rapid and robust upregulation of numerous inflammatory mediators, including cytokines, chemokines and interferons. Co-incident with the expression of these genes is the migration of DCs to draining lymph nodes (dLN) and the upregulation of factors important for T cell activation, such as MHC and co-stimulatory molecules. Thus, the process of PRR signaling leads to a shift in DC activities from a non-stimulatory (naïve) state to an "activated" state (K. Inaba, *et al. J. Exp. Med.*, vol. 191, no. 6, pp. 927–36, Mar. 2000 I. Mellman and R. M. Steinman, *Cell*, vol. 106, no. 3, pp. 255–8, Aug. 2001).

SUMMARY

There is a need to diversify current approaches to cancer immunotherapy. Accordingly, the present application is directed to methods of generating a population of therapeutic dendritic cells, methods of inducing an immune response in a subject, methods for treating cancer, methods of hyperactivating dendritic cells (DCs) which induce T helper type I (TH1) and cytotoxic T lymphocyte (CTL) responses in the absence of TH2 immunity. Hyperactivating stimuli drive T cell responses that protect against tumors that are sensitive or resistant to PD-1 inhibition. These protective responses depend on inflammasomes in DCs and can be generated using tumor lysates as immunogens.

In one aspect, the present disclosure refers to a method of generating a population of therapeutic dendritic cells, the method comprising:

priming the dendritic cells that have been obtained from a cell donor with a TLR ligand *ex vivo*;

culturing the primed dendritic cells with a non-canonical inflammasome-activating lipid *ex vivo*; and

loading the dendritic cells with an immunogen, thereby generating a population of therapeutic dendritic cells.

In one aspect, the present disclosure refers to use of a population of therapeutic dendritic cells in the manufacture of a medicament for inducing an immune response in a subject, wherein the population of therapeutic dendritic cells are living dendritic cells that have been obtained from a cell donor and primed with a TLR ligand *ex vivo*; and wherein the primed dendritic cells have been cultured with a non-canonical inflammasome-activating lipid *ex vivo* and loaded with an immunogen to thereby generate the population of therapeutic dendritic cells.

In one aspect, the present disclosure refers to use of a population of therapeutic dendritic cells in the manufacture of a medicament for treating cancer in a subject, wherein the population of therapeutic dendritic cells are living dendritic cells that have been obtained from a cell donor and primed with a TLR ligand *ex vivo*; and wherein the primed dendritic cells have been cultured with a non-canonical inflammasome-activating lipid *ex vivo* and loaded with an immunogen to thereby generate the population of therapeutic dendritic cells.

In certain ~~embodiment~~examples, a method of inducing a protective immune response to an immunogen in a subject, comprises obtaining dendritic cells, culturing the dendritic cells with an effective amount of a non-canonical inflammasome-activating lipid *ex vivo* and administering to the subject the living dendritic cells in an effective amount to enhance a protective immune response, thereby inducing a protective immune response. In some ~~embodiment~~examples, the therapeutically effective amount of the non-canonical inflammasome-activating lipid hyperactivates the dendritic cells.

In certain ~~embodiment~~examples, the dendritic cells are optionally cultured with an immunogen *ex vivo*. In certain ~~embodiment~~examples, the dendritic cells are optionally cultured with a cytokine *ex vivo*.

In certain ~~embodiment~~examples, the non-canonical inflammasome-activating lipid comprises: 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphorylcholine (PAPC), oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (oxPAPC), species of oxPAPC, components thereof or combinations thereof.

In certain ~~embodiment~~examples, the method further comprises administering a chemotherapeutic agent.

In certain ~~embodiment~~examples, the treatment approaches disclosed herein could also be combined with any of the following therapies: radiation, chemotherapy, surgery, therapeutic antibodies, immunomodulatory agents, proteasome inhibitors, pan-deacetylase (DAC) inhibitors, histone deacetylase (HDAC) inhibitors, checkpoint inhibitors, adoptive cell therapies include CAR T and NK cell therapy and vaccines.

