during or after any one of steps (c)-(g); and optimizing one or more parameters of any one of steps (c)-(g) to alter the one or more molecular characteristics of the cell culture.

[0007a] In another aspect, the present disclosure refers to a method for assessing and optimizing cellular quality of a cell-based therapy, comprising:

(a) determining one or more molecular characteristics of a pre-modified cell culture;

(b) optimizing one or more parameters of an automated cell engineering systems to alter one the one or more molecular characteristics of the pre-modified cell culture;

(c) activating the pre-modified cell culture with an activation reagent to produce an activated cell culture;

(d) transducing the activated immune cell culture with a vector, to produce a transduced cell culture;

(e) expanding the transduced cell culture;

(f) concentrating the expanded cell culture of (e);

(g) harvesting the concentrated cell culture of (f) to produce a genetically modified cell culture;

(h) determining the one or more molecular characteristics of the cell culture selected from the group consisting of a gene expression, a protein expression, an mRNA expression, and a copy number variation during or after any one of steps (c)-(g); and

(i) optimizing one or more parameters of any one of steps (c)-(g) to alter the one or more molecular characteristics of the cell culture.

[0008] In additional examples, a method for assessing and optimizing cellular quality of a chimeric antigen receptor T (CAR T) cell culture is provided, comprising: determining one or more molecular characteristics of a pre-modified T-cell culture; optimizing one or more parameters of an automated cell engineering system to alter one the one or more molecular characteristics of the pre-modified T-cell culture; activating the pre-modified T-cell culture with an activation reagent to produce an activated T-cell culture; transducing the activated T-cell culture with a vector encoding a chimeric antigen receptor, to produce a CAR T-cell culture; expanding the CAR T-cell culture; concentrating the expanded CAR T-cell culture of (e); harvesting the concentrated CAR-T cell culture of (f); determining the one or more molecular characteristics of the CAR T-cell culture during or after any one of steps (c)-(g); and optimizing one or more parameters of any one of steps (c)-(g) to alter the one or more molecular characteristics of the CAR T-cell culture.

[0008a] In yet another aspect, the present disclosure refers to a method for assessing and optimizing cellular quality of a chimeric antigen receptor T (CAR T) cell culture, comprising:

(a) determining one or more molecular characteristics of a pre-modified T-cell culture;
(b) optimizing one or more parameters of an automated cell engineering system to alter one the one or more molecular characteristics of the pre-modified T-cell culture;
(c) activating the pre-modified T-cell culture with an activation reagent to produce an activated T-cell culture;
(d) transducing the activated T-cell culture with a vector encoding a chimeric antigen receptor, to produce a CAR T-cell culture;
(e) expanding the CAR T-cell culture;
(f) concentrating the expanded CAR T-cell culture of (e);
(g) harvesting the concentrated CAR-T cell culture of (f);
(h) determining the one or more molecular characteristics of the CAR T-cell culture during or after any one of steps (c)-(g); and
(i) optimizing one or more parameters of any one of steps (c)-(g) to alter the one or more molecular characteristics of the CAR T-cell culture.

[0009] Also provided herein is a method for assessing and optimizing cellular quality of a cell culture, comprising: determining one or more molecular characteristics of a pre-modified cell culture; genetically modifying the cell culture via an automated cell engineering system; determining the one or more molecular characteristics of the modified cell culture during and after the genetically modifying; and optimizing one or more parameters of the automated cell engineering system to alter the one or more molecular characteristics of the modified cell culture.

Brief Description of the Figures

[0010] FIG. 1 shows a generalized manufacturing process for producing a cell-based therapy as described herein.

[0011] FIG. 2 shows a lab space containing exemplary cell engineering systems as described in embodiments herein.

[0012] FIG. 3 shows a cell-based therapy production process that can be performed in a cell engineering system as described in embodiments herein.

[0013] FIG. 4 shows process flow legend for an automated cell engineering system.

CLAIMS

1. A method for assessing and optimizing cellular quality of a cell-based therapy, comprising:
    (a) determining one or more molecular characteristics of a pre-modified cell culture;
    (b) genetically modifying the cell culture via an automated cell engineering system;
    (c) determining the one or more molecular characteristics of the modified cell culture during and after the genetically modifying; and
    (d) optimizing one or more parameters of the automated cell engineering system to alter the one or more molecular characteristics of the modified cell culture before, during, and/or after the genetically modifying.

2. The method of claim 1, wherein the one or more molecular characteristics are selected from the group consisting of a gene expression, a protein expression, an mRNA expression, and a copy number variation.

3. The method of claim 1 or claim 2, wherein the cell culture is an immune cell culture, a natural killer cell culture, and a cell culture for a neurodegenerative therapy.

4. The method of claim 3, wherein the immune cell culture is a T-cell culture, including wherein the T cell culture is a chimeric antigen receptor T (CAR T) cell culture.

5. The method of claim 4, wherein the one or more molecular characteristics include T-cell activation, metabolism, exhaustion, and T-cell receptor diversity.

