target antigens, the ability of NK cells to bind to the constant region of antibodies, enables NK cells to specifically kill the cells that are tagged with antibodies. The NK cell's recognition of the antibodies is mediated by Fc receptor (of the NK cell) binding to the Fc portion of the antibody. This type of killing is called, antibody-dependent cell cytotoxicity (ADCC). NK cells can also kill cells independent of the mechanism of ADCC, where this killing requires expression of MHC class I to be lost or deficient in the target cell (see, e.g., Caligiuri (2008) Blood 112:461-469).

**[0007]** The technique of "delayed type hypersensitivity response" can be used to distinguish between immune responses that mainly involve cellular immunity or mainly involve humoral immunity. A positive signal from the delayed type hypersensitivity response indicates a cellular response (see, e.g., Roychowdhury, et al. (2005) AAPS J. E834-E846).

**[0008]** 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.

## Summary

**[0008a]** In one aspect, there is provided a composition for use in the treatment of a cancer other than melanoma derived from a tissue of one or more of endodermal origin, mesodermal origin, and ectodermal origin, the composition comprising a population of mammalian dendritic cells comprising cancer-specific peptides derived from one or more cancer cells that are obtained from a mammalian subject that has the cancer; wherein the cancer-specific peptides have been acquired from the cancer cells in vitro by contact of the population of mammalian dendritic cells with the cancer cells; wherein:

the cancer cells are whole cells;

the cancer cells are MHC class II expressing;

the cancer cells have not been treated in vitro with interferon-gamma (IFN-gamma) or with an IFN-gamma mimetic;

the cancer cells are metabolically active and incapable of cell division;

cancer cells that are autophagic and non-apoptotic have been selected and cancer cells that are non-autophagic and apoptotic have been rejected and

the dendritic cells and cancer cells are from the same subject.~~for treating cancer comprising a population of dendritic cells comprising cancer-specific peptides derived from a population of cancer cells taken from a subject that has cancer;~~

~~wherein~~

~~(a) the cancer-specific peptides are acquired by contacting in vitro the population of dendritic cells with the population of cancer cells;~~

~~(b) the population of cancer cells are not treated in vitro with interferon-gamma (IFN-gamma) or are not treated in vitro with an IFN-gamma mimetic;~~

~~(c) the cancer cells are selected for being greater than 60 percent (%) autophagic and non-apoptotic, and,~~

~~(d) the dendritic cells and cancer cells are from the same subject.~~

**[0008b]** In another aspect, there is provided a cancer vaccine ~~for the subject as defined above,~~ comprising the composition as defined above.

**[0008c]** In another ~~aspect~~embodiment, there is provided a melanoma vaccine comprising:

at least one dendritic cell obtained from a subject that has melanoma;

wherein the at least one dendritic cell has been contacted in vitro with a population of cancer tumor cells from the same subject,

wherein the population of cancer tumor cells is selected for being greater than 60% autophagic and non-apoptotic wherein the population of melanoma tumor cells is non-dividing and not treated in vitro with IFN-gamma or a IFN-gamma mimetic, and wherein the vaccine (DC) is effective to provide superior overall survival rates in patients with documented metastatic melanoma compared to patients with irradiated tumor cells (TC) alone.

**[0008d]** In another aspect, there is provided a use of the composition as defined above in the manufacture of a medicament for treating a cancer derived from a tissue of one or more of endodermal origin, mesodermal origin, and ectodermal origin ~~comprising cancer cells~~ by stimulating an immune response against a cancer-specific antigen.

**[0008e]** In another aspect, there is provided a method for preparing a cancer vaccine~~, comprising cancer cells and dendritic cells from the same subject, the method~~ comprising:

(a) preparing cancer cells that are MHC class II expressing from a subject by:

(i) harvesting one or more cancer cells from a tumor other than melanoma derived from a tissue of one or more of endodermal origin, mesodermal origin, and ectodermal origin comprising at least one cancer-specific peptide antigen;

(ii) selecting cancer cells that are autophagic and non-apoptotic, and rejecting cancer cells that are non-autophagic and apoptotic; wherein the one or more cancer cells are not treated in vitro with interferon-gamma (IFN-gamma) or with an IFN-gamma mimetic

iii) treating the one or more cancer cells with an agent that prevents cell division while preserving metabolic activity;

