subject and the compositions demonstrate an increased ability to produce a positive clinical outcome.

SUMMARY

This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

In one aspect, the present disclosure refers to an *in vitro* method for determining the immunotherapeutic potency of an activated and not fully mature human dendritic cell composition, the method comprising the steps of:

i) preparing activated human dendritic cells that have not fully matured as measured by retaining the ability to uptake and process antigen;

ii) determining the relative amounts of Interleukin 6 (IL-6), Interleukin 8 (IL-8), Interleukin 12 p40 (IL-12 p40), and tumor necrosis factor α (TNFα) produced by the activated and not fully mature human dendritic cell compositioncells;

iii) comparing the determined amount of IL-6, IL-8, IL-12 p40 and TNFα to a threshold amount; and

iv) determining that the activated and not fully mature human dendritic cell composition is of low immunotherapeutic potency if the combinationall of IL-6, IL-8, IL-12 p40, and TNFα is are below threshold; or that the activated and not fully mature dendritic cell composition is of high immunotherapeutic potency if the combinationall of IL-6, IL-8, IL-12 p40, and TNFα is are above threshold.

In yet another aspect, the present disclosure refers to an *in vitro* method for selecting a patient having a solid tumor that will respond to administration of activated and not fully mature human dendritic cells by determining the immunotherapeutic potency of an activated and not fully mature human dendritic cell composition derived from the patient, the method comprising the steps of:

i) preparing a composition comprising activated and not fully mature human dendritic cells as measured by the ability to efficiently uptake and process antigen;

ii) determining the relative amounts of IL-6, IL-8, IL-12 p40, and TNFα produced by the activated and not fully mature human dendritic cells;

iii) comparing the determined amount of IL-6, IL-8, IL-12 p40 and TNFα to a threshold amount; and

iv) determining that the and not fully mature human activated dendritic cell composition is of low immunotherapeutic potency ~~if the combination of, and/or~~ all of IL-6, IL-8, IL-12 p40 and~~/or~~ TNFα is below threshold, or that the activated and not fully mature human dendritic cell composition is of high immunotherapeutic potency if ~~the combination~~all of IL-6, IL-8, IL-12 p40 and TNFα ~~is~~are above threshold and selecting those patients with activated and not fully mature human dendritic cells with IL-6, IL-8, IL-12 p40, and TNFα that are above the threshold as patients that will respond.

In yet another aspect, the present disclosure refers to an *in vitro* method for selecting a patient having a solid tumor that will not respond to administration of activated and not fully mature human dendritic cells by determining the immunotherapeutic potency of an activated and not fully mature human dendritic cell composition derived from the patient, the method comprising the steps of:

i) preparing activated and not fully mature human dendritic cells as measured by the ability to efficiently uptake and process antigen;

ii) determining the relative amounts of IL-6, IL-8, IL-12 p40 and TNFα produced by the activated and not fully mature human dendritic cells;

iii) comparing the determined amount of IL-6, IL-8, IL-12 p40 and TNFα to a threshold amount; and

iv) determining that the activated and not fully mature human dendritic cell composition is of low immunotherapeutic potency if ~~one or any combination of, and/or~~ all of IL-6, IL-8, IL-12 p40 and~~/or~~ TNFα is below threshold, or that the activated and not fully mature human dendritic cell composition is of high immunotherapeutic potency if ~~the combination~~all of IL-6, IL-8, IL-12 p40 and TNFα is above threshold and selecting those patients with the activated and not fully mature human dendritic cells with IL-6, IL-8, IL-12 p40, and TNFα that are below the threshold as patients that will not respond.

