hypersensitivity response indicates a cellular response (see, e.g., Roychowdhury, et al. (2005) AAPS J. E834-E846).

[0007] Autophagy is a homeostatic process by which cytosolic components and organelles are delivered to the lysosome for degradation. Autophagy is also associated with innate and adaptive immune responses to intracellular pathogens whereby cytosolic antigens are loaded onto major histocompatibility complex (MHC) class II molecules for CD4* T-cell recognition.

**Description**

[0008] As used herein, including the appended claims, the singular forms of words such as "a," "an," and "the" include their corresponding plural references unless the context clearly dictates otherwise. All references cited herein are incorporated by reference to the same extent as if each individual publication, patent, published patent application, and sequence listing, as well as figures and drawings in said publications and patent documents, was specifically and individually indicated to be incorporated by reference.

**Summary of the Disclosure**

**[0009]** In one aspect there is provided a melanoma vaccine comprising:

at least one dendritic cell from a subject that has melanoma,

wherein the at least one dendritic cell has been contacted *in vitro* with at least one melanoma tumor cell from the same subject

wherein the at least melanoma tumor cell is non-dividing, autophagic, and non-apoptotic

**[0009a]** In one aspect there is provided a composition comprising:

interferon-gamma (IFN-gamma) treated melanoma cells from a subject, and antigen presenting cells (APCs) from the same subject, wherein the melanoma cells are autophagic; and non-apoptotic.

**[0009b]** In one aspect there is provided use of an immunologically effective amount of the composition as described herein or the melanoma vaccine as described herein in the manufacture of a medicament for stimulating an immune response against a melanoma-specific antigen in a subject having melanoma.

**[0009c]** In one aspect there is provided a method for preparing a melanoma vaccine, comprising melanoma cells and dendritic cells from the same subject, the method comprising.

treating one or more melanoma cells is with an agent that prevents cell division;

treating the one or more melanoma cells *in vitro* with interferon-gamma (IFN-gamma) or with an IFN-gamma mimetic; selecting the melanoma cells that are autophagic and non-apoptotic;

rejecting melanoma cells that are non-autophagic and apoptotic;

contacting the melanoma cells that are autophagic and non-apoptotic with one or more autologous dendritic cells; and

allowing uptake of at least one melanoma-derived peptide from the melanoma cells by the one or more autologous dendritic cells to produce a melanoma vaccine.

**[0009d]** In one aspect there is provided a method for preparing a melanoma vaccine, comprising:

treating melanoma cells obtained from a subject with melanoma with an agent that prevents cell division;

treating the melanoma cells with IFN-gamma or an IFN-gamma mimetic;

selecting melanoma cells that are autophagic and non-apoptotic; and

contacting the selected melanoma cells with autologous dendritic cells from the same subject to form a melanoma vaccine.

**[0009e]** In one aspect there is provided a composition comprising a melanoma vaccine prepared by the method as described herein.

**[0009f]** In one aspect there is provided use of the melanoma vaccine as described herein in the manufacture of a medicament for stimulating an immune response against a melanoma-specific antigen in a subject with melanoma.

**[0009g]** In one aspect there is provided use of the composition as described herein in the manufacture of a medicament for stimulating an immune response against a melanoma-associated antigen in a subject with melanoma.

**[0009h]** In one aspect there is provided use of the composition as described herein in the manufacture of a medicament for the treatment of melanoma.

**[0009i]** In one aspect there is provided use of the melanoma vaccine as described herein in the manufacture of a medicament for the treatment of melanoma.

**[0009j]** In one aspect there is provided use of an immune-stimulatory amount of the melanoma vaccine as described herein, in the manufacture of a medicament for treating melanoma in a subject in need thereof.

**[0009k]** In one aspect there is provided use of an immune-stimulatory amount of the composition as described herein in the manufacture of a medicament for treating melanoma in a subject in need thereof.

