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 ~~of the Invention~~

[0006] In some example~~embodiment~~s 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 example~~embodiment~~s, 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 example~~embodiment~~s, 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 T cell culture is a chimeric antigen receptor T (CAR T) cell culture.~~

~~6.~~5. The method of claim ~~5~~4, wherein the one or more molecular characteristics include T-cell activation, metabolism, exhaustion, and T-cell receptor diversity.

~~7. The method of any one of claims 1-6, wherein the optimizing in (d) occurs before, during, and/or after the genetically modifying.~~

~~8.~~6. The method of any one of claims 1-~~7~~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.

~~9.~~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.

~~10.~~8. The method of claim ~~9~~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.

~~11.~~9. The method of claim ~~8~~9 ~~or claim 10~~, wherein the cell culture is an immune cell culture, a natural killer cell culture, and a cell culture for a neurodegenerative therapy.

~~12.~~10. The method of claim ~~11~~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.

~~13. The method of claim 12, wherein T-cell culture is a chimeric antigen receptor T (CAR T) cell culture.~~

~~14.~~11. The method of claim ~~13~~10, wherein the one or more molecular characteristics include T-cell activation, metabolism, exhaustion, and T-cell receptor diversity.

~~15.~~12. The method of any one of claims ~~9-14~~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.

~~16.~~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.

~~17. The method of claim 16, wherein the method produces at least about 100 million viable CAR T-cells~~

~~18. The method of claim 16, wherein the method produces at least about 2 billion viable CAR T-cells~~

~~19.~~14. The method of ~~any one of~~ claim~~s~~ ~~16-19~~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.

~~20. The method of any one of claims 16-19, wherein the T-cell culture comprises at least one accessory cell.~~

~~21.~~15. The method of claim ~~20~~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.

~~22. The method of claim 20, wherein the accessory cell comprises antigens for a T-cell receptor, including CD28, CD40, CD2, CD40L and/or ICOS.~~

~~23. The method of any one of claims 16-22, wherein the activation reagent comprises an antibody or a dendritic cell.~~

~~24.~~16. The method of claim ~~23~~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.

~~25. The method of claim 24, wherein the surface is a surface of a bead.~~

~~26. The method of claim 23, wherein the antibody is a soluble antibody.~~

~~27. The method of claim 23, wherein the antibody comprises at least one of an anti-CD3 antibody and an anti-CD28 antibody.~~

~~28.~~17. The method of any one of claims ~~16~~13-~~27~~16, wherein the transducing comprises viral infection, electroporation, membrane disruption, or combinations thereof, and wherein the vector is a lentiviral vector or a retrovirus.

~~29. The method of any one of claims 16-28, wherein the vector is a lentiviral vector or a retrovirus.~~

~~30.~~18. The method of any one of claims ~~16~~13-~~29~~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.

~~31.~~19. The method of any one of claims ~~16~~13-~~30~~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.

~~32. The method of claim 31, wherein at least about 500 gene expressions are determined.~~

~~33. The method of claim 31, wherein at least about 700 gene expressions are determined.~~

~~34. The method of claim 31, wherein about 780 gene expressions are determined.~~

~~35.~~20. The method of any one of claims ~~16~~13-~~34~~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.

~~36. A method for assessing and optimizing cellular quality of a cell culture, 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.~~