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(54) **OVARIAN CANCER VACCINES AND VACCINATION METHODS**

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(52) **U.S. Cl.**

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USPC **424/185.1**; 435/325

(57) **ABSTRACT**

Compositions of multipeptide vaccines including tumor associated antigens, compositions of antigen presenting cell (e.g., dendritic cell) based vaccines presenting epitopes from tumor associated antigens, and methods of making same, are provided herein. Also, disclosed are methods for treating ovarian cancers using such vaccines.

FIG. 1A

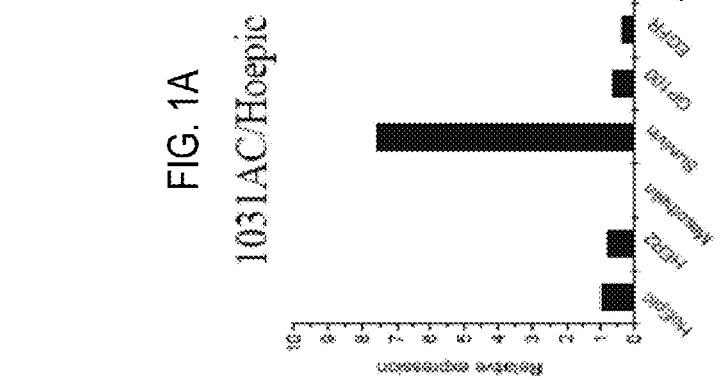


FIG. 1B

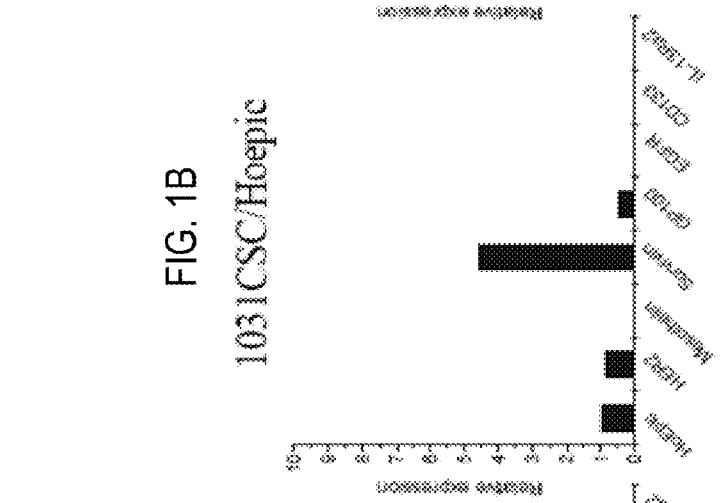
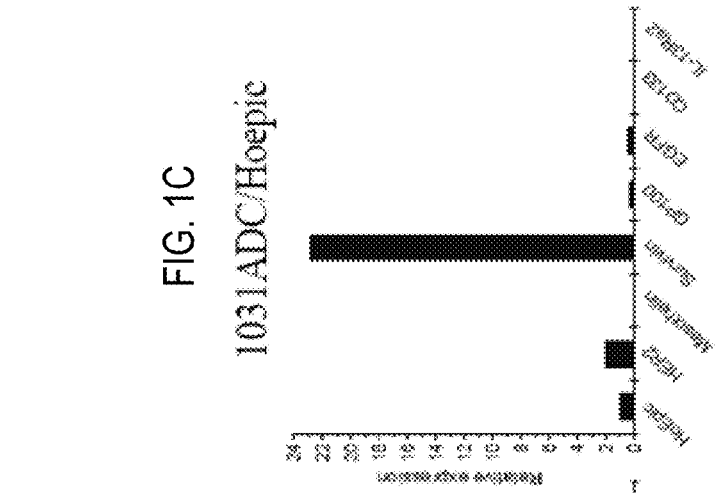


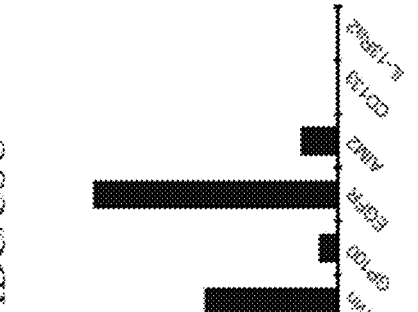
FIG. 1C



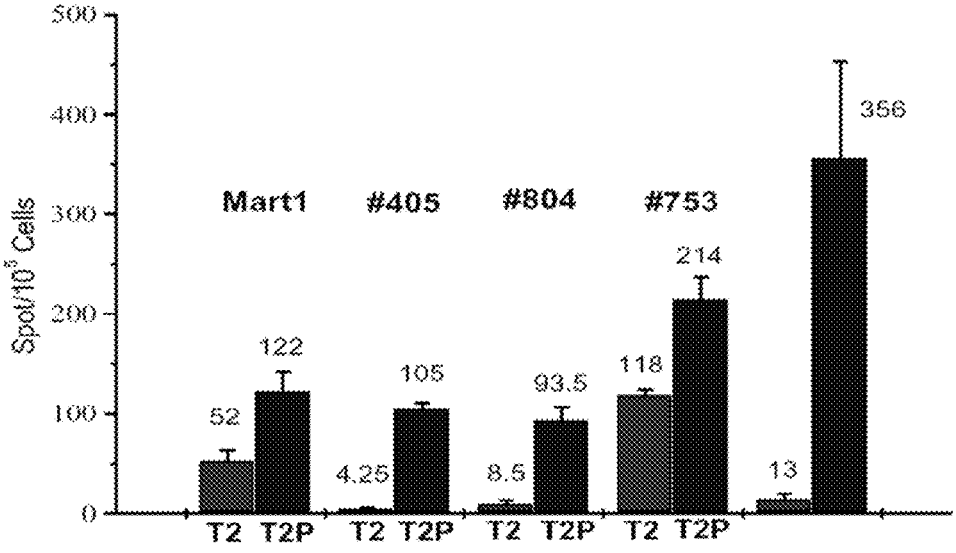
	HoEpic(Ct)	1031AC(Ct)	1031CSC(Ct)	1031ADC(Ct)	1031AC/Hoepic 2^- Δ(ΔCt)	1031CSC/Hoepic 2^- Δ(ΔCt)	1031ADC/Hoepic 2^- Δ(ΔCt)
GAPDH	22.27	22.05	21.13	22.4			
HER2	27.6	27.83	26.89	27.9	0.8312	0.8467	1.02
Mesothelin	29.28	38.43	36.66	37.48	0.0015	0.0027	0.00372
Survivin	34.83	31.68	31.49	30.45	7.6	4.59	22.94
GP100	36.13	36.55	36.11	37.97	0.6387	0.4579	0.305
EGFR	27.23	28.38	30.38	28.44	0.385	0.0518	0.475
AIM2	N/A	32.2	32.65	32.29			
CD133	31.9	N/A	38.15	N/A		0.00586	
IL-13ra2	33.44	N/A	N/A	N/A			

FIG. 2A

FIG. 2B

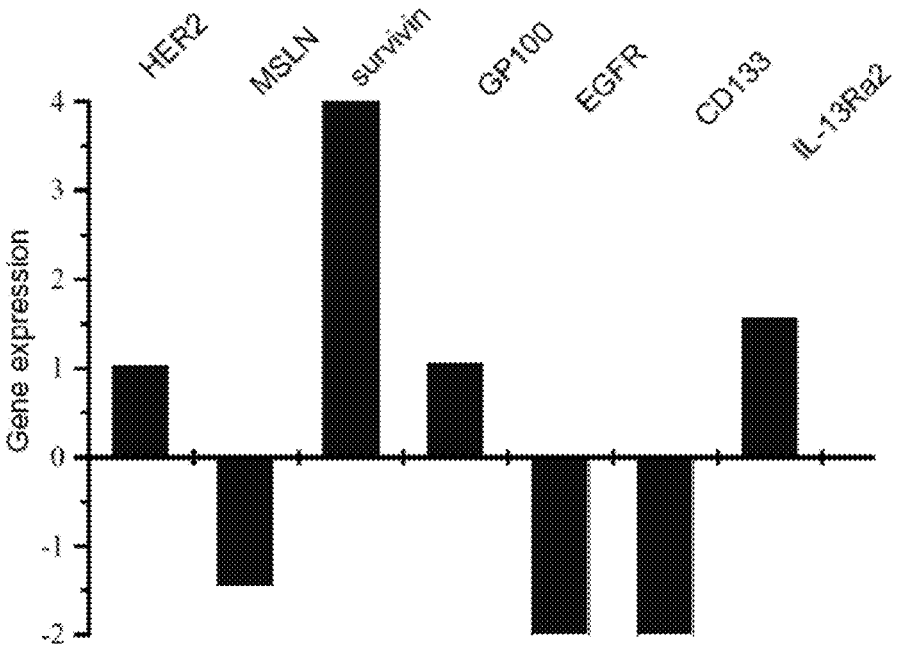


	1031AC(Ct)	1031CSC(Ct)	1031ADC(Ct)	1031CSC/AC 2^- Δ(ΔCt)	1031ADC/CSC 2^- Δ(ΔCt)
GAPDH	22.05	21.13	22.4		
HER2	27.83	26.89	27.9	1.02	1.203
Mesothelin	38.43	36.66	37.48	1.81	1.37
Survivin	31.68	31.49	30.45	0.6057	4.98
GP100	36.55	36.11	37.97	0.717	0.6659
EGFR	28.38	30.36	28.44	0.1346	9.17
AIM2	32.2	32.65	32.29	1.04	1.37
CD133	N/A	38.15	N/A		
IL-13ra2	N/A	N/A	N/A		



1. Mart1/T2: 52±11.64
2. Mart1/T2-Mart1: 122.25±19.32
3. #405/T2: 4.25±2.06
4. #405/T2-405:105 ±5.94
5. #804/T2: 8.5±4.32
6. #804/T2-804: 93.5 ± 13.64
7. #753/T2:118.75 ±5.62
8. #753/T2-753: 214.25 ±23.15
9. CD8 T cells+T2: 13.25 ±6.13
10. CD8 T cell+PHA: 356 ±97.44

FIG. 3



Gene name	Affymetrix probe ID	RNA expression
HER2	216836_s_at	1.025
MSLN	204885_s_at	-1.464
survivin	202095_s_at	11.29
GP100	209848_s_at	1.06
EGFR	201983_s_at	-2.552
CD133	204304_s_at	-4.331
IL-13Ra2	206172_at	1.463