In yet another aspect, the present disclosure refers to an *in vitro* method for selecting a dendritic cell maturation agent~~s~~ for producing activated and not fully mature human dendritic cells with increased immunotherapeutic potency, the method comprising the steps of:

i) preparing activated and not fully mature human dendritic cells by contacting immature human dendritic cells with a test dendritic cell maturation agent agent for a time sufficient to induce maturation of the immature dendritic cells but not allowing the dendritic cells to fully mature as determined by the ability of the dendritic cells to efficiently uptake and process antigen;

ii) determining the relative amounts of IL-6, IL-8, IL-12 p40 and TNFα ~~produced by the activated dendritic cells~~;

iii) comparing the determined amount of IL-6, IL-8, IL-12 p40 and TNFα to a threshold amount; and

iv) determining that the activated and not fully mature human dendritic cell composition is of low immunotherapeutic potency if ~~one or any combination of, and/or~~ all of IL-6, IL-8, IL-12 p40 and/or TNFα ~~is~~ are below threshold, or that the activated and not fully mature human dendritic cell composition is of high immunotherapeutic potency if ~~the combination~~ all of IL-6, IL-8, IL-12 p40 and TNFα are~~is~~ above threshold and selecting the dendritic cell maturation agent that induces the *in vitro* ~~the~~ production of the activated and not fully mature human dendritic cell~~s~~ composition with IL-6, IL-8, IL-12 p40, and TNFα that are above the threshold.

DESCRIPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

CLAIMS

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. An *in vitro* method for determining the immunotherapeutic potency of an activated and not fully mature human dendritic cell composition, the method comprising the steps of:

i) preparing activated human dendritic cells that have not fully matured as measured by retaining the ability to uptake and process antigen;

ii) determining the relative amounts of Interleukin 6 (IL-6), Interleukin 8 (IL-8), Interleukin 12 p40 (IL-12 p40), and tumor necrosis factor α (TNFα) produced by the activated and not fully mature human dendritic cell compositioncells;

iii) comparing the determined amount of IL-6, IL-8, IL-12 p40 and TNFα to a threshold amount; and

iv) determining that the activated and not fully mature human dendritic cell composition is of low immunotherapeutic potency if the combination all of IL-6, IL-8, IL-12 p40, and/or TNFα is are below threshold; or that the activated and not fully mature human dendritic cell composition is of high immunotherapeutic potency if the combinationall of IL-6, IL-8, IL-12 p40, and TNFα is are above threshold.

2. An *in vitro* method for selecting a patient having a solid tumor that will respond to administration of activated and not fully mature human dendritic cells by determining the immunotherapeutic potency of an activated and not fully mature human dendritic cell composition derived from the patient, the method comprising the steps of:

i) preparing a composition comprising activated and not fully mature human dendritic cells as measured by the ability to efficiently uptake and process antigen;

ii) determining the relative amounts of IL-6, IL-8, IL-12 p40, and TNFα produced by the activated and not fully mature human dendritic cells;

iii) comparing the determined amount of IL-6, IL-8, IL-12 p40 and TNFα to a threshold amount; and

iv) determining that the activated and not fully mature human dendritic cell composition is of low immunotherapeutic potency if ~~the combination of, and/or~~ all of IL-6, IL-8, IL-12 p40 and~~/or~~ TNFα ~~is~~ are below threshold, or that the activated and not fully mature human dendritic cell composition is of high immunotherapeutic potency if ~~the combination~~ all of IL-6, IL-8, IL-12 p40, and TNFα ~~is~~ are above threshold and selecting those patients with activated and not fully mature human dendritic cells with IL-6, IL-8, IL-12 p40, and TNFα that are above the threshold as patients that will respond.