6. The method of any one of claims 1-5, wherein the optimizing includes one or more of increasing or decreasing a flow rate of cell media, increasing or decreasing oxygen concentration, increasing or decreasing carbon dioxide concentration,

increasing or decreasing a glucose level, increasing or decreasing the temperature of a cell expansion, increasing or decreasing the pH of a cell media, modifying a cell transduction procedure, modifying a vector for use in a transduction procedure, and modifying a cell isolation procedure.

7. A method for assessing and optimizing cellular quality of a cell-based therapy, comprising:
   (a) determining one or more molecular characteristics of a pre-modified cell culture;
   (b) optimizing one or more parameters of an automated cell engineering systems to alter one the one or more molecular characteristics of the pre-modified cell culture;
   (c) activating the pre-modified cell culture with an activation reagent to produce an activated cell culture;
   (d) transducing the activated immune cell culture with a vector, to produce a transduced cell culture;
   (e) expanding the transduced cell culture;
   (f) concentrating the expanded cell culture of (e);
   (g) harvesting the concentrated cell culture of (f) to produce a genetically modified cell culture;
   (h) determining the one or more molecular characteristics of the cell culture selected from the group consisting of a gene expression, a protein expression, an mRNA expression, and a copy number variation during or after any one of steps (c)-(g); and
   (i) optimizing one or more parameters of any one of steps (c)-(g) to alter the one or more molecular characteristics of the cell culture.

8. The method of claim 7, wherein the one or more molecular characteristics are selected from the group consisting of a gene expression, a protein expression, an mRNA expression, and a copy number variation.

9. The method of claim 8, wherein the cell culture is an immune cell culture, a natural killer cell culture, and a cell culture for a neurodegenerative therapy.

10. The method of claim 9, wherein the immune cell culture is a T-cell culture, including wherein T-cell culture is a chimeric antigen receptor T (CAR T) cell culture.

11. The method of claim 10, wherein the one or more molecular characteristics include T-cell activation, metabolism, exhaustion, and T-cell receptor diversity.

12. The method of any one of claims 7-11, wherein the optimizing includes one or more of increasing or decreasing a flow rate of cell media, increasing or decreasing oxygen concentration, increasing or decreasing carbon dioxide concentration, increasing or decrease a glucose level, increasing or decreasing the temperature of a cell expansion, increasing or decreasing the pH of a cell media, modifying a cell transduction procedure, modifying a vector for use in a transduction procedure, and modifying a cell isolation procedure.

13. A method for assessing and optimizing cellular quality of a chimeric antigen receptor T (CAR T) cell culture, comprising:

(a) determining one or more molecular characteristics of a pre-modified T-cell culture;

(b) optimizing one or more parameters of an automated cell engineering system to alter one the one or more molecular characteristics of the pre-modified T-cell culture;

(c) activating the pre-modified T-cell culture with an activation reagent to produce an activated T-cell culture;

(d) transducing the activated T-cell culture with a vector encoding a chimeric antigen receptor, to produce a CAR T-cell culture;

(e) expanding the CAR T-cell culture;

(f) concentrating the expanded CAR T-cell culture of (e);

(g) harvesting the concentrated CAR-T cell culture of (f);

(h) determining the one or more molecular characteristics of the CAR T-cell culture during or after any one of steps (c)-(g); and

(i) optimizing one or more parameters of any one of steps (c)-(g) to alter the one or more molecular characteristics of the CAR T-cell culture.

14. The method of claim 13, wherein the T-cell culture comprises at least one accessory cell, peripheral blood mononuclear cells and/or purified T-cells, and wherein the activation reagent comprises an antibody of a dendritic cell.

15. The method of claim 15, wherein the accessory cell comprises a monocyte or a monocyte-derived cell and antigens for a T-cell receptor, including CD28, CD40, CD2, CD40L and/or ICOS.

16. The method of claim 15, wherein the antibody is a soluble antibody, comprises at least one of an anti-CD3 antibody and an anti0CD28 antibody and/or the antibody is immobilized on a surface.

17. The method of any one of claims 13-16, wherein the transducing comprises viral infection, electroporation, membrane disruption, or combinations thereof, and wherein the vector is a lentiviral vector or a retrovirus.

18. The method of any one of claims 13-17, wherein the one or more molecular characteristics include T-cell activation, metabolism, exhaustion, and T-cell receptor diversity, wherein the one or more molecular characteristics are selected from the group consisting of a gene expression, a protein expression, an mRNA expression, and a copy number variation, and wherein about 500 gene expressions are determined.

19. The method of any one of claims 13-18, wherein the one or more molecular characteristics are selected from the group consisting of a gene expression, a protein expression, an mRNA expression, and a copy number variation.

20. The method of any one of claims 13-19, wherein the optimizing includes one or more of increasing or decreasing a flow rate of cell media, increasing or decreasing oxygen concentration, increasing or decreasing carbon dioxide concentration, increasing or decrease a glucose level, increasing or decreasing the temperature of a cell expansion, increasing or decreasing the pH of a cell media, modifying a cell transduction procedure, modifying a vector for use in a transduction procedure, and modifying a cell isolation procedure.