**[0009l]** The present disclosure provides a method for preparing a dendritic cell vaccine, comprising treating melanoma cells acquired from a first subject with an agent that prevents cell division, treating the melanoma cells with IFN-gamma or an IFN-gamma mimetic, selecting

melanoma cells that are autophagic and non-apoptotic melanoma cells, and contacting the selected melanoma cells with autologous dendritic cells from the same first subject. Also provided is a composition that comprises a dendritic cell vaccine, as prepared by the above method. What is also provided by the disclosure is a method for stimulating immune response against a melanoma-specific antigen, comprising administering the above composition to a subject that has melanoma.

[0010] A vaccine comprising a population of mammalian dendritic cells from a given subject that is diseased with melanoma and wherein the subject comprises melanoma cells, wherein the melanoma cells comprised by the subject include melanoma cells that are autophagic and non-apoptotic when treated in vitro with IFN-gamma or IFN-gamma mimetic, wherein the dendritic cells comprise melanoma-specific peptide antigens, wherein said melanoma-specific peptide antigens are

**What is claimed is:**

Claim 1. A population of dendritic cells from a subject comprising:

melanoma-specific peptides from melanoma cells taken from a subject that has melanoma,

wherein said melanoma-specific peptides are acquired in vitro by dendritic cells from said melanoma cells that are treated in vitro with IFN-gamma or IFN-gamma mimetic,

wherein greater than 60 percent (%) of said melanoma cells that are treated in vitro with IFN-gamma or IFN-gamma mimetic are autophagic and non-apoptotic, and

wherein the dendritic cells and melanoma cells are from the same subject.

Claim 2. The population of dendritic cells of claim 1, wherein:

greater than 80% of said melanoma cells that are treated in vitro with IFN-gamma or IFN-gamma mimetic are autophagic and non-apoptotic.

Claim 3. A vaccine for the subject of claim 1, comprising the population of mammalian dendritic cells of claim 1.

Claim 4. The dendritic cells of claim 1, wherein essentially all of the melanoma-specific peptides that are acquired in vitro, are from melanoma cells that are incapable of cell division

Claim 5. The dendritic cells of claim 1, wherein essentially all of the melanoma-specific peptides are from melanoma cells that are incapable of cell division because the melanoma cells are irradiated.

Claim 6. The dendritic cells of claim 1, wherein essentially all of the melanoma-specific peptides are from melanoma cells that are incapable of cell division because the chromosomes of the melanoma cells are cross-linked by a nucleic acid cross-linking agent.

Claim 7. The dendritic cells of claim 1 comprising melanoma-specific peptides that are from melanoma cells that are treated with IFN-gamma and radiation.

Claim 8. The dendritic cells of claim 1 comprising melanoma-specific peptides, wherein all of said peptides are from melanoma cells that are treated with IFN-gamma and radiation.

Claim 9. The population of dendritic cells of claim 1, wherein the autophagic and non-apoptotic melanoma cells are acquired by adhesion to a surface.

Claim 10. The dendritic cells of claim 1, wherein essentially all of the melanoma-specific peptides are from melanoma cells that are treated in vitro to be incapable of cell division.

Claim 11. The dendritic cells of claim 1, wherein the subject is a human subject.

Claim 12. The dendritic cells of claim 1, wherein the subject is a mammal that is not human.

Claim 13. A melanoma vaccine comprising at least one mature dendritic cell from a subject that has melanoma,

wherein the at least one mature dendritic cell is prepared by contacting with at least one melanoma tumor cell from the same subject,

wherein the at least melanoma tumor cell that is contacted with the at least one mature dendritic cell is non-dividing, autophagic, and non-apoptotic.

Claim 14. A method for stimulating immune response against a melanoma-specific antigen comprising administering an immune-stimulatory amount of the dendritic cells of claim 1 to the subject of claim 1, or administering an immune-stimulatory amount of the vaccine of claim 13 to the subject of claim 13.

Claim 15. The method of claim 14, wherein the immune response that is stimulated comprises one or more of $CD4^+$ T cell response, $CD8^+$ T cell response, and B cell response.