(b) generating a population of dendritic cells from the subject;

(c) contacting the cancer cells from the tumor derived from a tissue of one or more of endodermal origin, mesodermal origin, and ectodermal origin comprising at least one cancer-specific peptide antigen that are non-autophagic and apoptotic of (a)(ii) with the population of dendritic cells of (b) in vitro; wherein the cancer-specific peptides are acquired in vitro by the dendritic cells and processed by the dendritic cells; and

(d) harvesting the population of dendritic cells of step (c) to form the cancer vaccine. ~~(a) preparing cancer cells comprising at least one cancer-specific peptide by:~~

~~(i) treating one or more cancer cells with an agent that prevents cell division; wherein the one or more cancer cells are not treated in vitro with interferon-gamma (IFN-gamma) or with an IFN-gamma mimetic;~~

~~(ii) selecting cancer cells that are autophagic and non-apoptotic;~~

~~(iii) rejecting cancer cells that are non-autophagic and apoptotic;~~

~~(b) generating the dendritic cells; and~~

~~(c) contacting the selected cancer cells of (a) that are autophagic and non-apoptotic with the dendritic cells of (b) in vitro;~~

~~wherein the contacting is sufficient for the dendritic cells to acquire the at least one cancer-derived peptide from the cancer cells, wherein the acquired peptide is effective for the dendritic cells to generate an immunostimulatory amount of a cancer vaccine; wherein the immunostimulatory amount of the cancer vaccine (DC) is effective to provide superior overall survival rates in patients with documented cancer compared to patients injected with irradiated tumor cells (TC) alone~~

**[0008f]** In another aspect, there is provided a composition comprising: at least one antigen presenting cell (APC) derived from a subject diseased with a cancer other than melanoma derived from a tissue of one or more of endodermal origin, mesodermal origin, and ectodermal origin comprising cancer-specific peptides acquired by contact with at least one cancer cell from the same subject in vitro, wherein the cancer cell is metabolically active and incapable of cell division, MHC class II expressing, autophagic, and non-apoptotic, and has not been treated in vitro with interferon-gamma (IFN-gamma) or with an IFN-gamma mimetic.

~~at least one cancer cell obtained from a subject with cancer, the cancer cell comprising at least one cancer specific peptide, wherein the at least one cancer cell is not treated in vitro with interferon-gamma (IFN-gamma) and the at least one cancer cell is selected for being autophagic and non-apoptotic; and at least one antigen presenting cell (APC) obtained from the same subject with cancer, wherein the APC comprises the at least one cancer specific peptide loaded in vitro from the at least one cancer cell.~~

**[0008g]** In another aspect, there is provided a use of the composition as defined above in the manufacture of a medicament for treating a cancer derived from a tissue of one or more of endodermal origin, mesodermal origin, and ectodermal origin ~~comprising cancer cells~~ by stimulating an immune response against a cancer-specific antigen of the cancer cells in a subject diseased with a cancer.

**Description**

**[0009]** 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.

## DISCLOSURE

**[0010]** The present disclosure provides a population of mammalian dendritic cells comprising: cancer-specific peptides from cancer cells taken from a subject that has a cancer; wherein the cancer-specific peptides are acquired in vitro by dendritic cells from said cancer cells; wherein the cancer cells are not treated in vitro with interferon-gamma (IFN-gamma) or with IFN-gamma mimetic; wherein the majority of the cancer cells are not treated in vitro with a interferon-gamma (IFN-gamma) or

What is claimed is:

Claim 1. A composition for use in the treatment of a cancer other than melanoma derived from a tissue of one or more of endodermal origin, mesodermal origin, and ectodermal origin, the composition comprising a population of mammalian dendritic cells comprising cancer-specific peptides derived from one or more cancer cells that are obtained from a mammalian subject that has the cancer;

wherein the cancer-specific peptides have been acquired from the cancer cells in vitro by contact of the population of mammalian dendritic cells with the cancer cells;

wherein:

the cancer cells are whole cells;

the cancer cells are MHC class II expressing;

the cancer cells have not been treated in vitro with interferon-gamma (IFN-gamma) or with an IFN-gamma mimetic;

the cancer cells are metabolically active and incapable of cell division;

cancer cells that are autophagic and non-apoptotic have been selected and cancer cells that are non-autophagic and apoptotic have been rejected and

the dendritic cells and cancer cells are from the same subject.