FIG. 4

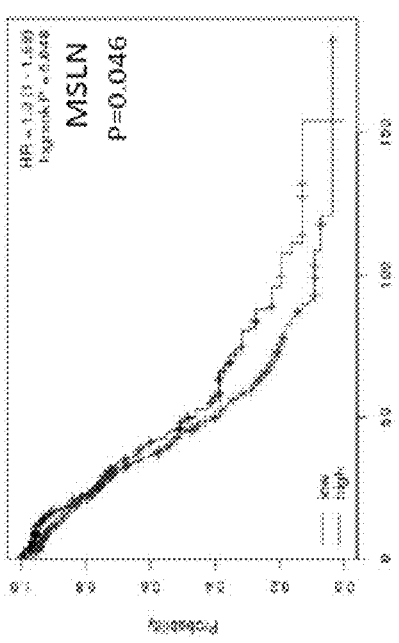


FIG. 5B

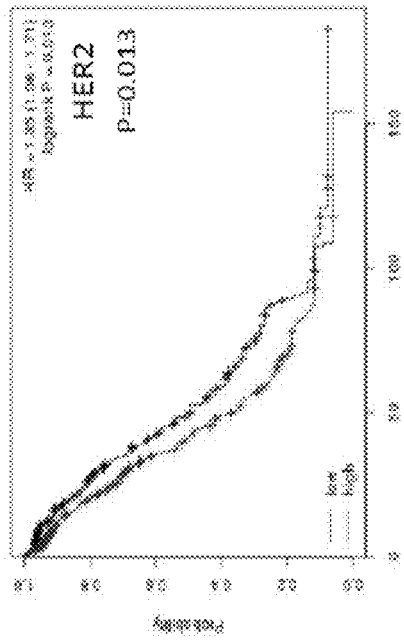


FIG. 5A

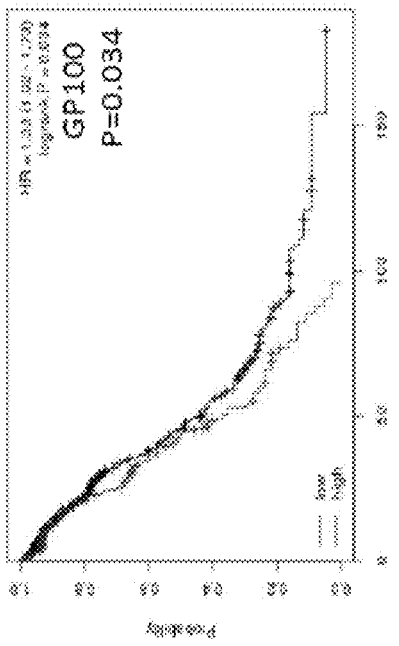


FIG. 5D

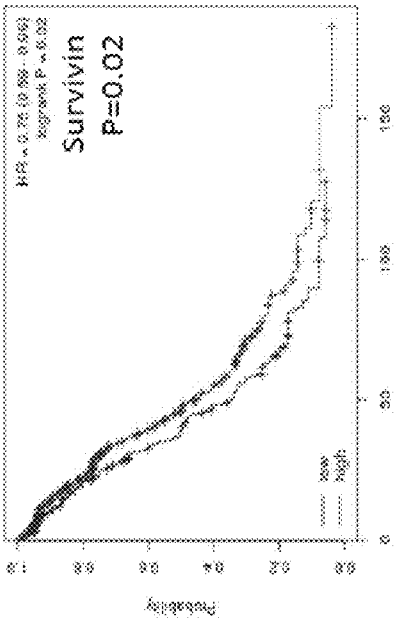


FIG. 5C

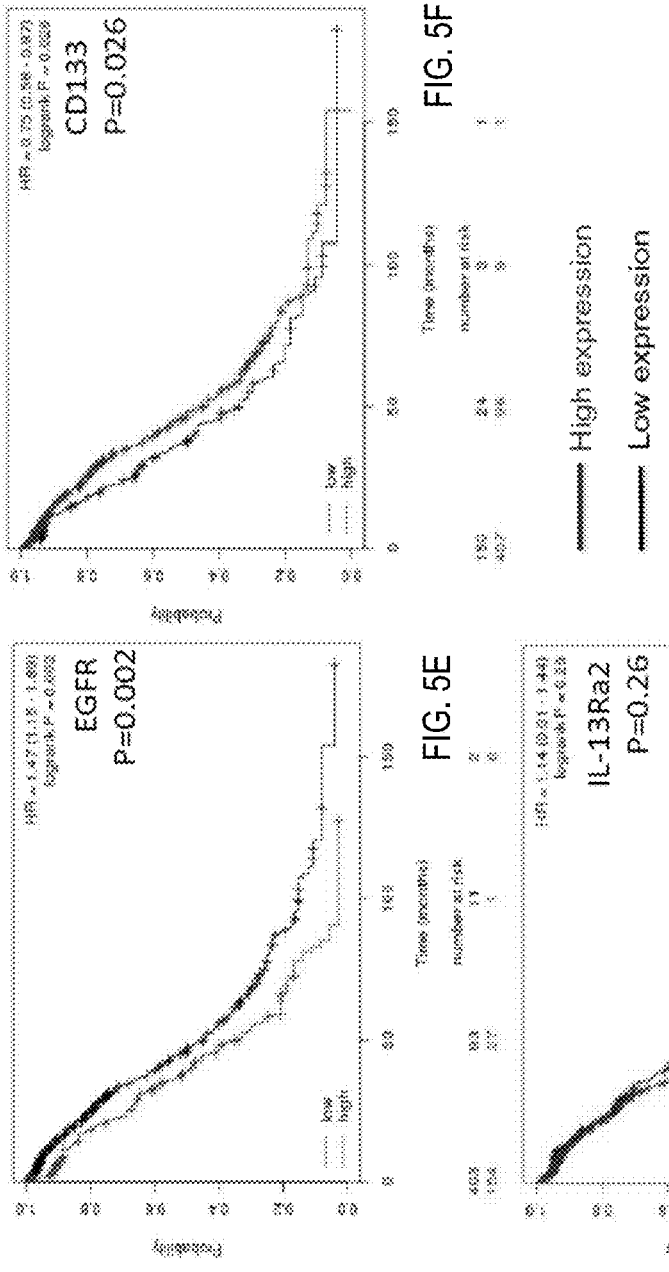


FIG. 5F

FIG. 5E

FIG. 5G

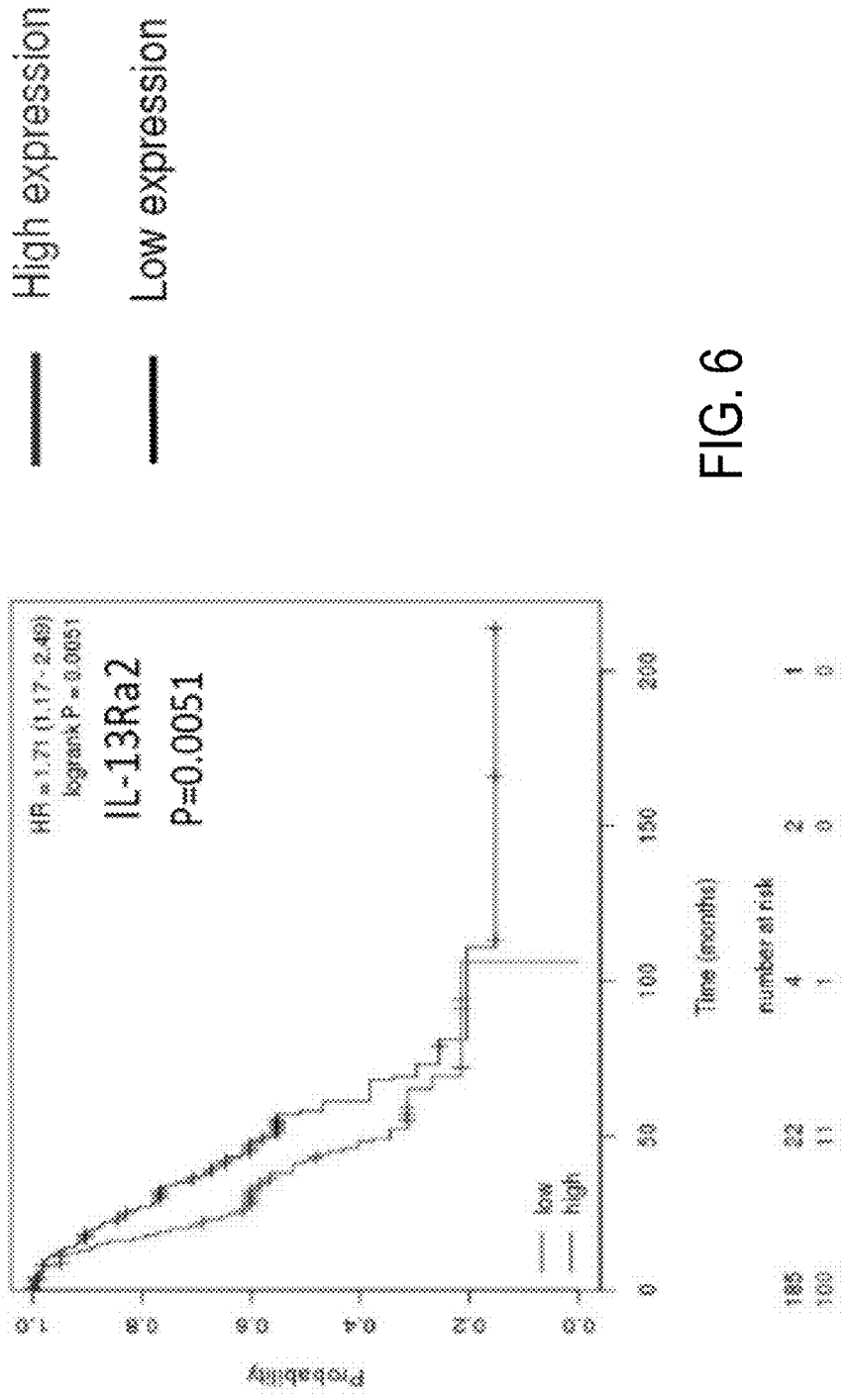

High expression
Low expression
IL-13Ra2
P=0.0051
Probability
Time (months)
number at risk
low
high

FIG. 6

OVARIAN CANCER VACCINES AND VACCINATION METHODS

TECHNICAL FIELD

[0001] The disclosure relates generally to multivalent vaccine compositions, methods of making such compositions, and methods for the treatment of ovarian cancers.

BACKGROUND

[0002] Epithelial ovarian cancer (EOC) is the most frequent cause of gynecologic cancer-related mortality in women (Jemal, A., et al., Global cancer statistics. CA Cancer J Clin, 2011, 61(2): p. 69-90). It was estimated that in 2008 (the most recent year numbers are available), approximately 21,204 women were diagnosed and 14,362 women died of disease in the US (see, www.cdc.gov/cancer/ovarian/statistics/index.htm). It is estimated that approximately 190,000 new cases will be diagnosed and 115,000 women will die from ovarian cancer per year world-wide. While advances in chemotherapy have been made over the past three decades, the overall 5 year survival for advanced stage disease remains less than 35%.

[0003] Initial response rates of advanced ovarian cancer to the standard upfront paclitaxel and carboplatin treatment approach is 75%, with complete clinical response rates near 55%. Unfortunately over 75% of subjects with complete clinical response are destined to relapse and succumb to their disease (Coukos, G. and S. C. Rubin, Chemotherapy resistance in ovarian cancer: new molecular perspectives. Obstet Gynecol, 1998, 91(5 Pt 1): p. 783-92). For most subjects, ovarian cancer will recur within two years, with median time to progression of 20-24 months for optimally surgically cytoreduced subjects and 12-18 months for subjects with suboptimal reduction. Response rates to second line chemotherapy are significantly lower, between 15-30%, depending on the length of progression free survival and the number of previous treatments. Once ovarian cancer has recurred, it is not considered curable and progression to death is usually inevitable, despite aggressive chemotherapy strategies. These facts elucidate the enormous unmet need for the development of alternate therapies in ovarian cancer (Coukos, G. and S. C. Rubin, Gene therapy for ovarian cancer. Oncology (Williston Park), 2001, 15(9): p. 1197-204, 1207; discussion 1207-8; Coukos, G., et al., Immunotherapy for gynaecological malignancies. Expert Opin Biol Ther, 2005, 5(9): p. 1193-210; Coukos, G., M. C. Courreges, and F. Benencia, Intraperitoneal oncolytic and tumor vaccination therapy with replication-competent recombinant virus: the herpes paradigm. Curr Gene Ther, 2003, 3(2): p. 113-25).

[0004] Immunotherapy is a form of cancer treatment that activates the immune system to attack and eradicate cancer cells. Cytotoxic T lymphocytes ("CTL") are critical to a successful antitumor immune response. T cells that attack cancer cells require the presentation of tumor antigens to naïve T cells that undergo activation, clonal expansion, and ultimately exert their cytolytic effector function. Effective antigen presentation is essential to successful CTL effector function. Thus, the development of a successful strategy to initiate presentation of tumor antigens to T cells can be important to an immunotherapeutic strategy for cancer treatment.

[0005] With the clinical outcome of ovarian cancers being from poor to lethal, there exists a significant need for the development of novel therapeutic treatments.

SUMMARY

[0006] This disclosure is based, at least in part, on the identification of antigens present on ovarian cancer stem cells. The identification of these antigens provides a method of targeting ovarian cancer stem cells within ovarian cancer by using a multivalent vaccine that stimulates T cells that recognize epitopes from these antigens thereby eliminating the cancer stem cell population within ovarian cancer. Targeting ovarian cancer stem cells can prevent recurrence of ovarian cancer. By using a multivalent vaccine comprising a combination of peptide epitopes, the methods described herein also provide a way of preventing or reducing the development of escape mutants.

[0007] Accordingly, compositions and methods for inducing immune responses in ovarian cancer patients against tumor antigens are provided herein. The compositions include multipeptide vaccines comprising HLA class I epitopes from at least five (e.g., 5, 6, 7 or 8) of the following antigens: mesothelin, HER-2/neu, IL-13 receptor α2, survivin, CD133, gp100, AIM-2, and epidermal growth factor receptor (EGFR). In certain embodiments, the at least five antigens are HER2, EGFR, IL13Rα2, survivin, and mesothelin. In certain embodiments, the at least five antigens are HER2, EGFR, IL13Rα2, survivin, and gp100. In certain embodiments, the at least five antigens are HER2, EGFR, IL13Rα2, survivin, and CD133. In certain embodiments, the at least five antigens are HER2, EGFR, IL13Rα2, survivin, and AIM2. In certain embodiments, the multipeptide vaccines described above further comprise an HLA class I epitope from RANBP2. The compositions also include antigen presenting cells (e.g., dendritic cells) that present epitopes comprising HLA class I epitopes from at least five of the above-listed eight tumor associated antigens. In certain embodiments, the at least five antigens are HER2, survivin, gp100, IL13Rα2, EGFR, and mesothelin. In certain embodiments, the at least five antigens are HER2, survivin, gp100, IL13Rα2, EGFR, and CD133. In certain embodiments, the at least five antigens are HER2, survivin, gp100, IL13Rα2, EGFR, and AIM2. In certain embodiments, the at least five antigens are HER2, survivin, gp100, IL13Rα2, EGFR, AIM2, and CD133. In certain embodiments, the at least five antigens are mesothelin, HER-2/neu, IL-13 receptor α2, survivin, CD133, gp100, AIM-2, and epidermal growth factor receptor (EGFR). In certain embodiments, the multipeptide vaccines described above further comprise an HLA class I epitope from RANBP2. It is believed that at least one epitope from each of five of the above-listed eight tumor associated antigens will give rise to an efficacious therapeutic. Accordingly, the methods described herein make use of such vaccines for the treatment of ovarian cancer.

[0008] In one aspect, the disclosure features a composition comprising at least one major histocompatibility complex (MHC) class I peptide epitope of at least five (5, 6, 7, or 8) antigens selected from the group consisting of mesothelin, HER-2/neu, IL-13 receptor α2, survivin, CD133, gp100, AIM-2, and epidermal growth factor receptor (EGFR). In certain embodiments, the at least five antigens are HER2, EGFR, IL13Rα2, survivin, and mesothelin. In certain embodiments, the at least five antigens are HER2, EGFR, IL13Rα2, survivin, and gp100. In certain embodiments, the at least five antigens are HER2, EGFR, IL13Rα2, survivin, and CD133. In certain embodiments, the at least five antigens are HER2, EGFR, IL13Rα2, survivin, and AIM2. In certain embodiments, the at least five antigens includes RANBP2.

The epitopes of the at least five antigens may be stored individually or stored as a mixture of these epitopes. In some embodiments, the composition features at least one major histocompatibility complex (MHC) class I peptide epitope of at least six antigens, at least seven antigens, or eight antigens. In certain embodiments, the at least five antigens are HER2, survivin, gp100, IL13Rα2, EGFR, and mesothelin. In certain embodiments, the at least five antigens are HER2, survivin, gp100, IL13Rα2, EGFR, and CD133. In certain embodiments, the at least five antigens are HER2, survivin, gp100, IL13Rα2, EGFR, and AIM2. In certain embodiments, the at least five antigens are HER2, survivin, gp100, IL13Rα2, EGFR, AIM2, and CD133. In certain embodiments, the at least five antigens are mesothelin, HER-2/neu, IL-13 receptor α2, survivin, CD133, gp100, AIM-2, and epidermal growth factor receptor (EGFR). In certain embodiments, the at least five antigens further comprise an HLA class I epitope from RANBP2. In certain embodiments of this aspect, the MHC class I peptide epitope is an HLA-A2 epitope. In a specific embodiment, the MHC class I peptide epitope is an HLA-A0201 epitope. In certain embodiments, the peptides are synthetic. In some embodiments, the composition further comprises at least one MHC class II peptide epitope. In some embodiments, the composition further comprises an adjuvant. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier.

[0009] In another aspect, the disclosure features a composition comprising isolated dendritic cells, wherein the dendritic cells present peptide sequences on their cell surface, wherein the peptide sequences comprise at least one major histocompatibility complex (MHC) class I peptide epitope of at least five antigens selected from the group consisting of mesothelin, HER-2/neu, IL-13 receptor α2, survivin, CD133, gp100, AIM-2, and epidermal growth factor receptor (EGFR). In certain embodiments, the at least five antigens are HER2, EGFR, IL13Rα2, survivin, and mesothelin. In certain embodiments, the at least five antigens are HER2, EGFR, IL13Rα2, survivin, and gp100. In certain embodiments, the at least five antigens are HER2, EGFR, IL13Rα2, survivin, and CD133. In certain embodiments, the at least five antigens are HER2, EGFR, IL13Rα2, survivin, and AIM2. In certain embodiments, the at least five antigens further comprise an HLA class I epitope from RANBP2. In some embodiments, the composition features at least one major histocompatibility complex (MHC) class I peptide epitope of at least six antigens, at least seven antigens, or eight antigens. In certain embodiments, the at least five antigens are HER2, survivin, gp100, IL13Rα2, EGFR, and mesothelin. In certain embodiments, the at least five antigens are HER2, survivin, gp100, IL13Rα2, EGFR, and CD133. In certain embodiments, the at least five antigens are HER2, survivin, gp100, IL13Rα2, EGFR, and AIM2. In certain embodiments, the at least five antigens are HER2, survivin, gp100, IL13Rα2, EGFR, AIM2, and CD 133. In certain embodiments, the at least five antigens are mesothelin, HER-2/neu, IL-13 receptor α2, survivin, CD133, gp100, AIM-2, and epidermal growth factor receptor (EGFR). In certain embodiments, the at least five antigens further comprise an HLA class I epitope from RANBP2. In certain embodiments of this aspect, the MHC class I peptide epitope is an HLA-A2 epitope. In a specific embodiment, the MHC class I peptide epitope is an HLA-A0201 epitope. In certain embodiments, the peptides are synthetic. In some embodiments, the composition further comprises at least one MHC class II peptide epitope. In some embodiments, the composition further comprises an adjuvant. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier. In certain embodiments, the dendritic cells acquired the peptide epitopes in vitro by exposure to synthetic peptides comprising the peptide epitopes.