3. An *in vitro* method for selecting a patient having a solid tumor that will not respond to administration of ~~activated dendritic cells~~activated and not fully mature human dendritic cells by determining the immunotherapeutic potency of an activated and not fully mature human dendritic cell composition derived from the patient, the method comprising the steps of:

i) preparing activated and not fully mature human dendritic cells as measure by the ability to efficiently uptake and process antigen;

ii) determining the relative amounts of IL-6, IL-8,~~ and~~ IL-12 p40, and TNFα produced by the ~~activated dendritic cells~~activated and not fully mature human dendritic cells;

iii) comparing the determined amount of IL-6, IL-8, IL-12 p40 and TNFα to a threshold amount;~~ and~~

iv) determining that the activated and not fully mature human dendritic cell composition is of low immunotherapeutic potency if ~~one or any combination of, and/or~~ all of IL-6, IL-8, IL-12 p40, and~~/or~~ TNFα ~~is~~ are below threshold, or that the activated and not fully mature human dendritic cell composition is of high immunotherapeutic potency if ~~the combination~~all of IL-6, IL-8, IL-12 p40, and TNFα are~~is~~ above threshold and selecting those patients with the ~~activated dendritic cells~~activated and not fully mature human dendritic cells with IL-6, IL-8, IL-12 p40, and TNFα that are below the threshold as patients that will not respond.

4. An *in vitro* method for selecting a dendritic cell maturation agent~~s~~ for producing ~~activated dendritic cells~~activated and not fully mature human dendritic cells with

increased immunotherapeutic potency for use in treating a solid tumor, the method comprising the steps of:

i) preparing activated and not fully mature human dendritic cells by contacting immature human dendritic cells with a test dendritic cell maturation agent for a time sufficient to induce maturation of the immature dendritic cells but not allowing the dendritic cells to fully mature as determined by the ability of the dendritic cells to efficiently uptake and process antigen;

ii) determining the relative amounts of IL-6, IL-8, IL-12 p40, and TNFα ~~produced by the activated dendritic cells~~;

iii) comparing the determined amount of IL-6, IL-8, IL-12 p40, and TNFα to a threshold amount; and

iv) determining that the activated and not fully mature human dendritic cell composition is of low immunotherapeutic potency if ~~one or any combination of, and/or~~ all of IL-6, IL-8, IL-12 p40 and~~/or~~ TNFα ~~is~~ are below threshold, or that the activated and not fully mature human dendritic cell composition is of high immunotherapeutic potency if ~~the combination~~ all of IL-6, IL-8, IL-12 p40, and TNFα ~~is~~ are above threshold and selecting the dendritic cell maturation agent that induces the *in vitro* production of ~~activated dendritic cells~~the activated and not fully mature human dendritic cell composition with IL-6, IL-8, IL-12 p40, and TNFα that are above the threshold.

5. The *in vitro* method of any one of claims 1 through 4, wherein the ~~activated dendritic cells~~activated and not fully mature human dendritic cells produce 50 to 200 ng/1 million cells/24 hours of IL-6; 500 to 2000 ng/1 million cells/24 hours of IL-8; and/or at least 75 to 100 ng/1 million cells/24 hours of the IL-12 p40.

6. The *in vitro* method according to claim 5, wherein the activated and not fully mature human dendritic cells produce 75 to 150 ng/1 million cells/24 hours of IL-6; 750 to 1500 ng/1 million cells/24 hours of IL-8; and/or at least 100 ng/1 million cells/24 hours of IL-12 p40.

7. The *in vitro* method according to claim 6, wherein the ~~activated dendritic cells~~activated and not fully mature human dendritic cells produce 100 ng/1 million cells/24

hours of IL-6, and 1000 ng/1 million cells/24 hours of IL-8, and/or at least 100 ng/1 million cells/24 hours of IL-12 p40.

8. The *in vitro* method according to any one of claims 1 through 47, wherein the ~~activated dendritic cells~~activated and not fully mature human dendritic cells are prepared by the following steps:

i) isolating a cell population comprising human peripheral blood mononuclear cells (PBMCs) from peripheral blood;

ii) enriching the cell population comprising human PBMCs for human monocytic dendritic cell precursors;

iii) culturing the cell population enriched for human monocytic dendritic cell precursors with a tissue culture medium supplemented with an effective amount of a dendritic cell differentiation agent for a time period sufficient to differentiate the human monocytic dendritic cell precursors into immature human dendritic cells;

iv) culturing the cell population enriched for immature human dendritic cells with an effective amount of a dendritic cell maturation agent to activate and not fully mature the immature human dendritic cells as determined by the efficiency of the human dendritic cells to uptake and process antigen; and

v) isolating and washing the activated and not fully mature human dendritic cells.