Preferably, the methods described herein inhibit the growth or progression of cancer, e.g., a tumor, or a viral infection in a subject. For example, the methods described herein inhibit the growth of a tumor by at least 1%, e.g., by at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or 100%. In other cases, the methods described herein reduce the size of a tumor by at least 1 mm in diameter, e.g., by at least 2 mm in diameter, by at least 3 mm in diameter, by at least 4 mm in diameter, by at least 5 mm in diameter, by at least 6 mm in diameter, by at least 7 mm in diameter, by at least 8 mm in diameter, by at least 9 mm in diameter, by at least 10 mm in diameter, by at least 11 mm in diameter, by at least 12 mm in diameter, by a least 13 mm in diameter, by at least 14 mm in diameter, by at least 15 mm in diameter, by at least 20 mm in diameter, by at least 25 mm in diameter, by at least 30 mm in diameter, by at least 40 mm in diameter, by at least 50 mm in diameter or more. In some cases, the subject has had the bulk of the tumor resected.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this ~~invention~~disclosure belongs. Methods and materials are described herein for use in the present ~~invention~~disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the ~~invention~~disclosure will be apparent from the following detailed description and figures, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1H are a series of graphs demonstrating that hyperactive DCs are superior antigen-presenting cells and drive TH1-skewed immune responses, with no evidence of TH2

What is claimed:

1. A method of generating a population of therapeutic dendritic cells, the method comprising:

~~obtaining living dendritic cells from a cell donor;~~

priming the dendritic cells that have been obtained from a cell donor with a TLR ligand *ex vivo*;

culturing the primed dendritic cells with a non-canonical inflammasome-activating lipid *ex vivo*; and

loading the dendritic cells with an immunogen, thereby generating a population of therapeutic dendritic cells.

2. Use of a population of therapeutic dendritic cells in the manufacture of a medicament for~~A method of~~ inducing an immune response in a subject, wherein the population of therapeutic dendritic cells are~~comprising:~~

~~obtaining~~ living dendritic cells that have been obtained from a cell donor~~;~~ and

~~priming the dendritic cells~~primed with a TLR ligand *ex vivo*; and wherein

~~culturing~~ the primed dendritic cells have been cultured with a non-canonical inflammasome-activating lipid *ex vivo*~~;~~ and

~~loading the dendritic cells~~loaded with an immunogen~~,~~ to thereby generat~~e~~~~ing~~ ~~a~~ the population of therapeutic dendritic cells~~; and~~

~~administering the living dendritic cells to the subject, thereby inducing an immune response in a subject~~.

3. Use of a population of therapeutic dendritic cells in the manufacture of a medicament for~~A method of~~ treating cancer in a subject, wherein the population of therapeutic dendritic cells are~~comprising:~~

~~obtaining~~ living dendritic cells that have been obtained from a cell donor~~;~~ and

~~priming the dendritic cells~~primed with a TLR ligand *ex vivo*; and wherein

~~culturing~~ the primed dendritic cells have been cultured with a non-canonical inflammasome-activating lipid *ex vivo*~~;~~ and

~~loading the dendritic cells~~loaded with an immunogen~~,~~ to thereby generat~~e~~~~ing~~ ~~a~~ the population of therapeutic dendritic cells~~; and~~

~~administering the living dendritic cells to the subject, thereby treating cancer in a subject~~.

4. The method or use of any one of claims 1–3, wherein the cell donor and/or subject is a mammalian subject.

5. The method or use of claim 4, wherein the cell donor and/or subject is a human subject.

6. The method or use of any one of claims 1–5, wherein ~~obtaining~~ the dendritic cells have been obtained from a cell donor ~~comprises~~ by:

harvesting progenitor cells from the cell donor; and

culturing the progenitor cells *ex vivo* under conditions effective to induce differentiation, thereby obtaining dendritic cells from the cell donor.

7. The method or use of any one of claims 1–5, wherein ~~obtaining~~ the dendritic cells have been obtained from a subject ~~comprises~~ by harvesting *in vivo* differentiated dendritic cells from the cell donor.