[0010] In another aspect, the disclosure features a method of treating an ovarian cancer, comprising administering to a subject in need thereof an effective amount of a composition described herein.

[0011] In yet another aspect, the disclosure features a method of killing ovarian cancer stem cells, comprising administering to a subject in need thereof an effective amount of a composition described herein.

[0012] In certain embodiments of the above two aspects, the methods further comprise administering a second agent prior to administering the subject with the composition, wherein the second agent is any agent that is useful in the treatment of ovarian cancer. Combination therapy may allow lower doses of multiple agents and/or modified dosing regimens, thus reducing the overall incidence of adverse effects. In some embodiments, the method further involves administering a chemotherapeutic agent prior to administering the subject with the composition. In certain embodiments, the subject is administered the chemotherapeutic agent half an hour to 3 days (e.g., 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 12 hours, 1 day, 1.5 days, 2 days, 2.5 days, 3 days) prior to administering the subject with the composition. In a specific embodiment, the chemotherapeutic agent is cyclophosphamide. In other embodiments, the chemotherapeutic agent is paclitaxel, altretamine, capecitabine, etoposide, gemcitabine, ifosfamide, irinotecan, doxorubicin, melphalan, pemetrexed, toptecan, or vinorelbine.

[0013] In yet another aspect, the disclosure features a process comprising the steps of: obtaining bone marrow derived mononuclear cells from a patient; culturing the mononuclear cells in vitro under conditions in which mononuclear cells become adherent to a culture vessel; selecting adherent mononuclear cells; culturing the adherent mononuclear cells in the presence of one or more cytokines under conditions in which the cells differentiate into antigen presenting cells; culturing the antigen presenting cells in the presence of peptides under conditions in which the antigen presenting cells present the peptides on major histocompatibility class I molecules. In certain embodiments, the peptides comprise amino acid sequences corresponding to at least one MHC class I peptide epitope of at least five, at least six, at least seven, or eight of the following antigens: mesothelin, HER-2/neu, IL-13 receptor α2, survivin, CD133, gp100, AIM-2, and EGFR. In some embodiments, the at least one MHC class I peptide epitope is a HLA-A2 epitope. In a specific embodiment, the HLA-A2 epitope is an HLA-A0201 epitope. In some embodiments, the one or more cytokines comprise granulocyte macrophage colony stimulating factor and interleukin-4 (IL-4). In some embodiments, the one or more cytokines comprise tumor necrosis factor-α (TNF-α). In certain embodiments, the bone marrow derived cells are obtained from a patient diagnosed with epithelial ovarian cancer.

[0014] "Epitope" means a short peptide derived from a protein antigen, wherein the peptide binds to a major histocompatibility complex (MHC) molecule and is recognized in

the MHC-bound context by a T cell. The epitope may bind an MHC class I molecule (e.g., HLA-A1, HLA-A2 or HLA-A3) or an MHC class II molecule.

[0015] By "peptide" is meant not only molecules in which amino acid residues are joined by peptide (—CO—NH—) linkages, but also molecules in which the peptide bond is reversed. Such retro-inverso peptidomimetics may be made using methods known in the art, for example such as those described in Meziere et al., J. Immunol. 159, 3230-3237 (1997), incorporated herein by reference. This approach involves making pseudopeptides containing changes involving the backbone, and not the orientation of side chains. Retro-inverse peptides, which contain NH—CO bonds instead of CO—NH peptide bonds, are much more resistant to proteolysis. In addition, the term "peptide" also includes molecules where the peptide bond may be dispensed with altogether provided that an appropriate linker moiety which retains the spacing between the Cα atoms of the amino acid residues is used; it is particularly preferred if the linker moiety has substantially the same charge distribution and substantially the same planarity of a peptide bond.

[0016] "Treatment" and "treating," as used herein refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to inhibit or slow down (lessen) the targeted disorder (e.g., cancer, e.g., ovarian cancer) or symptom of the disorder, or to improve a symptom, even if the treatment is partial or ultimately unsuccessful. Those in need of treatment include those already diagnosed with the disorder as well as those prone or predisposed to contract the disorder or those in whom the disorder is to be prevented. For example, in tumor (e.g., cancer) treatment, a therapeutic agent can directly decrease the pathology of tumor cells, or render the tumor cells more susceptible to treatment by other therapeutic agents or by the subject's own immune system.

[0017] A "dendritic cell" or "DC" is an antigen presenting cell (APC) that typically expresses high levels of MHC molecules and co-stimulatory molecules, and lacks expression of (or has low expression of) markers specific for granulocytes, NK cells, B lymphocytes, and T lymphocytes, but can vary depending on the source of the dendritic cell. DCs are able to initiate antigen specific primary T lymphocyte responses in vitro and in vivo, and direct a strong mixed leukocyte reaction (MLR) compared to peripheral blood leukocytes, splenocytes, B cells and monocytes. Generally, DCs ingest antigen by phagocytosis or pinocytosis, degrade it, present fragments of the antigen at their surface and secrete cytokines.

[0018] By "ovarian cancer" is meant a cancerous growth arising from the ovaries. The term encompasses epithelial ovarian tumors, germ cell ovarian tumors, sex cord stromal ovarian tumors as well as metastatic cancers that spread to the ovaries.

[0019] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Singleton et al., Dictionary of Microbiology and Molecular Biology 3rd ed., J. Wiley & Sons (New York, N.Y. 2001); March, Advanced Organic Chemistry Reactions, Mechanisms and Structure 5th ed., J. Wiley & Sons (New York, N.Y. 2001); Sambrook and Russel, Molecular Cloning: A Laboratory Manual 3rd ed., Cold Spring Harbor Laboratory Press (Cold Spring Harbor, N.Y. 2001); and Lutz et al., Handbook of Dendritic Cells: Biology, Diseases and Therapies, J. Wiley & Sons (New York, N.Y. 2006), provide one skilled in the art with a general guide to many of the terms used in the present application. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated herein by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0020] Other features and advantages of the invention will be apparent from the following detailed description, the drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIG. **1**A is a bar graph showing RNA expression of antigens in human ovarian cancer cell (1031AC) relative to human ovarian epithelial cell (HoEpic) based on quantitative real-time PCR analysis.

[0022] FIG. **1**B is a bar graph showing RNA expression of antigens in cancer stem cell (1031CSC) relative to human ovarian epithelial cell (HoEpic) based on quantitative real-time PCR analysis.

[0023] FIG. **1**C is a bar graph showing RNA expression of antigens in ovarian cancer daughter cells (1031ADC) relative to human ovarian epithelial cell (HoEpic) based on quantitative real-time PCR analysis.

[0024] FIG. **2**A is a bar graph showing gene expression in human ovarian cancer stem cell (1031CSC) relative to human ovarian cancer cell (1031AC)

[0025] FIG. **2**B is a bar graph showing gene expression in ovarian cancer daughter cell (1031ADC) relative to cancer stem cell (1031CSC).

[0026] FIG. **3** is a bar graph displaying the results of an IFN-γ ELISPOT assay of the antigen-specific T cell response to the T2 pulsed with CD133 HLA-A2 peptides of CD133p405, CD133p753, and CD133p804.

[0027] FIG. **4** is a bar graph showing the RNA expression of the indicated genes in a TCGA dataset (586 patient samples).

[0028] FIG. **5**A is a graph depicting overall survival (OS) and RNA expression of the HER2 gene in human ovarian cancer (Dataset: TCGA, 557 human ovarian cancer patients).

[0029] FIG. **5**B is a graph depicting overall survival (OS) and RNA expression of the MSLN gene in human ovarian cancer (Dataset: TCGA, 557 human ovarian cancer patients).

[0030] FIG. **5**C is a graph depicting overall survival (OS) and RNA expression of the survivin gene in human ovarian cancer (Dataset: TCGA, 557 human ovarian cancer patients).

[0031] FIG. **5**D is a graph depicting overall survival (OS) and RNA expression of the gp100 gene in human ovarian cancer (Dataset: TCGA, 557 human ovarian cancer patients).

[0032] FIG. **5**E is a graph depicting overall survival (OS) and RNA expression of the EGFR gene in human ovarian cancer (Dataset: TCGA, 557 human ovarian cancer patients).

[0033] FIG. **5**F is a graph depicting overall survival (OS) and RNA expression of the CD133 gene in human ovarian cancer (Dataset: TCGA, 557 human ovarian cancer patients).

[0034] FIG. **5**G is a graph depicting overall survival (OS) and RNA expression of the IL-13Rα2 gene in human ovarian cancer (Dataset: TCGA, 557 human ovarian cancer patients).

[0035] FIG. **6** is a graph showing overall survival (OS) and RNA expression of IL-13Rα2 in human ovarian cancer patients (Dataset GSE 9891, 285 human ovarian cancer patients).

DETAILED DESCRIPTION

[0036] This disclosure relates in part to compositions that are useful to treat ovarian cancers. The compositions described herein include antigen presenting cells (e.g., dendritic cells) presenting peptide epitopes from five or more (e.g., 5, 6, 7, 8) tumor-associated antigens that are expressed on ovarian cancer stem cells or expressed at a higher levels on ovarian cancer stem cells than on differentiated ovarian tumor cells. Examples of such tumor-associated antigens include mesothelin, HER-2/neu, IL-13 receptor α2, survivin, CD133, gp100, AIM-2, and epidermal growth factor receptor (EGFR). The compositions described herein also include multipeptide mixtures of HLA epitopes of five or more of the above-listed tumor-associated antigens. Such multivalent vaccines are useful to prevent the development of escape mutants. Often, a tumor will evolve to turn off the expression of a particular tumor associated antigen, creating "escape mutants." Thus, an immune response against multiple tumor antigens is more likely to provide effective therapy to deal with such mutants, and can provide significant therapeutic benefits for various patient populations. The multivalent vaccine compositions (multipeptide vaccines and APC vaccines) described herein are useful to raise a cytolytic T cell response against ovarian cancer stem cells thereby killing the cancer stem cells. The vaccines described can be used for the treatment of ovarian cancer and for the prevention or reduction of recurrence of ovarian cancer.

[0037] The compositions and methods of this disclosure feature at least five of the following antigens: mesothelin, HER-2/neu, IL-13 receptor α2, survivin, CD133, gp100, AIM-2, and epidermal growth factor receptor (EGFR). In one embodiment, the epitopes are MHC class I epitopes. In a specific embodiment, the epitopes are peptides that bind HLA-A2. The compositions and methods described herein may also feature one or more epitopes of other tumor associated antigens that are expressed on ovarian cancer stem cells; these epitopes may be MHC class I (e.g., HLA-A2) and/or class II epitopes.

[0038] This disclosure features the use of the peptides described herein (or polynucleotides encoding them) for active in vivo vaccination; for contacting autologous dendritic cells in vitro followed by introduction of the contacted dendritic cells in vivo to activate CTL responses; to activate autologous CTL in vitro followed by adoptive therapy (i.e., introducing the activated autologous CTL into a patient); and to activate CTL from healthy donors (MHC matched or mismatched) in vitro followed by adoptive therapy.

Antigens

[0039] Mesothelin

[0040] Mesothelin is a differentiation antigen present on normal mesothelial cells and overexpressed in several human tumors, including mesothelioma, ovarian cancer, and pancreatic adenocarcinoma. The mesothelin gene encodes a precursor protein that is processed to yield the 40-kDa protein, mesothelin, which is attached to the cell membrane by a glycosylphosphatidyl inositol linkage and a 31-kDa shed fragment named megakaryocyte-potentiating factor. This protein is thought to play a role in cancer metastasis by mediating cell adhesion by binding to MUC16/CA-125.

[0041] Table 1 provides an amino acid sequence of the 622 amino acid human mesothelin protein (also available in GenBank under accession no. NP_001170826.1). Exemplary sequences of mesothelin HLA epitopes are provided in Table 2.

[0042] HER-2

[0043] HER-2 (also known as HER-2/neu, and c-erbB2) is a 1255 amino acid transmembrane glycoprotein with tyrosine kinase activity. HER-2 is overexpressed in a variety of tumor types. This protein promotes tumor growth by activating a variety of cell signaling pathways including MAPK, PI3K/Akt, and PKC.

[0044] Table 1 provides an amino acid sequence of human HER-2 (also available in GenBank under accession no. NP_004439.2). Exemplary sequences of HER-2 HLA are listed in Table 2.

[0045] IL-13 Receptor α2

[0046] IL-13 receptor α2 is a non-signaling component of the multimeric IL-13 receptor. Stimulation of this receptor activates production of TGF-β1, which inhibits cytotoxic T cell function. The human IL-13 receptor α2 amino acid sequence, which is 380 amino acids in length, is shown in Table 1 (also available in Genbank under accession no. NP_000631.1). An exemplary sequence of an IL-13 receptor α2 HLA epitope is shown in Table 2.

[0047] Survivin

[0048] Survivin is a member of the inhibitor of apoptosis family. Survivin inhibits caspase activation, thereby leading to negative regulation of apoptosis or programmed cell death. Survivin is expressed highly in most human tumors and fetal tissue, but is completely absent in terminally differentiated cells. This fact makes survivin an ideal target for cancer therapy as cancer cells are targeted while normal cells are left alone.

[0049] Table 1 provides a sequence of human survivin which is 137 amino acids in length (also available in GenBank under accession no. NP_001012270.1). Exemplary HLA epitopes of survivin are listed in Table 2.

[0050] CD133

[0051] The cell surface marker CD133 (Prominin 1) is expressed in several human cancers including brain cancer, colon cancer, hepatocellular carcinoma, prostate cancer, multiple myeloma, and melanoma. Table 1 provides an amino acid sequence of human CD 133 (also available in GenBank under accession no. NP_001139319.1). Exemplary HLA epitopes of survivin are listed in Table 2.

[0052] gp100

[0053] gp100 is a glycoprotein preferentially expressed in melanocytes. Table 1 provides an amino acid sequence of human gp100 (also available in GenBank under accession no. NP_008859.1). Table 2 lists exemplary HLA epitopes from gp100.

