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 treating cancer comprising a population of ~~mammalian~~ 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 ~~in vitro~~ by contacting in vitro the population of dendritic cells ~~from said~~ with the population of cancer cells;

(b) ~~wherein~~ 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) ~~wherein~~ the cancer cells are selected for being greater than ~~about~~ 60 percent (%) ~~of said cancer cells that are not treated in vitro with IFN-gamma or IFN-gamma mimetic, are~~ autophagic and non-apoptotic, and,

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

[0008b] In another aspect, there is provided a vaccine for the subject as defined above, comprising the composition ~~population of dendritic cells~~ as defined above.

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

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

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

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

[0008d] In another aspect, there is provided a use of the composition as defined above ~~an immune-stimulatory amount of the population of dendritic cells as defined above for~~ in the ~~preparation~~ manufacture of a medicament for treating a cancer comprising cancer cells by stimulating an immune response against a cancer-specific antigen~~, wherein the medicament is to be administered to a subject~~.

[0008e] In another aspect, there is provided a method for preparing a ~~dendritic cell~~ cancer vaccine, ~~involving~~ 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 ~~is treated~~ 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 ~~are selected~~;

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

(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 ~~and, wherein~~

~~the cancer cells that are autophagic and non-apoptotic are provided to one or more autologous dendritic cells, or wherein peptides derived from the cancer cells that are autophagic and non-apoptotic are provided to one or more autologous dendritic cells.~~

[0008f] In another aspect, there is provided 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 ~~that~~ is not treated in vitro with interferon-gamma (IFN-gamma) ~~from a first subject~~ 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 ~~first~~ subject with cancer, wherein the APC comprises the at least one cancer specific peptide loaded in vitro from the at least one cancer cell ~~is: autophagic; and, non-apoptotic~~.

[0008g] In another aspect, there is provided a use of ~~an immunology effective amount of~~ the composition as defined above ~~for~~ in the ~~preparation~~ 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~~in a subject having a cancer and comprising cancer cells, wherein the medicament is to be administered to said subject, and wherein the subject is the same subject as the first subject~~.

~~**[0008h]** In another aspect, there is provided a method for manufacturing the vaccine as defined above or the composition as defined above, comprising contacting at least one cancer tumor cell to at least one antigen presenting cell (APC), wherein the at least one cancer tumor cell is from a first human subject, and wherein the at least one APC is from the same first human subject.~~

~~**[0008i]** In another aspect, there is provided a method for preparing a dendritic cell vaccine, comprising: treating cancer cells acquired from a first subject with an agent that prevents cell division; wherein the cancer cells are not treated in vitro with IFN-gamma or an IFN-gamma mimetic; selecting cancer cells that are autophagic and non-apoptotic; and, contacting the selected cancer cells with autologous dendritic cells from the same first subject.~~

~~**[0008j]** In another aspect, there is provided a composition that comprises a dendritic cell vaccine, as prepared by the method as defined above.~~

~~**[0008k]** In another aspect, there is provided a use of the composition as defined above for the preparation of a medicament for stimulating an immune response against a cancer-specific antigen, wherein the medicament is to be administered to a subject that has 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 treating cancer comprising a population of ~~mammalian~~ 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 ~~in vitro~~ by contacting in vitro the population of dendritic cells ~~from said~~ with the population of cancer cells;

(b)~~wherein~~ 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) ~~wherein~~ the cancer cells are selected for being greater than ~~about~~ 60 percent (%) ~~of said cancer cells that are not treated in vitro with IFN-gamma or IFN-gamma mimetic, are~~ autophagic and non-apoptotic, and,

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

Claim 2. The ~~population of dendritic cells~~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 ~~population of dendritic cells~~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 ~~population of dendritic cells~~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 ~~population of dendritic cells~~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 ~~population of dendritic cells~~ composition of claim 1.

Claim 7. The ~~population of dendritic cells~~ 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 ~~population of dendritic cells~~ 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 ~~population of dendritic cells~~ 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 ~~population of dendritic cells~~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 ~~population of dendritic cells~~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 ~~population of dendritic cells~~composition of claim 1, wherein the given subject is a human subject.

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

Claim 14. A melanoma vaccine comprising:

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

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

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

Claim 15. Use ~~an immune-stimulatory amount~~ of the ~~population of dendritic cells~~ composition of claim 1 ~~for the~~in the manufacture ~~preparation~~ of a medicament for treating a cancer comprising cancer cells by stimulating an immune response against a cancer-specific antigen~~, wherein the medicament is to be administered to a subject~~.

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 ~~dendritic cell~~cancer vaccine, ~~involving~~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 ~~is treated~~ 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 ~~are selected~~;

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

(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~~and, wherein the cancer cells that are autophagic and non-apoptotic are provided to one or more autologous dendritic cells, or wherein peptides derived from the cancer cells that are autophagic and non-apoptotic are provided to one or more autologous dendritic cells~~.

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~~that~~ is not treated in vitro with interferon-gamma (IFN-gamma) ~~from a first subject~~ 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 ~~first~~ subject with cancer, wherein the APC comprises the at least one cancer specific peptide loaded in vitro from the at least one cancer cell ~~is~~:

~~autophagic; and,~~

~~non-apoptotic~~.

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 an immunology effective amount~~ of the composition of claim 22 ~~for the~~ in the manufacture ~~preparation~~ of a medicament for treating a cancer comprising cancer cells by stimulating an immune response against a cancer-specific antigen of the cancer cells~~in a subject having a cancer and comprising cancer cells, wherein the medicament is to be administered to said subject, and wherein the subject is the same subject as the first subject~~.

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.

~~Claim 33. A method for manufacturing the vaccine of claim 1 or the composition of claim 19, comprising contacting at least one cancer tumor cell to at least one antigen presenting cell (APC), wherein the at least one cancer tumor cell is from a first human subject, and wherein the at least one APC is from the same first human subject.~~

~~Claim 34. A method for preparing a dendritic cell vaccine, comprising: treating cancer cells acquired from a first subject with an agent that prevents cell division; wherein the cancer cells are not treated in vitro with IFN-gamma or an IFN-gamma mimetic; selecting cancer cells that are autophagic and non-apoptotic; and, contacting the selected cancer cells with autologous dendritic cells from the same first subject.~~

~~Claim 35. A composition that comprises a dendritic cell vaccine, as prepared by the method of claim 34.~~

~~Claim 36. Use of the composition of claim 35 for the preparation of a medicament for stimulating an immune response against a cancer-specific antigen, wherein the medicament is to be administered to a subject that has cancer.~~