9. The *in vitro* method according to any one of claims 1 through ~~4,~~7, wherein the ~~activated dendritic cells~~activated and not fully mature human dendritic cells are prepared by the following steps:

i) isolating a cell population comprising human monocytic dendritic cell precursors;

ii) culturing the cell population enriched for ~~–~~human monocytic dendritic cell precursors with a tissue culture medium supplemented with an effective amount of a dendritic cell differentiation agent for a time period sufficient to differentiate the human monocytic dendritic cell precursors into immature human dendritic cells;

iii) culturing the cell population enriched for immature human dendritic cells with an effective amount of a dendritic cell maturation agent to activate and induce the maturation of the immature human dendritic cells; and

iv) isolating and washing the activated and not fully mature human dendritic cells.

10. The *in vitro* method according to claim 9, wherein the monocytic dendritic cell precursors are obtained from skin, spleen, bone marrow, thymus, lymph nodes, umbilical cord blood, or peripheral blood.

11. The *in vitro* method according to any one of claims ~~8~~ 9 - 10, wherein the monocytic dendritic cell precursor cells are non-activated monocytic dendritic cell precursors.

12. The *in vitro* method according to any one of claims ~~8~~ 9 - 11, wherein the monocytic dendritic cell precursors are obtained from the individual subject to be treated.

13. The *in vitro* method according to any one of claims 8 - 11, wherein the peripheral blood mononuclear cells or the monocytic dendritic cell precursors are obtained from a healthy individual subject HLA-matched to the individual subject to be treated.

14. The *in vitro* method according to any one of claims 8 and 9, wherein the dendritic cell differentiation agent is GM-CSF without any other cytokine, or GM-CSF in combination with IL-4, IL-7, IL-13 or IL-15.

15. The *in vitro* method according to any one of claims 8 and 9, wherein the dendritic cell maturation agent is inactivated Bacillus Calmette-Guerin (BCG), interferon γ (IFNγ), lipopolysaccharide (LPS), tumor necrosis factor α (TNFα), an imidazoquinoline compound, a synthetic double stranded polyribonucleotide, a agonist of a Toll-like receptor (TLR), a sequence of nucleic acids containing unmethylated CpG motifs known to induce the maturation of dendritic cells, or any combination thereof; preferably wherein the dendritic cell maturation agent is a combination of BCG and IFNγ.

16. The *in vitro* method according to claim 15, wherein the inactivated BCG comprises whole BCG, cell wall constituents of BCG, BCG-derived lipoarabidomannans, or BCG components

17. The *in vitro* method according to claim 16, wherein the inactivated BCG is heat-inactivated BCG, formalin-treated BCG, or heat-inactivated and formalin treated BCG.

18. The *in vitro* method according to any one of claims 15-17, wherein the effective amount of BCG is about 10^5 to 10^7 cfu per milliliter of tissue culture media and the effective amount of IFNγ is about 100 to about 1,000 Units per milliliter of tissue culture media.

19. The *in vitro* method according to claim 15, wherein the imidazoquinoline compound is an imidazoquinoline-4-amine compound.

20. The *in vitro* method according to claim 19, wherein the imidazoquinoline-4-amine compound is 4-amino-2-ethoxymethyl-α,α-dimethyl-lH-imidazol[4,5-c]quinolin-l-5 ethanol or 1-(2-methylpropyl)-1H-imidazo[4,5-c]quinolin-4-amine, or a derivative thereof.

21. The *in vitro* method according to claim 15, wherein the synthetic double stranded polyribonucleotide is poly[I]:poly[C(12)U].