[0054] AIM-2

[0055] AIM-2 is expressed in a variety of tumor types, including neuroectodermal tumors, and breast, ovarian and colon carcinomas. Table 1 provides an amino acid sequence of human AIM-2 (also available in GenBank under accession no. AAD51813.1). An exemplary sequence of an AIM-2 HLA epitope is shown in Table 2.

[0056] Epidermal Growth Factor Receptor (EGFR)

[0057] The epidermal growth factor receptor (EGFR; ErbB-1; HER1 in humans) is the cell-surface receptor for

members of the epidermal growth factor family (EGF-family) of extracellular protein ligands. EGFR exists on the cell surface and is activated by binding of its specific ligands, including epidermal growth factor and transforming growth factor α (TGFα). Table 1 provides an amino acid sequence of human EGFR (also available in GenBank under accession no. NP_005219.2). An exemplary sequence of an EGFR HLA epitope is listed in Table 2.

TABLE 1

Tumor Antigen	Amino Acid Sequence
Mesothelin	MALPTARPLL GSCGTPALGS LLFLLFSLGW VQPSRTLAGE TGQEAAPLDG VLANPPNISS LSPRQLLGFP CAEVSGLSTE RVRELAVALA QKNVKLSTEQ LRCLAHRLSE PPEDLDALPL DLLLFLNPDA FSGPQACTRF FSRITKANVD LLPRGAPERQ RLLPAALACW GVRGSLLSEA DVRALGGLAC DLPGRFVAES AEVLLPRLVS CPGPLDQDQQ EAARAALQGG GPPYGPPSTW SVSTMDALRG LLPVLGQPII RSIPQGIVAA WRQRSSRDPS WRQPERTILR PRFRREVEKT ACPSGKKARE IDESLIFYKK WELEACVDAA LLATQMDRVN AIPFTYEQLD VLKHKLDELY PQGYPESVIQ HLGYLFLKMS PEDIRKWNVT SLETLKALLE VNKGHEMSPQ VATLIDRFVK GRGQLDKDTL DTLTAFYPGY LCSLSPEELS SVPPSSIWAV RPQDLDTCDP RQLDVLYPKA RLAFQNMNGS EYFVKIQSFL GGAPTEDLKA LSQQNVSMDL ATFMKLRTDA VLPLTVAEVQ KLLGPHVEGL KAEERHRPVR DWILRQRQDD LDTLGLGLQG GIPNGYLVLD LSMQEALSGT PCLLGPGPVL TVLALLLAST LA (SEQ ID NO: 1)
HER-2	MELAALCRWG LLLALLPPGA ASTQVCTGTD MKLRLPASPE THLDMLRHLY QGCQVVQGNL ELTYLPTNAS LSFLQDIQEV QGYVLIAHNQ VRQVPLQRLR IVRGTQLFED NYALAVLDNG DPLNNTTPVT GASPGGLREL QLRSLTEILK GGVLIQRNPQ LCYQDTILWK DIFHKNNQLA LTLIDTNRSR ACHPCSPMCK GSRCWGESSE DCQSLTRTVC AGGCARCKGP LPTDCCHEQC AAGCTGPKHS DCLACLHFNH SGICELHCPA LVTYNTDTFE SMPNPEGRYT FGASCVTACP YNYLSTDVGS CTLVCPLHNQ EVTAEDGTQR CEKCSKPCAR VCYGLGMEHL REVRAVTSAN IQEFAGCKKI FGSLAFLPES FDGDPASNTA PLQPEQLQVF ETLEEITGYL YISAWPDSLP DLSVFQNLQV IRGRILHNGA YSLTLQGLGI SWLGLRSLRE LGSGLALIHH NTHLCFVHTV PWDQLFRNPH QALLHTANRP EDECVGEGLA CHQLCARGHC WGPGPTQCVN CSQFLRGQEC VEECRVLQGL PREYVNARHC LPCHPECQPQ NGSVTCFGPE ADQCVACAHY KDPPFCVARC PSGVKPDLSY MPIWKFPDEE GACQPCPINC THSCVDLDDK GCPAEQRASP LTSIISAVVG ILLVVVLGVV FGILIKRRQQ KIRKYTMRRL LQETELVEPL TPSGAMPNQA QMRILKETEL RKVKVLGSGA FGTVYKGIWI PDGENVKIPV AIKVLRENTS PKANKEILDE AYVMAGVGSP YVSRLLGICL TSTVQLVTQL MPYGCLLDHV RENRGRLGSQ DLLNWCMQIA KGMSYLEDVR LVHRDLAARN VLVKSPNHVK ITDFGLARLL DIDETEYHAD GGKVPIKWMA LESILRRRFT HQSDVWSYGV TVWELMTFGA KPYDGIPARE IPDLLEKGER LPQPPICTID VYMIMVKCWM IDSECRPRFR ELVSEFSRMA RDPQRFVVIQ NEDLGPASPL DSTFYRSLLE DDDMGDLVDA EEYLVPQQGF FCPDPAPGAG GMVHHRHRSS STRSGGGDLT LGLEPSEEEA PRSPLAPSEG AGSDVFDGDL GMGAAKGLQS LPTHDPSPLQ RYSEDPTVPL PSETDGYVAP LTCSPQPEYV NQPDVRPQPP SPREGPLPAA RPAGATLERP KTLSPGKNGV VKDVFAFGGA VENPEYLTPQ GGAAPQPHPP PAFSPAFDNL YYWDQDPPER GAPPSTFKGT PTAENPEYLG LDVPV (SEQ ID NO: 2)
IL-13 receptor α2	MAFVCLAIGC LYTFLISTTF GCTSSSDTEI KVNPPQDFEI VDPGYLGYLY LQWQPPLSLD HFKECTVEYE LKYRNIGSET WKTIITKNLH YKDGFDLNKG IEAKIHTLLP WQCTNGSEVQ SSWAETTYWI SPQGIPETKV QDMDCVYYNW QYLLCSWKPG IGVLLDTNYN LFYWYEGLDH ALQCVDYIKA DGQNIGCRFP YLEASDYKDF YICVNGSSEN KPIRSSYFTF QLQNIVKPLP PVYLTFTRES SCEIKLKWSI PLGPIPARCF DYEIEIREDD TTLVTATVEN ETYTLKTTNE TRQLCFVVRS KVNIYCSDDG IWSEWSDKQC WEGEDLSKKT LLRFWLPFGF ILILVIFVTG LLLRKPNTYP KMIPEFFCDT (SEQ ID NO: 3)
Survivin	MGAPTLPPAW QPFLKDHRIS TFKNWPFLEG CACTPERMAE AGFIHCPTEN EPDLAQCFFC FKELEGWEPD DDPMQRKPTI RRKNLRKLRR KCAVPSSSWL PWIEASGRSC LVPEWLHHFQ GLFPGATSLP VGPLAMS (SEQ ID NO: 4)
CD133	MALVLGSLLL LGLCGNSFSG GQPSSTDAPK AWNYELPATN YETQDSHKAG PIGILFELVH IFLYVVQPRD FPEDTLRKFL QKAYESKIDY DKIVYYEAGI ILCCVLGLLF IILMPLVGYF FCMCRCCNKC GGEMHQRQKE NGPFLRKCFA ISLLVICIII SIGIFYGFVA NHQVRTRIKR SRKLADSNFK DLRTLLNETP EQIKYILAQY NTTKDKAFTD LNSINSVLGG GILDRLRPNI IPVLDEIKSM ATAIKETKEA LENMNSTLKS LHQQSTQLSS SLTSVKTSLR SSLNDPLCLV HPSSETCNSI RLSLSQLNSN PELRQLPPVD AELDNVNNVL RTDLDGLVQQ GYQSLNDIPD RVQRQTTTVV AGIKRVLNSI GSDIDNVTQR LPIQDILSAF SVYVNNTESY IHRNLPTLEE YDSYWWLGGL VICSLLTLIV IFYYLGLLCG VCGYDRHATP TTRGCVSNTG GVFLMVGVGL SFLFCWILMI IVVLTFVFGA NVEKLICEPY TSKELFRVLD TPYLLNEDWE YYLSGKLFNK SKMKLTPEQV YSDCKKNRGT YGTLHLQNSF NISEHLNINE HTGSISSELE SLKVNLNIFL LGAAGRKNLQ DFAACGIDRM NYDSYLAQTG KSPAGVNLLS FAYDLEAKAN SLPPGNLRNS LKRDAQTIKT IHQQRVLPIE QSLSTLYQSV KILQRTGNGL LERVTRILAS LDFAQNFITN NTSSVIIEET KKYGRTIIGY FEHYLQWIEF SISEKVASCK PVATALDTAV DVFLCSYIID PLNLFWFGIG KATVFLLPAL IFAVKLAKYY RRMDSEDVYD DVETIPMKNM ENGNNGYHKD HVYGIHNPVM TSPSQH (SEQ ID NO: 5)
gp100	MDLVLKRCLL HLAVIGALLA VGATKVPRNQ DWLGVSRQLR TKAWNRQLYP EWTEAQRLDC WRGGQVSLKV SNDGPTLIGA NASFSIALNF PGSQKVLPDG QVIWVNNTII NGSQVWGGQP VYPQETDDAC IFPDGGPCPS GSWSQKRSFV YVWKTWGQYW QVLGGPVSGL SIGTGRAMLG THTMEVTVYH RRGSRSYVPL AHSSSAFTIT DQVPFSVSVS QLRALDGGNK HFLRNQPLTF ALQLHDPSGY LAEADLSYTW DFGDSSGTLI SRALVVTHTY LEPGPVTAQV VLQAAIPLTS

TABLE 1-continued

Tumor Antigen	Amino Acid Sequence
	CGSSPVPGTT DGHRPTAEAP NTTAGQVPTT EVVGTTPGQA PTAEPSGTTS VQVPTTEVIS TAPVQMPTAE STGMTPEKVP VSEVMGTTLA EMSTPEATGM TPAEVSIVVL SGTTAAQVTT TEWVETTARE LPIPEPEGPD ASSIMSTESI TGSLGPLLDG TATLRLVKRQ VPLDCVLYRY GSFSVTLDIV QGIESAEILQ AVPSGEGDAF ELTVSCQGGL PKEACMEISS PGCQPPAQRL CQPVLPSPAC QLVLHQILKG GSGTYCLNVS LADTNSLAVV STQLIMPGQE AGLGQVPLIV GILLVLMAVV LASLIYRRRL MKQDFSVPQL PHSSSHWLRL PRIFCSCPIG ENSPLLSGQQ V (SEQ ID NO: 6)
AIM-2	MVVLGMQTEE GHCIMLRGLA PSLGGTQVIC KVVGLPSSIG FNTSSHLLFP ATLQGAPTHF PCRWRQGGST DNPPA (SEQ ID NO: 7)
EGFR	MRPSGTAGAA LLALLAALCP ASRALEEKKV CQGTSNKLTQ LGTFEDHFLS LQRMFNNCEV VLGNLEITYV QRNYDLSFLK TIQEVAGYVL IALNTVERIP LENLQIIRGN MYYENSYALA VLSNYDANKT GLKELPMRNL QEILHGAVRF SNNPALCNVE SIQWRDIVSS DFLSNMSMDF QNHLGSCQKC DPSCPNGSCW GAGEENCQKL TKIICAQQCS GRCRGKSPSD CCHNQCAAGC TGPRESDCLV CRKFRDEATC KDTCPPLMLY NPTTYQMDVN PEGKYSFGAT CVKKCPRNYV VTDHGSCVRA CGADSYEMEE DGVRKCKKCE GPCRKVCNGI GIGEFKDSLS INATNIKHFK NCTSISGDLH ILPVAFRGDS FTHTPPLDPQ ELDILKTVKE ITGFLLIQAW PENRTDLHAF ENLEIIRGRT KQHGQFSLAV VSLNITSLGL RSLKEISDGD VIISGNKNLC YANTINWKKL FGTSGQKTKI ISNRGENSCK ATGQVCHALC SPEGCWGPEP RDCVSCRNVS RGRECVDKCN LLEGEPREFV ENSECIQCHP ECLPQAMNIT CTGRGPDNCI QCAHYIDGPH CVKTCPAGVM GENNTLVWKY ADAGHVCHLC HPNCTYGCTG PGLEGCPTNG PKIPSIATGM VGALLLLLVV ALGIGLFMRR RHIVRKRTLR RLLQERELVE PLTPSGEAPN QALLRILKET EFKKIKVLGS GAFGTVYKGL WIPEGEKVKI PVAIKELREA TSPKANKEIL DEAYVMASVD NPHVCRLLGI CLTSTVQLIT QLMPFGCLLD YVREHKDNIG SQYLLNWCVQ IAKGMNYLED RRLVHRDLAA RNVLVKTPQH VKITDFGLAK LLGAEEKEYH AEGGKVPIKW MALESILHRI YTHQSDVWSY GVTVWELMTF GSKPYDGIPA SEISSILEKG ERLPQPPICT IDVYMIMVKC WMIDADSRPK FRELIIEFSK MARDPQRYLV IQGDERMHLP SPTDSNFYRA LMDEEDMDDV VDADEYLIPQ QGFFSSPSTS RTPLLSSLSA TSNNSTVACI DRNGLQSCPI KEDSFLQRYS SDPTGALTED SIDDTFLPVP EYINQSVPKR PAGSVQNPVY HNQPLNPAPS RDPHYQDPHS TAVGNPEYLN TVQPTCVNST FDSPAHWAQK GSHQISLDNP DYQQDFFPKE AKPNGIFKGS TAENAEYLRV APQSSEFIGA (SEQ ID NO: 8)

TABLE 2

Tumor Antigen Peptide Epitopes

Tumor Antigen	Position in Sequence	Peptide Sequence
Mesothelin	20-28	SLLFLLFSL (SEQ ID NO: 9)
Mesothelin	23-31	FLLFSLGWV (SEQ ID NO: 10)
Mesothelin	530-538	VLPLTVAEV (SEQ ID NO: 11)
Mesothelin	547-556 (wt)	KLLGPHVEGL (SEQ ID NO: 12)
Mesothelin	547-556 (554L)	KLLGPHV**L**GL (SEQ ID NO: 13)
Mesothelin	547-556 (554L/556V))	KLLGPHV**LGV** (SEQ ID NO: 14)
Mesothelin	547-556 (548M/554L/556V	K**M**LGPHV**LGV** (SEQ ID NO: 15)
Mesothelin	547-556 (548M/554L)	K**M**LGPHV**L**GL (SEQ ID NO: 16)
Mesothelin	547-556 (548I/554L)	K**I**LGPHV**L**GL (SEQ ID NO: 17)
Mesothelin	547-556 (547Y/554L/556V)	**Y**LLGPHV**LGV** (SEQ ID NO: 18)
Mesothelin	547-556 (547Y/554L)	**Y**LLGPHV**L**GL (SEQ ID NO: 19)
HER-2	5-13	ALCRWGLLL (SEQ ID NO: 20)
HER-2	8-16	RWGLLLALL (SEQ ID NO: 21)