Claim 2. The composition for use according to claim 1, wherein the cancer is colon cancer of endoderm origin, renal cancer of mesoderm origin, glioblastoma of ectoderm origin, or ovarian cancer of mixed mesoderm plus extra-embryonic origin.

Claim 3. The composition for use according to claim 1, wherein greater than 80% of said cancer cells that are not treated in vitro with IFN-gamma or an IFN-gamma mimetic are autophagic and non-apoptotic.

Claim 4. A cancer vaccine comprising the composition of claim 1.

Claim 5. The composition for use according to claim 1, wherein essentially all of the cancer cells are incapable of cell division.

Claim 6. The composition for use according to claim 1, wherein the cancer cells are incapable of cell division after treatment with irradiation or with a nucleic acid cross-linking agent.

Claim 7. The composition for use according to claim 1, wherein the mammalian subject is a human subject.

Claim 8. Use of the composition of claim 1 in the manufacture of a medicament for treating a cancer derived from a tissue of one or more of endodermal origin, mesodermal origin, and ectodermal origin by stimulating an immune response against a cancer-specific antigen.

Claim 9. The use of claim 8, 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 10. The use of claim 9, 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 11. The use of claim 8, wherein the medicament is in a form for subcutaneous injection.

Claim 12. The use of claim 8, wherein the medicament is in a form for injections given weekly for three months and then monthly for five months.

Claim 13. A method for preparing a cancer vaccine comprising:

(a) preparing cancer cells that are MHC class II expressing from a subject by:

(i) harvesting one or more cancer cells from a tumor other than melanoma derived from a tissue of one or more of endodermal origin, mesodermal origin, and ectodermal origin comprising at least one cancer-specific peptide antigen;

(ii) selecting cancer cells that are autophagic and non-apoptotic, and rejecting cancer cells that are non-autophagic and apoptotic; wherein the one

or more cancer cells are not treated in vitro with interferon-gamma (IFN-gamma) or with an IFN-gamma mimetic

(iii) treating the one or more cancer cells with an agent that prevents cell division while preserving metabolic activity;

(b) generating a population of dendritic cells from the subject;

(c) contacting the cancer cells from the tumor derived from a tissue of one or more of endodermal origin, mesodermal origin, and ectodermal origin comprising at least one cancer-specific peptide antigen that are non-autophagic and apoptotic of (a)(ii) with the population of dendritic cells of (b) in vitro; wherein the cancer-specific peptides are acquired in vitro by the dendritic cells and processed by the dendritic cells; and

(d) harvesting the population of dendritic cells of step (c) to form the cancer vaccine.

Claim 14. A composition comprising:
at least one antigen presenting cell (APC) derived from a subject diseased with a cancer other than melanoma derived from a tissue of one or more of endodermal origin, mesodermal origin, and ectodermal origin comprising cancer-specific peptides acquired by contact with at least one cancer cell from the same subject in vitro,

wherein the cancer cell is metabolically active and incapable of cell division, MHC class II expressing, autophagic, and non-apoptotic, and has not been treated in vitro with interferon-gamma (IFN-gamma) or with an IFN-gamma mimetic.

Claim 15. The composition of claim 14, wherein the APC is a dendritic cell, a macrophage, or a B cell.

Claim 16. The composition of claim 14, wherein the cancer-specific peptides are substantially not contained in said APCs and are substantially not processed by said APCs.

Claim 17. The composition of claim 14, wherein the cancer-specific peptides are substantially contained in said APCs and are partially or substantially processed in said APCs.

Claim 18. The composition of claim 14, wherein the cancer cell is loaded into the APC.

Claim 19. The composition of claim 14, wherein the cancer cell is not loaded into the APC.

Claim 20. The composition of claim 14, wherein the autophagy is demonstrated by a test that assays microtubule-associated protein light chain 3 (LC3).