8. The method or use of any one of claims 1–7, wherein the immunogen is an immunogen from an infectious agent associated with the development of cancer.

9. The method or use of any one of claims 1–7, wherein the immunogen is a cancer antigen.

10. The method or use of any one of claims 1–7, wherein the immunogen is whole tumor lysate.

11. The method or use of claim 9 or claim 10, wherein the immunogen is autologous.

12. The method or use of any one of claims 1–11, wherein said priming and said culturing occur simultaneously.

13. The method or use of any one of claims 1–11, wherein said priming occurs before said culturing.

14. The method or use of any one of claims 1–13, wherein the TLR ligand is selected from a TLR1 ligand, a TLR2 ligand, a TLR3 ligand, a TLR4 ligand, a TLR5 ligand, a TLR6 ligand, a TLR7 ligand, a TLR8 ligand, a TLR9 ligand, a TLR10 ligand, a TLR11 ligand, a TLR12 ligand, a TLR13 ligand, and combinations thereof.

15. The method or use of any one of claims 1–13, wherein the TLR ligand is a TLR4 ligand.

16. The method or use of claim 15, wherein the TLR4 ligand is selected from monophosphoryl lipid A (MPLA), lipopolysaccharide (LPS), or combinations thereof.

17. The method or use of any one of claims 1–16, wherein the non-canonical inflammasome-activating lipid comprises a species of oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (oxPAPC).

18. The method or use of any one of claims 1–16, wherein the non-canonical inflammasome-activating lipid comprises 2-[[(2R)-2-[(E)-7-carboxy-5-hydroxyhept-6-enoyl]oxy-3-hexadecanoyloxypropoxy]-hydroxyphosphoryl]oxyethyl-trimethylazanium (**HOdiA-PC**), [(2R)-2-[(E)-7-carboxy-5-oxohept-6-enoyl]oxy-3-hexadecanoyloxypropyl] 2-(trimethylazaniumyl)ethyl phosphate (**KOdiA-PC**), 1-palmitoyl-2-(5-hydroxy-8-oxo-octenoyl)-*sn*-glycero-3-phosphorylcholine (**HOOA-PC**), 2-[[(2R)-2-[(E)-5,8-dioxooct-6-enoyl]oxy-3-hexadecanoyloxypropoxy]-hydroxyphosphoryl]oxyethyl-trimethylazanium (**KOOA-PC**), [(2R)-3-hexadecanoyloxy-2-(5-oxopentanoyloxy)propyl] 2-(trimethylazaniumyl)ethyl phosphate (**POVPC**), [(2R)-2-(4-carboxybutanoyloxy)-3-hexadecanoyloxypropyl] 2-(trimethylazaniumyl)ethyl phosphate (**PGPC**), [(2R)-3-hexadecanoyloxy-2-[4-[3-[(E)-[2-[(Z)-oct-2-enyl]-5-oxocyclopent-3-en-1-ylidene]methyl]oxiran-2-yl]butanoyloxy]propyl] 2-(trimethylazaniumyl)ethyl phosphate (**PECPC**), [(2R)-3-hexadecanoyloxy-2-[4-[3-[(E)-[3-hydroxy-2-[(Z)-oct-2-enyl]-5-

oxocyclopentylidene]methyl]oxiran-2-yl]butanoyloxy]propyl] 2-(trimethylazaniumyl)ethyl phosphate (**PEIPC**), or a combination thereof.

19. The method or use of any one of claims 1–16, wherein the non-canonical inflammasome-activating lipid comprises [(2R)-2-(4-carboxybutanoyloxy)-3-hexadecanoyloxypropyl] 2-(trimethylazaniumyl)ethyl phosphate (**PGPC**).

20. The ~~method~~ use of any one of claims 2–19, wherein ~~further comprising administering~~ an anti-cancer agent is to be further administered to the subject.

21. The ~~method~~ use of claim 20, wherein the anti-cancer agent is a chemotherapeutic agent.