TABLE 2-continued

Tumor Antigen Peptide Epitopes

Tumor Antigen	Position in Sequence	Peptide Sequence
HER-2	63-71	TYLPTNASL (SEQ ID NO: 22)
HER-2	106-114	QLFEDNYAL (SEQ ID NO: 23)
HER-2	369-377	KIFGSLAFL (SEQ ID NO: 24)
HER-2	435-443	ILHNGAYSL (SEQ ID NO: 25)
HER-2	654-662	IISAVVGIL (SEQ ID NO: 26)
HER-2	665-673	VVLGVVFGI (SEQ ID NO: 27)
HER-2	689-697	RLLQETELV (SEQ ID NO: 28)
HER-2	754-762	VLRENTSPK (SEQ ID NO: 29)
HER-2	773-782	VMAGVGSPYV (SEQ ID NO: 30)
HER-2	780-788	PYVSRLLGI (SEQ ID NO: 31)
HER-2	789-797	CLTSTVQLV (SEQ ID NO: 32)
HER-2	799-807	QLMPYGCLL (SEQ ID NO: 33)
HER-2	835-842	YLEDVRLV (SEQ ID NO: 34)
HER-2	851-859	VLVKSPNHV (SEQ ID NO: 35)
HER-2	883-899	KVPIKWMALESILRRRF (SEQ ID NO: 36)
HER-2	952-961	YMIMVKCWMI (SEQ ID NO: 37)
HER-2	971-979	ELVSEFSRM (SEQ ID NO: 38)
IL-13 receptor α2	345-354	WLPFGFILI (SEQ ID NO: 39)
Survivin	18-28	RISTFKNWPFL (SEQ ID NO: 40)
Survivin	53-67 M57	DLAQ**M**FFCFKELEGW (SEQ ID NO: 41)
Survivin	95-104	ELTLGEFLKL (SEQ ID NO: 42)
Survivin	96-104 wt	LTLGEFLKL (SEQ ID NO: 43)
Survivin	96-104 M2 m	L**M**LGEFLKL (SEQ ID NO: 44)
CD133	117-126	LLFIILMPLV (SEQ ID NO: 45)
CD133	301-309	SLNDPLCLV (SEQ ID NO: 46)
CD133	405-413	ILSAFSVYV (SEQ ID NO: 47)
CD133	708-716	GLLERVTRI (SEQ ID NO: 48)
CD133	804-813	FLLPALIFAV (SEQ ID NO: 49)
gp100	71-78	SNDGPTLI (SEQ ID NO: 50)
gp100	154-162	KTWGQYWQV (SEQ ID NO: 51)
gp100	209-217	ITDQVPFSV (SEQ ID NO: 52)
gp100	280-288	YLEPGPVTA (SEQ ID NO: 53)
gp100	613-622	SLIYRRRLMK (SEQ ID NO: 54)
gp100	614-622	LIYRRRLMK (SEQ ID NO: 55)
gp100	619-627	RLMKQDFSV (SEQ ID NO: 56)

TABLE 2-continued

Tumor Antigen Peptide Epitopes

Tumor Antigen	Position in Sequence	Peptide Sequence
gp100	639-647	RLPRIFCSC (SEQ ID NO: 57)
gp100	476-485	VLYRYGSFSV (SEQ ID NO: 58)
AIM-2		RSDSGQQARY (SEQ ID NO: 59)
EGFR	853-861	IXDFGLAKL (SEQ ID NO: 60)

[0058] As noted above, the epitopes listed in Table 2 are only exemplary. One of ordinary skill in the art would be able to identify other epitopes for these tumor associated antigens. In addition, the ordinary artisan would readily recognize that the epitopes listed in Table 2 can be modified by amino acid substitutions to alter HLA binding (e.g., to improve HLA binding). The epitopes may be modified at one, two, three, four, five, or six positions and tested for HLA binding activity. For instance, one or two of the amino acid residues are altered (for example by replacing them with the side chain of another naturally occurring amino acid residue or some other side chain) such that the peptide is still able to bind to an HLA molecule in substantially the same way as a peptide consisting of the given amino acid sequence.

[0059] For example, a peptide may be modified so that it at least maintains, if not improves, the ability to interact with and bind a suitable MHC molecule, such as HLA-A0201, and so that it at least maintains, if not improves, the ability to generate activated CTL which can recognize and kill ovarian cancer cells. Positions 2 and 9 of an HLA-A2-binding nonamer are typically anchor residues. Modifications of these and other residues involved in binding HLA-A2 may enhance binding without altering CTL recognition (Tourdot et al., J. Immunol., 159:2391-2398 (1997)). Based on routine binding assays, those with the desired binding activity and those capable of inducing suitable T cell responsiveness can be selected for use.

[0060] The antigenic peptides described herein can be used in multipeptide vaccines or for loading antigen presenting cells which can then be used for vaccination. These epitopes stimulate a T cell mediated immune response (e.g., a cytotoxic T cell response) by presentation to T cells on MHC molecules. Therefore, useful peptide epitopes of mesothelin, HER-2/neu, IL-13 receptor $\alpha 2$, survivin, CD133, gp100, AIM-2, and epidermal growth factor receptor (EGFR) include portions of their amino acid sequences that bind to MHC molecules and in that bound state are presented to T cells.

[0061] Humans have three different genetic loci that encode MHC class I molecules (designated human leukocyte antigens (HLA)): HLA-A, HLA-B, and HLA-C. HLA class I in humans, and equivalent systems in other animals, are genetically very complex. For example, there are at least 110 alleles of the HLA-B locus and at least 90 alleles of the HLA-A locus. Although any HLA class I (or equivalent) molecule is useful for purposes of this disclosure, it is preferred if the stimulator cell present epitopes in an HLA class I molecule which occurs at a reasonably high frequency in the human population. It is well known that the frequency of HLA class I alleles varies between different ethnic groupings such as Caucasian, African, and Chinese. For example, in Caucasian populations the HLA class I molecule is typically encoded by an HLA-A2 allele, an HLA-A1 allele, an HLA-A3 allele, or an HLA-B27 allele. HLA-A2 is particularly preferred. Combinations of HLA molecules may also be used. For example, a combination of HLA-A2 and HLA-A3 covers about 75% of the Caucasian population. Humans also have three different loci for MHC class II genes: HLA-DR, HLA-DQ, and HLA-DP. Peptides that bind to MHC class I molecules are generally 8-10 amino acids in length. Peptides that bind to MHC class II molecules are generally 13 amino acids or longer (e.g., 12-17 amino acids long).

[0062] T cell epitopes can be identified by a number of different methods. Naturally processed MHC epitopes can be identified by mass spectrophotometric analysis of peptides eluted from antigen-loaded APC (e.g., APC that have taken up antigen, or that have been engineered to produce the protein intracellularly). After incubation at 37° C., cells are lysed in detergent and the MHC protein is purified (e.g., by affinity chromatography). Treatment of the purified MHC with a suitable chemical medium (e.g., under acidic conditions, e.g., by boiling in 10% acetic acid, as described in Sanchez et al., Proc. Natl. Acad. Sci. USA, 94(9): 4626-4630, 1997) results in the elution of peptides from the MHC. This pool of peptides is separated and the profile compared with peptides from control APC treated in the same way. The peaks unique to the protein expressing/fed cells are analyzed (for example by mass spectrometry) and the peptide fragments identified. This protocol identifies peptides generated from a particular antigen by antigen processing, and provides a straightforward means of isolating these antigens.

[0063] Alternatively, T cell epitopes are identified by screening a synthetic library of peptides that overlap and span the length of the antigen in an in vitro assay. For example, peptides that are 9 amino acids in length and that overlap by 5 amino acids can be used. The peptides are tested in an antigen presentation system that includes antigen presenting cells and T cells. T cell activation in the presence of APCs presenting the peptide can be measured (e.g., by measuring T cell proliferation or cytokine production) and compared to controls, to determine whether a particular epitope is recognized by the T cells.

[0064] Another way to identify T cell epitopes is by algorithmic analysis of sequences that have predictive binding to HLA (see, e.g., www.immuneepitope.org) followed by binding studies and confirmation with in vitro induction of peptide specific CD8 T cells.

[0065] The T cell epitopes described herein can be modified to increase immunogenicity. One way of increasing immunogenicity is by the addition of dibasic amino acid

residues (e.g., Arg-Arg, Arg-Lys, Lys-Arg, or Lys-Lys) to the N- and C-termini of peptides. Taking mesothelin as an example, modified T cell epitopes would be RRKLLGPHVEGL, KLLGPHVEGLRR, and KK KLLGPHVEGL, KLLGPHVEGLKK, KR KLLGPHVEGL, KLLGPHVEGLKR, RK KLLGPHVEGL, KLLGPHVEGLRK. Another way of increasing immunogenicity is by amino acid substitutions to either enhance Major Histocompatibility Complex (MHC) binding by modifying anchor residues ("fixed anchor epitopes"), or enhance binding to the T cell receptor (TCR) by modifying TCR interaction sites ("heteroclitic epitopes") (see, e.g., Sette and Fikes, Current Opinion in Immunology, 2003, 15:461-5470). In some embodiments, the epitopes described herein can be modified at one, two, three, four, five, or six positions. Even non-immunogenic or low affinity peptides can be made immunogenic by modifying their sequence to introduce a tyrosine in the first position (see, e.g., Tourdot et al., Eur. J. Immunol., 2000, 30:3411-3421).

[0066] The peptides can also include internal mutations that render them "superantigens" or "superagonists" for T cell stimulation. Superantigen peptides can be generated by screening T cells with a positional scanning synthetic peptide combinatorial library (PS-CSL) as described in Pinilla et al., Biotechniques, 13(6):901-5, 1992; Borras et al., J. Immunol. Methods, 267(1):79-97, 2002; U.S. Publication No. 2004/0072246; and Lustgarten et al., J. Immun. 176:1796-1805, 2006. In some embodiments, a superagonist peptide is a peptide shown in Table 2, above, with one, two, three, or four amino acid substitutions which render the peptide a more potent immunogen.

[0067] Antigenic peptides can be obtained by chemical synthesis using a commercially available automated peptide synthesizer. Chemically synthesized peptides can be precipitated and further purified, for example by high performance liquid chromatography (HPLC). Alternatively, the peptides can be obtained by recombinant methods using host cell and vector expression systems. "Synthetic peptides" includes peptides obtained by chemical synthesis in vitro as well as peptides obtained by recombinant expression. When tumor antigen peptides are obtained synthetically, they can be incubated with antigen presenting cells in higher concentrations (e.g., higher concentrations than would be present in a tumor antigen cell lysates, which includes an abundance of peptides from non-immunogenic, normal cellular proteins). This permits higher levels of MHC-mediated presentation of the tumor antigen peptide of interest and induction of a more potent and specific immune response, and one less likely to cause undesirable autoimmune reactivity against healthy non-cancerous cells.

Multipeptide Vaccines

[0068] In formulating a multipeptide vaccine it is not only important to identify and characterize tumor-associated antigens expressed on the ovarian cancer, but also the combinations of different epitopes from the tumor-associated antigens that increase the likelihood of a response to more than one epitope for the patient. To counter the tumor's ability to evade therapies directed against it, the present disclosure utilizes epitopes from a variety of antigens in the vaccine. Specifically, in one embodiment, combinations or mixtures of at least one HLA epitope from one, two, three, four, five, six, seven, or eight of the following tumor-associated antigens are particularly useful for immunotherapeutic treatments: mesothelin, HER-2/neu, IL-13 receptor α2, survivin, CD133, gp100, AIM-2, and epidermal growth factor receptor (EGFR). More than one epitope from the same antigen can be used in the multipeptide vaccine. For example, the vaccine may contain at least one, at least two, at least three, or at least four different epitopes from any of the eight tumor associated antigens listed above. In addition one or more epitopes from antigens other than the eight listed above can also be used. Furthermore, a class II epitope(s) may also be included.

[0069] To induce CTL killing of ovarian cancer cells, or to treat ovarian cancer, or prevent or reduce recurrence of ovarian cancer, the multipeptide vaccines comprise at least one HLA epitope from at least five (e.g., five, six, seven, or eight) of the following antigens: mesothelin, HER-2/neu, IL-13 receptor α2, survivin, CD133, gp100, AIM-2, and epidermal growth factor receptor (EGFR). In some embodiments, the HLA epitopes are HLA-A2 epitopes.

[0070] Ovarian cancer stem cells can also be targeted for destruction by using multipeptide vaccines that comprise at least one HLA epitope from at least five (e.g., five, six, seven, or eight) of the following antigens: mesothelin, HER-2/neu, IL-13 receptor α2, survivin, CD133, gp100, AIM-2, and epidermal growth factor receptor (EGFR). In some embodiments, the HLA epitopes are HLA-A2 epitopes. These vaccines can not only induce CTL killing of ovarian cancer stem cells but also cells of the differentiated ovarian tumors.

[0071] In some embodiments, the multipeptide vaccines described herein comprise a mixture of peptides that include one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten eleven, twelve) HLA epitopes from five or more (e.g., five, six, seven, or eight) of the antigens listed in Table 2.

[0072] In certain embodiments, the multipeptide vaccines described herein comprise a mixture of peptides that include one or more of the following HLA epitopes (e.g., one, two, three) from five or more of the following antigens (e.g., five, six, seven, or eight):

[0073] KLLGPHVEGL (SEQ ID NO:12); KLLGPHVLGV (SEQ ID NO:14); SLLFLLFSL (SEQ ID NO:9); VLPLTVAEV (SEQ ID NO:11) from mesothelin;

[0074] LTLGEFLKL (SEQ ID NO:43); LMLGEFLKL (SEQ ID NO:44); ELTLGEFLKL (SEQ ID NO: 42); RISTFKNWPFL (SEQ ID NO:40); DLAQMFFCFKELEGW (SEQ ID NO:41) from survivin;

[0075] VMAGVGSPYV (SEQ ID NO:30); KIFGSLAFL (SEQ ID NO:24); IISAVVGIL (SEQ ID NO:26); ALCRWGLLL (SEQ ID NO:20); ILHNGAYSL (SEQ ID NO:25); RLLQETELV (SEQ ID NO:28); VVLGVVFGI (SEQ ID NO:27); YMIMVKCWMI (SEQ ID NO:37); HLYQGCQVV (SEQ ID NO:61); YLVPQQGFFC (SEQ ID NO:62); PLQPEQLQV (SEQ ID NO:63); TLEEITGYL (SEQ ID NO:64); ALIHHNTHL (SEQ ID NO:65); PLTSIISAV (SEQ ID NO:66) from HER-2/neu;

[0076] IMDQVPFSV (SEQ ID NO:67); KTWGQYWQV (SEQ ID NO:51); AMLGTHTMEV (SEQ ID NO:68); ITDQVPFSV (SEQ ID NO:52); YLEPGPVTA (SEQ ID NO:53); LLDGTATLRL (SEQ ID NO:69); VLYRYGSFSV (SEQ ID NO:58); SLADTNSLAV (SEQ ID NO:70); RLMKQDFSV (SEQ ID NO:56); RLPRIFCSC (SEQ ID NO:71) from gp100;

[0077] RSDSGQQARY (SEQ ID NO:59) from AIM-2;

[0078] WLPFGFILI (SEQ ID NO:39) from IL13Rα2;

[0079] ILSAFSVYV (SEQ ID NO:47); YLQWIEFSI (SEQ ID NO:72) from CD133; and

[0080] IXDFGLAKL (SEQ ID NO:60) from EGFR (where X is any amino acid).