Claim 16. The method of claim 17, wherein the $CD4^+$ T cell response, $CD8^+$ T cell response, or B cell response, can be measured by ELISPOT assays, by intracellular cytokine staining assays, by tetramer assays, or by detecting antigen-specific antibody production.

Claim 17. The method of claim 14, wherein the immune response comprises a survival time that comprises 2-year overall survival (OS), and where the 2-year overall survival is at least 60%.

Claim 18. The method of claim 14, wherein the administration comprises subcutaneous injections of the vaccine.

Claim 19. The method of claim 14, wherein the administration comprises injections of the vaccine given weekly for three months and then monthly for five months.

Claim 20. A method for preparing a dendritic cell vaccine, involving melanoma cells and dendritic cells from the same subject, the method comprising:

treating one or more melanoma cells is with an agent that prevents cell division;

treating the one or more melanoma cells in vitro with interferon-gamma (IFN-gamma) or with an IFN-gamma mimetic;

selecting the melanoma cells that are autophagic and non-apoptotic;

rejecting melanoma cells that are non-autophagic and apoptotic;

contacting the melanoma cells that are autophagic and non-apoptotic to one or more autologous dendritic cells; and

allowing uptake of at least one melanoma-derived peptide by the one or more autologous dendritic cells.

Claim 21. A composition comprising:

interferon-gamma (IFN-gamma) treated melanoma cells from a first subject, and antigen presenting cells (APCs) from the same first subject, wherein the melanoma cells are:

autophagic; and

non-apoptotic.

Claim 22. The composition of claim 21, wherein the melanoma cells are MHC class II-expressing.

Claim 23. The composition of claim 21, wherein the APCs are dendritic cells, macrophages, or B cells.

Claim 24. The composition of claim 21, wherein the melanoma cells comprise melanoma-specific peptides, and wherein the melanoma specific-peptides are not contained in the APCs.

Claim 25. The composition of claim 21, wherein the melanoma cells comprise melanoma-specific peptides, and wherein at least one of the melanoma-specific peptides is taken up by the APCs and is comprised by the APCs.

Claim 26. The composition of claim 21, wherein the IFN gamma-treated melanoma cells are loaded into the APC.

Claim 27. The composition of claim 21, wherein the IFN gamma-treated melanoma cells are not loaded into the APC.

Claim 28. The composition of claim 21, wherein autophagy of the melanoma cells is demonstrated by a test that assays microtubule-associated protein light chain 3 (LC3).

Claim 29. The composition of claim 21, wherein the melanoma cells are demonstrated to be non-apoptotic using at least one of the reagent, 7-aminoactinomycin D (7-ADD), or the reagent, annexin.

Claim 30. A method of stimulating immune response in a subject having melanoma and comprising melanoma cells, wherein the subject is the same subject as the first subject, comprising administering an immunology effective amount of the composition of claim 21.

Claim 31. A method for manufacturing the vaccine of claim 1 or the composition of claim 21, comprising contacting at least one autophagic, non-apoptotic melanoma tumor cell to at least one antigen presenting cell (APC), wherein the at least one autophagic, non-apoptotic melanoma tumor cell is from a first human subject, and wherein the at least one APC is from the same first human subject.

Claim 32. A method for preparing a dendritic cell vaccine, comprising: treating melanoma cells acquired from a first subject with an agent that prevents cell division; treating the melanoma cells with IFN-gamma or an IFN-gamma mimetic; selecting melanoma cells that are autophagic and non-apoptotic; and, contacting the selected melanoma cells with autologous dendritic cells from the same first subject.

Claim 33. A composition that comprises a dendritic cell vaccine, as prepared by the method of claim 32.

Claim 34. A method for stimulating immune response against a melanoma-specific antigen, comprising administering the composition of claim 33 to a subject that has melanoma.

What is claimed is:

1. A melanoma vaccine comprising:

at least one dendritic cell from a subject that has melanoma,

wherein the at least one dendritic cell has been contacted *in vitro* with at least one melanoma tumor cell from the same subject,

wherein the at least melanoma tumor cell is non-dividing, autophagic, and non-apoptotic.