Claim 21. The composition of claim 14, wherein the cells are demonstrated to be non-apoptotic using at least one of 7-aminoactinomycin D (7-ADD) or annexin.

Claim 22. Use of the composition of claim 14 in the manufacture of a medicament for treating a cancer derived from a tissue of one or more of endodermal origin, mesodermal origin, and ectodermal origin by stimulating an immune response against a cancer-specific antigen of the cancer cells in a subject diseased with a cancer.

Claim 23. The composition for use according to claim 1, wherein the population of cancer cells is enriched in cancer cells that are non-apoptotic by separation of autophagic and non-apoptotic cells adherent to a surface from non-autophagic apoptotic cells that are floating.

Claim 24. The method according to claim 13, further comprising enriching the population of cancer cells in cancer cells that are non-apoptotic by separating autophagic and non-apoptotic cells adherent to a surface from non-autophagic apoptotic cells that are floating.

Claim 25. The composition according to claim 14, wherein the population of cancer cells is enriched in cancer cells that are non-apoptotic by separation of autophagic and non-apoptotic cells adherent to a surface from non-autophagic apoptotic cells that are floating.

Claim 26. The method according to claim 13, wherein in step (iii) the cancer cells are incapable of cell division after treating with irradiation or with a nucleic acid cross-linking agent.

Claim 27. The composition according to claim 14, wherein the cancer cells are incapable of cell division after treatment with irradiation or with a nucleic acid cross-linking agent.

**What is claimed is**

Claim 1. A composition for treating cancer comprising a population of dendritic cells comprising cancer-specific peptides derived from a population of cancer cells taken from a subject that has cancer;

wherein

(a) the cancer-specific peptides are acquired by contacting in vitro the population of dendritic cells with the population of cancer cells;

(b) the population of cancer cells are not treated in vitro with interferon-gamma (IFN-gamma) or are not treated in vitro with an IFN-gamma mimetic;

(c) the cancer cells are selected for being greater than 60 percent (%) autophagic and non-apoptotic, and,

(d) the dendritic cells and cancer cells are from the same subject.

Claim 2. The composition of claim 1, wherein the cancer is from a tissue of one or more of endodermal origin, mesodermal origin, or ectodermal origin.

Claim 3. The composition of claim 1, wherein the cancer is from a tissue of neural crest origin, and wherein the neural crest is of ectodermal origin.

Claim 4. The composition of claim 1, wherein the cancer is from a tissue of one or more endodermal origin, mesodermal origin, or ectodermal origin, and where the cancer is melanoma or neural crest origin, colon cancer or endoderm origin, renal cancer of mesoderm origin, glioblastoma of ectoderm origin, or ovarian cancer of mixed mesoderm pluse extra-embryonic origin.

Claim 5. The composition of claim 1, wherein greater than 80% of said cancer cells that are not treated in vitro with IFN-gamma or IFN-gamma mimetic, are autophagic and non-apoptotic.

Claim 6. A vaccine for the subject of claim 1, comprising the composition of claim 1.

Claim 7. The composition of claim 1, wherein essentially all of the cancer cells that are not treated with IFN-gamma or IFN-gamma mimetic are incapable of cell division.

Claim 8. The composition of claim 1, wherein essentially all of the cancer that are not treated with IFN-gamma or IFN-gamma mimetic are irradiated and incapable of cell division.

Claim 9. The composition of claim 1, wherein at least 80% of the cancer cells that are not treated with IFN-gamma or IFN-gamma mimetic are irradiated and incapable of cell division.

Claim 10. The composition of claim 1, wherein at least 80% of the cancer cells that are not treated with IFN-gamma or IFN-gamma mimetic are treated with a nucleic acid cross-linker and are incapable of cell division.

Claim 11. The composition of claim 1 that comprise one or more peptides derived from a melanoma-specific antigen that is S-100, HMB-45, Mel-2, Melan-A, Mel-5, MAGE-1, MART-1, or Tyrosinase, and wherein the cancer is melanoma.

Claim 12. The composition of claim 1, wherein the given subject is a human subject.

Claim 13. The composition of claim 1, wherein the given subject is a mammal that is not human.