[0081] The multipeptide vaccines of the present disclosure can contain mixtures of epitopes from HLA-A2 restricted epitopes alone; HLA-A2 restricted epitopes in combination with at least one HLA-A1 or HLA-A3 restricted epitope; HLA-A2 restricted epitopes in combination with at least one HLA-DR, HLA-DQ, and/or HLA-DP restricted epitope; or HLA-A2 restricted epitopes in combination with at least one HLA-A1 or HLA-A3 restricted epitope and at least one HLA-DR, HLA-DQ, and/or HLA-DP restricted epitope. The MHC class I and MHC class II epitopes can be from the same antigen or different antigens.

[0082] The multipeptide mixture can be administered with adjuvants to render the composition more immunogenic. Adjuvants include, but are not limited to, Freund's adjuvant, GM-CSF, Montanide (e.g., Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, and Montanide ISA-51), 1018 ISS, aluminum salts, Amplivax®, AS15, BCG, CP-870,893, CpG7909, CyaA, dSLIM, flagellin or TLR5 ligands derived from flagellin, FLT3 ligand, IC30, IC31, Imiquimod (ALDARA®), resiquimod, ImuFact IMP321, Interleukins such as IL-2, IL-4, IL-7, IL-12, IL-13, IL-15, IL-21, IL-23, Interferon-α or -β, or pegylated derivatives thereof, IS Patch, ISS, ISCOMATRIX, ISCOMs, Juvlmmune, LipoVac, MALP2, MF59, monophosphoryl lipid A, water-in-oil and oil-in-water emulsions, OK-432, OM-174, OM-197-MP-EC, ONTAK, OspA, PepTel® vector system, poly(lactid co-glycolid) [PLG]-based and dextran microparticles, talactoferrin SRL172, virosomes and other virus-like particles, YF-17D, VEGF trap, R848, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, mycobacterial extracts and synthetic bacterial cell wall mimics, Ribi's Detox, Quil, Superfos, cyclophosphamide, sunitinib, bevacizumab, celebrex, NCX-4016, sildenafil, tadalafil, vardenafil, sorafenib, temozolomide, temsirolimus, XL-999, CP-547632, pazopanib, VEGF Trap, ZD2171, AZD2171, and anti-CTLA4 antibodies. CpG immunostimulatory oligonucleotides can be used to enhance the effects of adjuvants in a vaccine setting. In one embodiment, the multipeptide vaccine is administered with Montanide ISA-51 and/or GM-CSF.

[0083] The multipeptide compositions of the present disclosure can be administered parenterally (e.g., subcutaneous, intradermal, intramuscular, intraperitoneal) or orally. The peptides and optionally other molecules (e.g., adjuvants) can be dissolved or suspended in a pharmaceutically acceptable carrier. In addition, the multipeptide compositions of the present disclosure can contain buffers and/or excipients. The peptides can also be administered together with immune stimulating substances, such as cytokines. The peptides of the multipeptide vaccine can be administered at doses of between 1 mg and 500 mg of peptide. This disclosure also features polynucleotides encoding the peptides of the multivalent vaccine. As an alternative to administering a patient with multipeptide vaccines, polynucleotides encoding the desired HLA epitopes can also be administered to the patient in need of treatment for ovarian cancer.

[0084] The peptides for use in the vaccine can be synthesized, for example, by using the Fmoc-polyamide mode of solid-phase peptide synthesis which is disclosed by Lu et al (1981) J. Org. Chem. 46, 3433 and the references therein. The peptides described herein can be purified by any one, or a combination of, techniques such as recrystallization, size exclusion chromatography, ion-exchange chromatography, hydrophobic interaction chromatography, and reverse-phase high performance liquid chromatography using e.g. acetonitrile/water gradient separation. Analysis of peptides can be carried out using thin layer chromatography, electrophoresis, in particular capillary electrophoresis, solid phase extraction (CSPE), reverse-phase high performance liquid chromatography, amino-acid analysis after acid hydrolysis and by fast atom bombardment (FAB) mass spectrometric analysis, as well as MALDI and ESI-Q-TOF mass spectrometric analysis.

[0085] The peptides disclosed herein can have additional N- and/or C-terminally located stretches of amino acids that do not necessarily form part of the peptide that functions as the actual epitope for MHC molecules but can, nevertheless, be important for efficient introduction of the peptide into cells. The peptides described herein can also be modified to improve stability and/or binding to MHC molecules to elicit a stronger immune response. Methods for such an optimization of a peptide sequence are well known in the art and include, for example, the introduction of reverse peptide bonds or non-peptide bonds. Peptides comprising the sequences described herein can be synthesized with additional chemical groups present at their amino and/or carboxy termini, to enhance, for example, the stability, bioavailability, and/or affinity of the peptides. For example, hydrophobic groups such as carbobenzoxyl, dansyl, t-butyloxycarbonyl, acetyl, or a 9-fluorenylmethoxy-carbonyl group can be added to the peptides' amino terminus. Additionally, hydrophobic, t-butyloxycarbonyl, or amido groups can be added to the peptides' carboxy terminus. Further, all peptides described herein can be synthesized to alter their steric configuration. For example, the D-isomer of one or more of the amino acid residues of the peptides can be used, rather than the usual L-isomer. Still further, at least one of the amino acid residues of the peptides can be substituted by one of the well-known, non-naturally occurring amino acid residues. Alterations such as these can serve to increase the stability, bioavailability and/or binding action of the peptides of the disclosure. The peptides described herein can also be modified with polyethyleneglycol (PEG) and other polymers to extend their half-lives.

[0086] Once each peptide is prepared, it can be solubilized, sterile-filtered, and either stored by itself or mixed with the other peptides of the multipeptide vaccine and stored, at low temperatures (e.g., −80° C.) and protected from light.

Preparation of Antigen Presenting Cells

[0087] Antigen-presenting cells (APCs) are cells that display antigens complexed with major histocompatibility complex (MHC) proteins on their surfaces. T cells cannot recognize, and therefore do not react to, "free" antigen. APCs process antigens and present them to T cells. T cells may recognize these complexes using their T-cell receptors (TCRs). Examples of APCs include dendritic cells, macrophages, B cells, and certain activated epithelial cells. Dendritic cells (DCs) include myeloid dendritic cells and plasmacytoid dendritic cells. APCs, suitable for administration to subjects (e.g., cancer patients), can be isolated or obtained from any tissue in which such cells are found, or can be otherwise cultured and provided.

[0088] APCs (e.g., DCs) can be found, by way of example, in the bone marrow or PBMCs of a mammal, in the spleen of a mammal, or in the skin of a mammal (i.e., Langerhans cells, which possess certain qualities similar to that of DC, may be found in the skin) For example, bone marrow can be har-

vested from a mammal and cultured in a medium that promotes the growth of DC. GM-CSF, IL-4 and/or other cytokines (e.g., TNF-α), growth factors and supplements can be included in this medium. After a suitable amount of time in culture in medium containing appropriate cytokines (e.g., suitable to expand and differentiate the DCs into mature DCs, e.g., 4, 6, 8, 10, 12, or 14 days), clusters of DC are cultured in the presence of epitopes of antigens of interest (e.g., in the presence of a mixture of at least one epitope from at least five, six, seven, or eight, of the following antigens: mesothelin, HER-2/neu, IL-13 receptor α2, survivin, CD133, gp100, AIM-2, and epidermal growth factor receptor (EGFR)) and harvested for use in a cancer vaccine using standard techniques.

[0089] Examples of epitopes that can be used for culturing with the APCs are listed in Table 2. In some embodiments, the APCs (e.g., DCs) are cultured with a mixture of peptides that include one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve) of HLA epitopes from five or more (e.g., five, six, seven, or eight) of the antigens listed in Table 2.

[0090] In certain embodiments, the APCs (e.g., DCs) are cultured with a mixture of peptides that include one or more of the following HLA epitopes (e.g., one, two, or three) from five or more of the following antigens (e.g., five, six, seven, or eight):

[0091] KLLGPHVEGL (SEQ ID NO:12); KLLGPHVLGV (SEQ ID NO:14); SLLFLLFSL (SEQ ID NO:9); VLPLTVAEV (SEQ ID NO:11) from mesothelin;

[0092] LTLGEFLKL (SEQ ID NO:43); LMLGEFLKL (SEQ ID NO:44); ELTLGEFLKL (SEQ ID NO: 42); RISTFKNWPFL (SEQ ID NO:40); DLAQMFFCFKELEGW (SEQ ID NO:41) from survivin;

[0093] VMAGVGSPYV (SEQ ID NO:30); KIFGSLAFL (SEQ ID NO:24); IISAVVGIL (SEQ ID NO:26); ALCRWGLLL (SEQ ID NO:20); ILHNGAYSL (SEQ ID NO:25); RLLQETELV (SEQ ID NO:28); VVLGVVFGI (SEQ ID NO:27); YMIMVKCWMI (SEQ ID NO:37); HLYQGCQVV (SEQ ID NO:61); YLVPQQGFFC (SEQ ID NO:62); PLQPEQLQV (SEQ ID NO:63); TLEEITGYL (SEQ ID NO:64); ALIHHNTHL (SEQ ID NO:65); PLTSIISAV (SEQ ID NO:66) from HER-2/neu;

[0094] IMDQVPFSV (SEQ ID NO:67); KTWGQYWQV (SEQ ID NO:51); AMLGTHTMEV (SEQ ID NO:68); ITDQVPFSV (SEQ ID NO:52); YLEPGPVTA (SEQ ID NO:53); LLDGTATLRL (SEQ ID NO:69); VLYRYGSFSV (SEQ ID NO:58); SLADTNSLAV (SEQ ID NO:70); RLMKQDFSV (SEQ ID NO:56); RLPRIFCSC (SEQ ID NO:71) from gp100;

[0095] RSDSGQQARY (SEQ ID NO:59) from AIM-2;

[0096] WLPFGFILI (SEQ ID NO:39) from IL13Rα2;

[0097] ILSAFSVYV (SEQ ID NO:47); YLQWIEFSI (SEQ ID NO:72) from CD133; and

[0098] IXDFGLAKL (SEQ ID NO:60) from EGFR (where X is any amino acid).

[0099] In certain embodiments, the epitopes are cultured with an APC (e.g., DC) are HLA-A2 epitopes. In addition to the HLA-A2 epitopes, the APCs can also be expanded in the presence of MHC class II epitopes and/or other HLA epitopes (e.g., HLA-A1 and/or HLA-A3). Epitopes of the antigens (e.g., isolated, purified peptides, or synthetic peptides) can be added to cultures at a concentration of 1 μg/ml-50 μg/ml per epitope, e.g., 2, 5, 10, 15, 20, 25, 30, or 40 μg/ml per epitope. Subject-specific APC vaccines (e.g., DC vaccines) are produced, carefully labeled, and stored. Single doses of the peptide-loaded (e.g., 1 to 50×10^6 cells) APCs (e.g., DCs) can be cryopreserved in human serum albumin containing 10% dimethyl sulphoxide (DMSO) or in any other suitable medium for future use.

[0100] In one exemplary method of preparing APC (e.g., DC), the APC are isolated from a subject (e.g., a human) according to the following procedure. Mononuclear cells are isolated from blood using leukapheresis (e.g., using a COBE Spectra Apheresis System). The mononuclear cells are allowed to become adherent by incubation in tissue culture flasks for 2 hours at 37° C. Nonadherent cells are removed by washing. Adherent cells are cultured in medium supplemented with granulocyte macrophage colony stimulating factor (GM-CSF) (800 units/ml, clinical grade, Immunex, Seattle, Wash.) and interleukin-4 (IL-4) (500 units/ml, R&D Systems, Minneapolis, Minn.) for five days. On day five, TNF-α is added to the culture medium for another 3-4 days. On day 8 or 9, cells are harvested and washed, and incubated with peptide antigens for 16-20 hours on a tissue rotator. Peptide antigens are added to the cultures at a concentration of about 10 μg/ml to about 20 μg/ml per epitope.

[0101] Various other methods can be used to isolate the APCs, as would be recognized by one of skill in the art. DCs occur in low numbers in all tissues in which they reside, making isolation and enrichment of DCs a requirement. Any of a number of procedures entailing repetitive density gradient separation, fluorescence activated cell sorting techniques, positive selection, negative selection, or a combination thereof, are routinely used to obtain enriched populations of isolated DCs. Guidance on such methods for isolating DCs can be found, for example, in O'Doherty et al., J. Exp. Med., 178: 1067-1078, 1993; Young and Steinman, J. Exp. Med., 171: 1315-1332, 1990; Freudenthal and Steinman, Proc. Nat. Acad. Sci. USA, 57: 7698-7702, 1990; Macatonia et al., Immunol., 67: 285-289, 1989; Markowicz and Engleman, J. Clin. Invest., 85: 955-961, 1990; Mehta-Damani et al., J. Immunol., 153: 996-1003, 1994; and Thomas et al., J. Immunol., 151: 6840-6852, 1993. One method for isolating DCs from human peripheral blood is described in U.S. Pat. No. 5,643,786.

[0102] The DCs prepared according to methods described herein present epitopes corresponding to the antigens at a higher average density than epitopes present on dendritic cells exposed to a tumor lysate (e.g., an ovarian cancer lysate). The relative density of one or more antigens on antigen presenting cells can be determined by both indirect and direct means. The primary immune response of naïve animals is roughly proportional to the antigen density of antigen presenting cells (Bullock et al., J. Immunol., 170:1822-1829, 2003). Relative antigen density between two populations of antigen presenting cells can therefore be estimated by immunizing an animal with each population, isolating B or T cells, and monitoring the specific immune response against the specific antigen by, e.g., tetramer assays, ELISPOT, or quantitative PCR.

[0103] Relative antigen density can also be measured directly. In one method, the antigen presenting cells are stained with an antibody that binds specifically to the MHC-antigen complex, and the cells are then analyzed to determine the relative amount of antibody binding to each cell (see, e.g., Gonzalez et al., Proc. Natl. Acad. Sci. USA, 102:4824-4829, 2005). Exemplary methods to analyze antibody binding include flow cytometry and fluorescence activated cell sort-

ing. The results of the analysis can be reported e.g., as the proportion of cells that are positive for staining for an individual MHC-antigen complex or the average relative amount of staining per cell. In some embodiments, a histogram of relative amount of staining per cell can be created.