2. A composition comprising:

interferon-gamma (IFN-gamma) treated melanoma cells from a subject, and antigen presenting cells (APCs) from the same subject, wherein the melanoma cells are autophagic; and non-apoptotic.

3. The composition of claim 2, wherein the melanoma cells express MHC class II.

4. The composition of claim 2, wherein the APCs are dendritic cells, macrophages, or B cells.

5. The composition of claim 2, wherein the melanoma cells comprise melanoma-specific peptides, and as a result of the contacting step at least one of the melanoma-specific peptides is taken up by the APCs and are partially or substantially processed by the APCs.

6. Use of an immunologically effective amount of the composition of claim 2 or the melanoma vaccine of claim 1 in the manufacture of a medicament for stimulating an immune response against a melanoma-specific antigen in a subject having melanoma.

7. The use of claim 6 wherein the immune response comprises one or more of a CD4+ T cell response, a CD8+ T cell response, and a B cell response.

8. The use of claim 7, wherein the CD4* T cell response, CD8+ T cell response, or B cell response is determined by ELISPOT assays, by intracellular cytokine staining assays, by tetramer assays, or by detecting antigen-specific antibody production.

9. The use of claim 6, wherein the immune response results in a 2-year overall survival of at least 60%.

10. The use of claim 6, wherein the administration comprises subcutaneous injections of the vaccine.

11. The use of claim 6, wherein the administration comprises injections of the vaccine given weekly for three months and then monthly for five months.

12. A method for preparing a melanoma vaccine, comprising melanoma cells and dendritic cells from the same subject, the method comprising:

treating one or more melanoma cells is with an agent that prevents cell division;

treating the one or more melanoma cells *in vitro* with interferon-gamma (IFN-gamma) or with an IFN-gamma mimetic;

selecting the melanoma cells that are autophagic and non-apoptotic;

rejecting melanoma cells that are non-autophagic and apoptotic;

contacting the melanoma cells that are autophagic and non-apoptotic with one or more autologous dendritic cells; and

allowing uptake of at least one melanoma-derived peptide from the melanoma cells by the one or more autologous dendritic cells to produce a melanoma vaccine.

13. A method for preparing a melanoma vaccine, comprising:

treating melanoma cells obtained from a subject with melanoma with an agent that prevents cell division;

treating the melanoma cells with IFN-gamma or an IFN-gamma mimetic;

selecting melanoma cells that are autophagic and non-apoptotic; and,

contacting the selected melanoma cells with autologous dendritic cells from the same subject to form a melanoma vaccine.

14. A composition comprising a melanoma vaccine prepared by the method of claim 13.

15. The melanoma vaccine of claim 1, wherein greater than 80% of said melanoma cells that are treated *in vitro* with IFN-gamma or a IFN-gamma mimetic are autophagic and non-apoptotic.

16. The melanoma vaccine of claim 1, wherein essentially all of the melanoma cells are incapable of cell division.

17. The melanoma vaccine of claim 1, wherein the melanoma cells are rendered incapable of cell divisional by irradiation or by a nucleic acid cross-linker.

18. Use of the melanoma vaccine of claim 1 in the manufacture of a medicament for stimulating an immune response against a melanoma-specific antigen in a subject with melanoma.

19. Use of the composition of claim 2 in the manufacture of a medicament for stimulating an immune response against a melanoma-associated antigen in a subject with melanoma.

20. Use of the composition of claim 2 in the manufacture of a medicament for the treatment of melanoma.

21. Use of the melanoma vaccine of claim 1 in the manufacture of a medicament for the treatment of melanoma.

22. Use of an immune-stimulatory amount of the melanoma vaccine of claim 1, in the manufacture of a medicament for treating melanoma in a subject in need thereof.

23. Use of an immune-stimulatory amount of the composition of claim 2 in the manufacture of a medicament for treating melanoma in a subject in need thereof.