Claim 14. A melanoma vaccine comprising:

at least one dendritic cell obtained from a subject that has melanoma;

wherein the at least one dendritic cell has been contacted in vitro with a population of cancer tumor cells from the same subject,

wherein the population of cancer tumor cells is selected for being greater than 60% autophagic and non-apoptotic wherein the population of melanoma tumor cells is non-dividing and not treated in vitro with IFN-gamma or a IFN-gamma mimetic, and wherein the vaccine (DC) is effective to provide superior overall survival rates in patients with documented metastatic melanoma compared to patients with irradiated tumor cells (TC) alone.

Claim 15. Use of the composition of claim 1 in the manufacture of a medicament for treating a cancer comprising cancer cells by stimulating an immune response against a cancer-specific antigen.

Claim 16. The use of claim 15, 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 17. The use of claim 16, 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 18. The use of claim 15, 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 about 60%.

Claim 19. The use of claim 15, wherein the administration comprises subcutaneous injections of the vaccine.

Claim 20. The use of claim 15, wherein the administration comprises injections of the vaccine given weekly for three months and then monthly for five months.

Claim 21. A method for preparing a cancer vaccine, comprising cancer cells and dendritic cells from the same subject, the method comprising:

(a) preparing cancer cells comprising at least one cancer-specific peptide by:

(i) treating one or more cancer cells with an agent that prevents cell division; wherein the one or more cancer cells are not treated in vitro with interferon-gamma (IFN-gamma) or with an IFN-gamma mimetic;

(ii) selecting cancer cells that are autophagic and non-apoptotic;

(iii) rejecting cancer cells that are non-autophagic and apoptotic;

(b) generating the dendritic cells; and

(c) contacting the selected cancer cells of (a) that are autophagic and non-apoptotic with the dendritic cells of (b) in vitro;

wherein the contacting is sufficient for the dendritic cells to acquire the at least one cancer-derived peptide from the cancer cells, wherein the acquired peptide is effective for the dendritic cells to generate an immunostimulatory amount of a cancer vaccine; wherein the immunostimulatory amount of the cancer vaccine (DC) is effective to provide superior overall survival rates in patients with documented cancer compared to patients injected with irradiated tumor cells (TC) alone.

Claim 22. A composition comprising:

at least one cancer cell obtained from a subject with cancer, the cancer cell comprising at least one cancer specific peptide, wherein the at least one cancer cell is not treated in vitro with interferon-gamma (IFN-gamma) and the at least one cancer cell is selected for being autophagic and non-apoptotic; and at least one antigen presenting cell (APC) obtained from the same subject with cancer, wherein the APC comprises the at least one cancer specific peptide loaded in vitro from the at least one cancer cell:

Claim 23. The composition of claim 22, wherein the cancer cell is MHC class II-expressing.

Claim 24. The composition of claim 22, wherein the APC is a dendritic cell, a macrophage, or a B cell.

Claim 25. The composition of claim 22, wherein the at least one cancer cell comprises cancer-specific peptides, and wherein the cancer-specific peptides are substantially not contained in said APCs and are substantially not processed by said APCs.

Claim 26. The composition of claim 22, wherein the cancer cells comprise melanoma-specific peptides, and wherein the cancer-specific peptides are substantially contained in said APCs and are partially or substantially processed in said APCs.

Claim 27. The composition of claim 22, wherein the cancer cell is loaded into the APC.

Claim 28. The composition of claim 22, wherein the cancer cell is not loaded into the APC.

Claim 29. The composition of claim 22, wherein the autophagy is demonstrated by a test that assays microtubule-associated protein light chain 3 (LC3).

Claim 30. The composition of claim 22, wherein the cells are demonstrated to be non-apoptotic using at least one of the reagent, 7-aminoactinomycin D (7-ADD), or the reagent annexin.

Claim 31. Use of the composition of claim 22 in the manufacture of a medicament for treating a cancer comprising cancer cells by stimulating an immune response against a cancer-specific antigen of the cancer cells.

Claim 32. The composition of claim 22, wherein at least 90% of the cancer cells are not treated in vitro with IFN-gamma, and less than 10% of the cancer cells are treated in vitro with IFN-gamma.