[0104] In some embodiments, antigen density can be measured directly by direct analysis of the peptides bound to MHC, e.g., by mass spectrometry (see, e.g., Purcell and Gorman, Mol. Cell. Proteomics, 3:193-208, 2004). Typically, MHC-bound peptides are isolated by one of several methods. In one method, cell lysates of antigen presenting cells are analyzed, often following ultrafiltration to enrich for small peptides (see, e.g., Falk et al., J. Exp. Med., 174:425-434, 1991; Rotzxhke et al., Nature, 348:252-254, 1990). In another method, MHC-bound peptides are isolated directly from the cell surface, e.g., by acid elution (see, e.g., Storkus et al., J. Immunother., 14:94-103, 1993; Storkus et al., J. Immunol., 151:3719-27, 1993). In another method, MHC-peptide complexes are immunoaffinity purified from antigen presenting cell lysates, and the MHC-bound peptides are then eluted by acid treatment (see, e.g., Falk et al., Nature, 351:290-296). Following isolation of MHC-bound peptides, the peptides are then analyzed by mass spectrometry, often following a separation step (e.g., liquid chromatography, capillary gel electrophoresis, or two-dimensional gel electrophoresis). The individual peptide antigens can be both identified and quantified using mass spectrometry to determine the relative average proportion of each antigen in a population of antigen presenting cells. In some methods, the relative amounts of a peptide in two populations of antigen presenting cells are compared using stable isotope labeling of one population, followed by mass spectrometry (see, e.g., Lemmel et al., Nat. Biotechnol., 22:450-454, 2004).

Administration of Antigen Presenting Cell-Based Vaccine

[0105] The APC-based vaccine can be delivered to a patient (e.g., a patient having a gynecological cancer or a peritoneal cancer) or test animal by any suitable delivery route, which can include injection, infusion, inoculation, direct surgical delivery, or any combination thereof. In some embodiments, the cancer vaccine is administered to a human in the deltoid region or axillary region. For example, the vaccine is administered into the axillary region as an intradermal injection. In other embodiments, the vaccine is administered intravenously.

[0106] An appropriate carrier for administering the cells can be selected by one of skill in the art by routine techniques. For example, the pharmaceutical carrier can be a buffered saline solution, e.g., cell culture media, and can include DMSO for preserving cell viability.

[0107] In certain embodiments, the cells are administered in an infusible cryopreservation medium. The composition comprising the cells can include DMSO and hetastarch as cryoprotectants, Plasmalyte A and/or dextrose solutions and human serum albumin as a protein component.

[0108] The quantity of APC appropriate for administration to a patient as a cancer vaccine to effect the methods described herein and the most convenient route of such administration are based upon a variety of factors, as can the formulation of the vaccine itself. Some of these factors include the physical characteristics of the patient (e.g., age, weight, and sex), the physical characteristics of the tumor (e.g., location, size, rate of growth, and accessibility), and the extent to which other therapeutic methodologies (e.g., chemotherapy, and beam radiation therapy) are being implemented in connection with an overall treatment regimen. Notwithstanding the variety of factors one should consider in implementing the methods of the present disclosure to treat a disease condition, a mammal can be administered with from about 10^5 to about 10^8 APC (e.g., 10^7 APC) in from about 0.05 mL to about 2 mL solution (e.g., saline) in a single administration. Additional administrations can be carried out, depending upon the above-described and other factors, such as the severity of tumor pathology. In one embodiment, from about one to about five administrations of about 10^6 APC is performed at two-week intervals.

[0109] DC vaccination can be accompanied by other treatments. For example, a patient receiving DC vaccination can also be receiving chemotherapy, radiation, and/or surgical therapy before, concurrently, or after DC vaccination. Chemotherapy is used to shrink and slow cancer growth. Chemotherapy is recommended for most women having ovarian cancer after the initial surgery for cancer; however, sometimes chemotherapy is given to shrink the cancer before surgery. The number of cycles of chemotherapy treatment depends on the stage of the disease. Chemotherapy may neutralize antitumor immune response generated through vaccine therapy. In addition, chemotherapy can be combined safely with immunotherapy, with possibly additive or synergistic effects, as long as combinations are designed rationally. Examples of chemotherapeutic agents that can be used in treatments of patients with ovarian cancers include, but are not limited to, carboplatin, cisplatin, cyclophosphamide, docetaxel, doxorubicin, etoposide, gemcitabine, oxaliplatin, paclitaxel, taxol, topotecan, and vinorelbine. In one embodiment, a patient is treated with cyclophosphamide (intravenously 200 mg/kg) prior to APC (e.g., DC) vaccination. For example, a patient can be intravenously injected with cyclophosphasmide (200 mg/kg) one day before, or between 24 hours and one hour before, APC (e.g., DC) vaccination. Cyclophosphamide is an alkylating drug that is used for treating several types of cancer. Cyclophosphamide is an inactive pro-drug; it is converted and activated by the liver into two chemicals, acrolein and phosphoramide. Acrolein and phosphoramide are the active compounds, and they slow the growth of cancer cells by interfering with the actions of deoxyribonucleic acid (DNA) within the cancerous cells. Cyclophosphamide is, therefore, referred to as a cytotoxic drug. Methods of treating cancer using DC vaccination in conjunction with chemotherapy are described, e.g., in Wheeler et al., U.S. Pat. No. 7,939,090. In some embodiments, a patient receiving DC vaccination has already received chemotherapy, radiation, and/or surgical treatment for the gynecological or peritoneal cancer.

[0110] In addition to, or separate from chemotherapeutic treatment, a patient receiving DC vaccination can be treated with any other treatments that are beneficial for ovarian cancer. For example, a patient having ovarian cancer can be treated prior to, concurrently, or after DC vaccination with a COX-2 inhibitor, as described, e.g., in Yu and Akasaki, WO 2005/037995. In another embodiment, a patient receiving DC vaccination can be treated with bevacizumab (Avastin®) prior to, concurrently, or after DC vaccination.

Immunological Testing

[0111] The antigen-specific cellular immune responses of vaccinated subjects can be monitored by a number of different assays, such as tetramer assays and ELISPOT. The fol-

lowing sections provide examples of protocols for detecting responses with these techniques. Additional methods and protocols are available. See e.g., Current Protocols in Immunology, Coligan, J. et al., Eds., (John Wiley & Sons, Inc.; New York, N.Y.).

Tetramer Assay

[0112] Tetramers comprised of recombinant MHC molecules complexed with a peptide can be used to identify populations of antigen-specific T cells. To detect T cells specific for antigens such as HER-2, FBP and mesothelin, fluorochrome labeled specific peptide tetramer complexes (e.g., phycoerythrin (PE)-tHLA) containing peptides from these antigens can be synthesized and provided by Beckman Coulter (San Diego, Calif.). Specific CTL clone CD8 cells can be resuspended in a buffer, e.g., at 10^5 cells/50 μl FACS buffer (phosphate buffer plus 1% inactivated FCS buffer). Cells can be incubated with 1 μl tHLA for a sufficient time, e.g., for 30 minutes at room temperature, and incubation can be continued for an additional time, e.g., 30 minutes at 4° C. with 10 μl anti-CD8 mAb (Becton Dickinson, San Jose, Calif.). Cells can be washed twice, e.g., in 2 ml cold FACS buffer, before analysis by FACS (Becton Dickinson).

ELISPOT Assay

[0113] ELISPOT assays can be used to detect cytokine secreting cells, e.g., to determine whether cells in a vaccinated patient secrete cytokine in response to antigen, thereby demonstrating whether antigen-specific responses have been elicited. ELISPOT assay kits are supplied, e.g., from R & D Systems (Minneapolis, Minn.) and can be performed as described by the manufacturer's instructions.

[0114] Responder (R) 1×10^5 patients' PBMC cells from before and after vaccination are plated in 96-well plates with nitrocellulose membrane inserts coated with capture Ab. Stimulator (S) cells (TAP-deficient T2 cells pulsed with antigen) are added at the R:S ratio of 1:1. After a 24-hour incubation, cells are removed by washing the plates 4 times. The detection Ab is added to each well. The plates are incubated at 4° C. overnight and the washing steps will be repeated. After a 2-hour incubation with streptavidin-AP, the plates are washed. Aliquots (100 μl) of BCIP/NBT chromogen are added to each well to develop the spots. The reaction is stopped, e.g., after 60 minutes, e.g., by washing with water. The spots can be scanned and counted with a computer-assisted image analysis (Cellular Technology Ltd, Cleveland, Ohio). When experimental values are significantly different from the mean number of spots against non-pulsed T2 cells (background values), as determined by a two-tailed Wilcoxon rank sum test, the background values can be subtracted from the experimental values.

In Vitro Induction of CTL in Patient-Derived PBMCs

[0115] The following protocol can be used to produce antigen specific CTL in vitro from patient-derived PBMC. To generate dendritic cells, the plastic adherent cells from PBMCs can be cultured in AIM-V medium supplemented with recombinant human GM-CSF and recombinant human IL-4 at 37° C. in a humidified CO_2 (5%) incubator. Six days later, the immature dendritic cells in the cultures can be stimulated with recombinant human TNF-α for maturation. Mature dendritic cells can then be harvested on day 8, resuspended in PBS at 1×106 per mL with peptide (2 μg/mL), and incubated for 2 hours at 37° C. Autologous CD8+ T cells can be enriched from PBMCs using magnetic microbeads (Miltenyi Biotech, Auburn, Calif.). CD8+ T cells (2×10^6 per well) can be co-cultured with 2×10^5 per well peptide-pulsed dendritic cells in 2 mL/well of AIM-V medium supplemented with 5% human AB serum and 10 units/mL rhIL-7 (Cell Sciences) in each well of 24-well tissue culture plates. About 20 U/ml of IL-2 can be added 24 h later at regular intervals, 2 days after each restimulation.

[0116] On day 7, lymphocytes can be restimulated with autologous dendritic cells pulsed with peptide in AIM-V medium supplemented with 5% human AB serum, rhIL-2, and rhIL-7 (10 units/mL each). About 20 U/ml of IL-2 can be added 24 h later at regular intervals, 2 days after each restimulation. On the seventh day, after the three rounds of restimulation, cells can be harvested and tested the activity of CTL. The stimulated CD8+ cultured cells (CTL) can be co-cultured with T2 cells (a human TAP-deficient cell line) pulsed with 2 μg/ml Her-2, FBP, mesothelin or IL13 receptor α2 peptides. After 24 hours incubation, IFN-γ in the medium can be measured by ELISA assay.

Pharmaceutical Compositions

[0117] In various embodiments, the present disclosure provides pharmaceutical compositions, e.g., including a pharmaceutically acceptable carrier along with a therapeutically effective amount of the vaccines described herein that include multipeptide vaccines and dendritic cells loaded with the antigens described herein. "Pharmaceutically acceptable carrier" as used herein refers to a pharmaceutically acceptable material, composition, or vehicle that is involved in carrying or transporting a compound of interest from one tissue, organ, or portion of the body to another tissue, organ, or portion of the body. For example, the carrier can be a liquid or solid filler, diluent, excipient, solvent, or encapsulating material, or a combination thereof. Each component of the carrier must be "pharmaceutically acceptable" in that it must be compatible with the other ingredients of the formulation. It must also be suitable for use in contact with any tissues or organs with which it can come in contact, meaning that it must not carry a risk of toxicity, irritation, allergic response, immunogenicity, or any other complication that excessively outweighs its therapeutic benefits.

[0118] In various embodiments, the pharmaceutical compositions described herein can be formulated for delivery via any route of administration. "Route of administration" can refer to any administration pathway, whether or not presently known in the art, including, but not limited to, aerosol, nasal, transmucosal, transdermal, or parenteral. "Parenteral" refers to a route of administration that is generally associated with injection, including intraorbital, infusion, intraarterial, intracapsular, intracardiac, intradermal, intramuscular, intraperitoneal, intrapulmonary, intraspinal, intrasternal, intrathecal, intrauterine, intravenous, subarachnoid, subcapsular, subcutaneous, transmucosal, or transtracheal. Via the parenteral route, the compositions can be in the form of solutions or suspensions for infusion or for injection, or as lyophilized powders.

[0119] The pharmaceutical compositions described herein can be delivered in a therapeutically effective amount. The precise therapeutically effective amount is that amount of the composition that will yield the most effective results in terms of efficacy of treatment in a given subject. This amount will vary depending upon a variety of factors, including but not

limited to the characteristics of the therapeutic compound (including activity, pharmacokinetics, pharmacodynamics, and bioavailability), the physiological condition of the subject (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage, and type of medication), the nature of the pharmaceutically acceptable carrier or carriers in the formulation, and the route of administration. One skilled in the clinical and pharmacological arts will be able to determine a therapeutically effective amount through routine experimentation, for instance, by monitoring a subject's response to administration of a compound and adjusting the dosage accordingly. For additional guidance, see Remington: The Science and Practice of Pharmacy (Gennaro ed. 21st edition, Williams & Wilkins PA, USA) (2005). In one embodiment, a therapeutically effective amount of the vaccine can comprise about 10^6 to about 10^8 tumor antigen-pulsed DC (e.g., 10^6, 0.5×10^7, 10^7, 0.5×10^8, 10^8). In some embodiments, a therapeutically effective amount is an amount sufficient to reduce or halt tumor growth, and/or to increase survival of a patient.

Kits

[0120] The present disclosure is also directed to kits to treat ovarian cancer. The kits are useful for practicing the inventive method of treating cancer with a vaccine comprising dendritic cells loaded with the antigens or multipeptide vaccines as described herein. The kit is an assemblage of materials or components, including at least one of the compositions described herein. Thus, in some embodiments, the kit includes a set of peptides for preparing cells for vaccination. The kit can also include agents for preparing cells (e.g., cytokines for inducing differentiation of DC in vitro). The disclosure also provides kits containing a composition including a vaccine comprising dendritic cells (e.g., cryopreserved dendritic cells) loaded with the antigens as described herein.

[0121] The exact nature of the components configured in the kits described herein depends on their intended purpose. For example, some embodiments are configured for the purpose of treating ovarian cancers. In one embodiment, the kit is configured particularly for the purpose of treating mammalian subjects. In another embodiment, the kit is configured particularly for the purpose of treating human subjects. In further embodiments, the kit is configured for veterinary applications, treating subjects such as, but not limited to, farm animals, domestic animals, and laboratory animals.

[0122] Optionally, the kit also contains other useful components, such as, diluents, buffers, pharmaceutically acceptable carriers, syringes, catheters, applicators, pipetting or measuring tools, or other useful paraphernalia as will be readily recognized by those of skill in the art.

