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:

culturing progenitor cells obtained from a cell donor *ex vivo* in the presence of transforming growth factor β (TGF-β), stem cell factor (SCF), or fms-like tyrosine kinase 3 ligand (FLT3-L), or a combination thereof under conditions effective to induce differentiation, thereby obtaining living dendritic cells from the cell donor;

priming the living dendritic cells with a TLR ligand *ex vivo*;

culturing the primed dendritic cells with a non-canonical inflammasome-activating lipid *ex vivo*, wherein the non-canonical inflammasome-activating lipid comprises a species of oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (oxPAPC); and

loading the primed and cultured 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,

wherein the non-canonical inflammasome-activating lipid comprises a species of oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC).

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,

wherein the non-canonical inflammasome-activating lipid comprises a species of oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC), and

wherein the immunogen is a cancer antigen or whole tumor lysate, or wherein the immunogen is an immunogen from an infectious agent associated with the development of cancer.

In certain 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 examples, the therapeutically effective amount of the non-canonical inflammasome-activating lipid hyperactivates the dendritic cells.

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

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

What is claimed:

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

culturing progenitor cells obtained from a cell donor *ex vivo* in the presence of transforming growth factor β (TGF-β), stem cell factor (SCF), or fms-like tyrosine kinase 3 ligand (FLT3-L), or a combination thereof under conditions effective to induce differentiation, thereby obtaining living dendritic cells from the cell donor;

priming the living dendritic cells with a TLR ligand *ex vivo*;

culturing the primed dendritic cells with a non-canonical inflammasome-activating lipid *ex vivo*, wherein the non-canonical inflammasome-activating lipid comprises a species of oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (oxPAPC); and

loading the primed and cultured 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 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,

wherein the non-canonical inflammasome-activating lipid comprises a species of oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC).

3. 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,

wherein the non-canonical inflammasome-activating lipid comprises a species of oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC), and

wherein the immunogen is a cancer antigen or whole tumor lysate, or wherein the immunogen is an immunogen from an infectious agent associated with the development of cancer.

4. The method or use of claim 2 or claim 3, wherein the subject is a mammalian subject.

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

6. The method or use of any one of claims 1–5, wherein the cell donor is a mammalian cell donor.

7. The method or use of claim 6, wherein the cell donor is a human cell donor.

8. The method or use of claim 1 or claim 2, wherein the immunogen is an immunogen from an infectious agent associated with the development of cancer.

9. The method or use of claim 1 or claim 2, wherein the immunogen is a cancer antigen.

10. The method or use of claim 1 or claim 2, 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 occurs before said culturing.

13. The method or use of any one of claims 1–12, 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.

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

15. The method or use of claim 14, wherein the TLR4 ligand is selected from monophosphoryl lipid A (MPLA), lipopolysaccharide (LPS), or combinations thereof.

16. The method or use of any one of claims 1-15, wherein the TLR ligand is a TLR agonist.

17. 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.

18. 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**).

19. The use of any one of claims 2–18, wherein an anti-cancer agent is to be further administered to the subject.

20. The use of claim 19, wherein the anti-cancer agent is a chemotherapeutic agent.