[0123] The materials or components assembled in the kit can be provided to the practitioner stored in any convenient and suitable ways that preserve their operability and utility. For example the components can be in dissolved, dehydrated, or lyophilized form; they can be provided at room, refrigerated or frozen temperatures. The components are typically contained in suitable packaging material(s). As employed herein, the phrase "packaging material" refers to one or more physical structures used to house the contents of the kit, such as inventive compositions and the like. The packaging material is constructed by well-known methods, preferably to provide a sterile, contaminant-free environment. The packaging materials employed in the kit are those customarily utilized in cancer treatments or in vaccinations. As used herein, the term "package" refers to a suitable solid matrix or material such as glass, plastic, paper, foil, and the like, capable of holding the individual kit components. Thus, for example, a package can be a glass vial used to contain suitable quantities of an inventive composition containing for example, a vaccine comprising dendritic cells loaded with epitopes from the antigens as described herein. The packaging material generally has an external label which indicates the contents and/or purpose of the kit and/or its components.

EXAMPLES

Example 1

Identification of Tumor Associated Antigens Expressed on Ovarian Cancer Stem Cells

SKOV-3 and Ovarian Cancer Cells (882, 1078, 1082) Culture

[0124] Human ovarian cancer cell line SKOV-3 and 882, 1078, 1082 were cultured in McCoy's 5A medium (Mediatech, Herndon, Va.) supplied with 10% fetal bovine serum (Omega Scientific, Inc), Pen Strep Glutamine (100×) (Invitrogen). All cells were cultured in 5% CO_2 and in a 37° C. cell incubator (Form a Scientific, Inc).

Human Ovarian Cancer Stem Cell Culture

[0125] Human ovarian cancers (882,1078 and 1082) cells were grown in Dulbecco's modified Eagle's medium DMEM/F12 medium (Invitrogen) containing 10% fetal bovine serum (FBS) as growth medium and plated at a density of 4×10^6 cells per 75 cm^2 cell culture flask (Corning). The cells attached and grew as a monolayer in the flasks. These monolayer growing human ovarian cancer cells were switched into DMEM/F12 medium supplemented with B-27 (Invitrogen, Carlsbad, Calif.), 20 ng/ml of basic fibroblast growth factor, and 20 ng/ml of endothelial-derived growth factor (Peprotech, Rocky Hill, N.J.).

Flow Cytometric Analysis

[0126] The human ovarian cancer cells (1×10^6) were resuspended in 1% FBS-PBS stained with following specific antibodies: anti-HER2, anti-IL-13RA2, anti-CD184, anti-CD44, anti-Survivin, anti-CD133, anti-mesothelin, anti-CD24, anti-EGFR, anti-EphA2, anti-FLOR1, anti-nestin, anti-NY-ESO-1, anti-MAGE-A1, and anti-TRP-2. These antibodies were purchased from commercial sources as direct conjugates to either PE or FITC.

[0127] For intracellular antigens (gp100, AIM-2) staining, cells were permeabilized using Cytofix/Cytoperm kit (BD Biosciences) and stained with PE-conjugated 2nd antibody.

[0128] Flow cytometric analysis was performed using a CyAn™ flow cytometer (Beckman Coulter) and the data was analyzed using Summit (Dako, Carpinteria, Calif., USA) software.

[0129] CSC are a defined subset of tumor cells capable of self-renewal and give rise to the proliferating bulk of rapidly proliferating and differentiating cells in a tumor. CSC are responsible for recurrence in many cancers including ovarian cancer. CSC in ovarian cancer are isolated by culturing under non-differentiating non-adherent conditions where they form spheroids. These spheroids have been shown to occur in vivo

and are related to metastases. Ovarian CSC from spheroid cultures have been characterized for their expression of stem cell related antigen.

[0130] Although others have characterized antigen expression on ovarian tumor cells, the antigens present on the CSC fraction of ovarian cancer cells have not been well characterized. Table 3 provides the results of experiments conducted to characterize antigens that are expressed or overexpressed on the CSC population from ovarian cancer.

TABLE 3

Per Cent Positive Expression of Antigen						
		882 line		1078 line		1082 line
Antigens	SKOV3	Adherent	Spheroid	Adherent	Spheroid	Adherent
HER2	99.9	89.9	93.6	91.94	82.18	99.07
IL-13R2	3.4	1.1	26.2	15.97	47.52	18.36
CD184	0.6	10.8	18.4	11.22	40.21	17.67
CD44	99.9	99.7	98.42	62.8	63.88	99.08
Survivin	6.7	1.9	21.4	11.9	67.17	23.19
CD133	6.7	83.4	5.6	93.04	88.17	7.19
Mesothelin	7.4			1.11	12.41	3.31
EGFR	1.55		8.05		6.04	1.65
CD24	31.32		24.05		78.78	39.75
Nestin	2.67		51.25		4.98	1.51
GP100	14.07	3.8	10.2	23.59	0.2	7.8
AIM-2	78.14	28.95	25.38	9.8	0.14	37.6
TRP-2	0.1	0.34	1.12	1.43	0.1	0.37
MAGE-A1	2.08	0.48	2.53	5.68	0.12	0.17
NY-ESO-1	0.09	0.26	0.24	0.95	0.17	0.66

[0131] As shown in Table 3, the antigens expressed on differentiated tumor (adherent) include: HER2, IL-13Rα2 (subset), CD184 (subset), CD44, survivin (subset), CD133, gp100 (subset), AIM2 (subset). The antigens expressed, or with an increased proportion, on CSC tumor (spheroid) include: HER2, IL-13Rα2 (increased), CD184 (increased), CD44, survivin (increased), CD133, mesothelin (increased), CD24, gp100, AIM2 (subset), nestin, and EGFR.

[0132] MHC epitopes of these antigens can be used in a multivalent vaccine for treatment of ovarian cancer.

Example 2

Preparation of Autologous Dendritic Cells (DC)

[0133] Human leukocyte antigen A2 (HLA-A2 or A2) positive patients with ovarian cancer are identified. Peripheral blood mononuclear cells (PBMCs) are isolated from such patients between days −30 to −15 using leukapheresis. The COBE Spectra Apheresis System is used to harvest the mononuclear cell layer. Leukapheresis yields about 10^{10} peripheral blood mononuclear cells (PBMC). If these cells are not to be processed to prepare DCs shortly after they are harvested, the product is packaged in insulated led containers with temperature monitors to ensure that a temperature range of 2-18° C. is maintained.

[0134] For processing the PBMCs to prepare DCs, the PBMCs are allowed to become adherent for two hours at 37° C. in a tissue culture flask and washed in HBSS. PBMC are seeded at a density of 1.4×10^6 cells/cm^2 in 185-cm^2 culture flasks (Nunc, Roskilde, Denmark) and allowed to adhere for 2 h at 37° C. Non-adherent cells are removed by washing four times. Adherent cells are cultured in RPMI 1640 supplemented with GM-CSF (Berlex) and IL-4 (R&D systems) for 5 days. On day 5, 50 ng/ml clinical grade TNF-α (R&D systems) is added to the culture medium for another 3-4 days. On days 8-9, DCs are harvested and washed three times. Ideally about 7×10^9 DCs are needed for treatment.

Example 3

Preparation of Vaccines

[0135] Dendritic cells, prepared as described in Example 2, are washed three times in dPBS, resuspended at 5-10×10^6 cells/ml in complete media and then co-incubated with tumor associated antigen peptides (20 μg/ml per antigen, reconstituted in 10% DMSO). The dendritic cells are incubated with the peptides at 37°/5% CO2 for 16-20 hours on a tissue rotator to facilitate interaction.

[0136] After production, each DC preparation is tested for viability and microbial growth, and undergoes additional quality testing prior to freezing. A certificate of analysis will be produced for each batch (one certificate of analysis for each patient). The DC preparation is then frozen as follows: DC are resuspended in cryo tubes at various concentrations (1×10^7 cells per ml in autologous freezing medium (10% DMSO and 90% autologous serum), then immediately transferred to 2 ml cryo tubes (cryo tube vials, Nunc, Brand Products, Roskilde, Denmark), slowly frozen to −80° C. by using a cryo-freezing container (Nalgene cryo 1° C. freezing container, rate of cooling −1° C./min (Fisher Scientific, CA)) and finally transferred into the gas phase of liquid nitrogen until use.

[0137] The study treatments will be labeled in such a way to clearly identify the patient. It is imperative that only the patient's own (autologous) study treatment be administered to the same individual patient. For these reasons, the blood specimen is procured and handled according to a strict protocol to ensure optimal quality of the specimen and minimum transport time to and from the processing facility, as well as to ensure the unique identification of the specimen at all times including injection back into the patient.

Example 4

Analysis of Expression of Tumor Antigens in Human Ovarian Tumor Samples

Purpose:

[0138] To determine if the antigens described in Example 1 are present on/in primary human ovarian cancer cells.

Materials & Methods:

[0139] Patients were entered into an Institutional Review Board-approved protocol and signed an informed consent prior to tissue collection. For enzymatic digestion of solid tumors, tumor specimen was diced into RPMI-1640, washed and centrifuged at 800 rpm for 5 min at 15-22° C., resuspended in enzymatic digestion buffer (0.2 mg/ml collagenase and 30 units/ml DNase in RPMI-1640) before overnight rotation at room temperature. Cells were then washed and cryopreserved as single cell suspensions for later use. Some solid tumor samples were physically dissociated using a Bellco Cellector device. For antigen profiling, seven solid tumor samples were enzymatically digested overnight and two were physically dissociated. On the day of study, cells were thawed and stained with indicated antibodies for extracellular protein analysis or fixed and permeabilized for staining of intracellular antigens. Multiparameter phenotypic analysis was performed on gated viable tumor cells (EpCAM+, 7AAD negative, CD45 negative) using antibodies specific for the following eight proteins: mesothelin, HER2/neu, IL-13Rα2, survivin, AIM2, RANBP2, gp100, and CD133 and compared to staining achieved using isotype antibody. Antigen positive established tumor cell lines were used as positive control whenever possible. Acquisition was performed on a BD Canto II flow cytometer and analysis performed using Flo-Jo software.

[0140] The antibodies used for the flow cytometric immunofluorescence analysis were as follows: antibodies against human CD45, EpCAM, HER2 and IL-13Rα2 were purchased from Biolegend (San Diego, Calif.); antibodies against mesothelin and survivin were from R&D Systems (Minneapolis, Minn.); antibodies against AIM2, RANBP2 and gp100 were from Abcam (Cambridge, Mass.); and antibody against CD133 was from Miltenyi Biotec (Auburn, Calif.). 7-AAD viability staining solution was purchased from BD Bioscience.

[0141] The flow cytometric immunofluorescence analysis was performed as follows: cells were resuspended in FACS buffer consisting of PBS with 2% FBS (Gemini Bioproducts). 10^6 cells in 100 μl were directly stained with fluoro-chrome-conjugated mAbs at 4° C. for 40 min in the dark. For unconjugated antibodies, second fluoro-chrome-conjugated antibodies were stained for another 20 minutes. For viability gating, cells were briefly stained with 7-AAD solution and analyzed for nonviable cell exclusion using a FACS Cantor II (BD Biosciences). Intracellular staining was according to eBiosciences protocol (San Diego, Calif.).

Results:

[0142] In the study of nine primary human ovarian cancers, 38.6%±13.4% of all viable cells from solid tumor cell suspensions were $EpCAM^+$ tumor cells, while 28.6%±15.3% were $CD45^+$ leukocytes (Table 4). Leukocytes were comprised of $CD14^+$ monocytes, T lymphocytes, and low numbers of B lymphocytes, as well as other (non-T, B, mono) cells not defined within the applied antibody cocktail.

TABLE 4

T.1 Composition of cells from primary solid ovarian

		% viable of total			% of viable cells			% of viable leukocytes			
Sample	Date collected	total	tumor	leuco	CD45+	EpCam+	CD45−	T cells	B cells	mono	other
1796	Mar. 21, 2011	20.6	19.6	55.1	10.2	39.5	89.8	4.7	1.6	60.1	33.6
1797	Mar. 22, 2011	56.5	51.3	72.0	34.5	29.1	65.5	24.4	7.6	36.0	31.9
1807	May 17, 2011	52.1	68.0	78.9	24.3	49.3	75.7	9.3	0.5	44.8	45.4
1836	Aug. 24, 2011	53.9	57.2	46.6	25.8	30.7	74.2	5.2	0.5	67.6	26.8
1884	Apr. 18, 2012	71.6	85.1	43.1	20.1	32.9	79.9	30.8	9.7	18.4	41.1
1913	Sep. 24, 2012	56.2	86.4	85.2	23.5	23.7	76.5	29.9	ND	27.9	24.9
1922*	Apr. 22, 2013	74.5	67.3	81.5	51.3	35.6	48.7	62.4	ND	14.7	12.8
1934	Dec. 12, 2012	85.1	86.1	88.5	13.7	68.4	86.3	47.5	ND	20.3	21.5
1938*	Apr. 22, 2013	47.1	30.5	79.5	54.0	38.0	46.0	23.8	ND	61.5	6.7
	average	57.5	61.3	70.0	28.6	38.6	71.4	26.4	4.0	39.0	27.2
	STDEV	18.59	24.23	17.22	15.33	13.32	15.33	19.35	4.35	20.31	12.52
	SEM	6.20	8.08	5.74	5.11	4.44	5.11	6.45	1.45	6.77	4.17

Note:
This table contains samples prepared by enzyme digestion of solid tumor only except as noted (*)

[0143] Among viable tumor cells, a variety of cell surface or intracellular antigens were detected by flow cytometry. The expression of the antigens is shown in Table 5 below.

TABLE 5

Expression of tumor antigens in human ovarian tumor samples (%)

Tumor	Mesothelin	Her-2	IL-13Rα2	Survivin	AIM2	RANBP2	gp100	CD133
1796 TuTcE	4.72	72.30	35.60	79.00	0.13	95.00	0.45	4.07
1797 TuTcE	4.29	99.80	24.60	97.00	0.16	92.70	2.19	12.2
1807 TuTcE	6.53	89.90	34.00	99.30	0.28	90.20	1.87	12.2
1836 TuTcE	61.50	93.90	34.30	93.90	0.06	76.10	0.48	13.5
1884 TuTcE	4.50	42.00	18.60	68.40	0.61	46.70	1.98	0.12
1913 TuTcE	28.30	85.60	35.60	90.20	0.32	59.90	0.87	1.95
1922 Bellco	20.40	82.50	20.60	75.30	1.40	46.60	3.19	1.26
1934 TuTcE	2.58	12.30	3.42	45.90	1.93	64.30	2.09	0.26

TABLE 5-continued

Expression of tumor antigens in human ovarian tumor samples (%)

Tumor	Mesothelin	Her-2	IL-13Rα2	Survivin	AIM2	RANBP2	gp100	CD133
1938 Bellco	14.50	96.70	62.40	70.70	0.97	96.00	14.40	0.14
average	16.37	75.00	29.90	79.97	0.65	74.17	3.06	5.08
SD	17.98	27.61	15.28	16.21	0.62	19.24	4.10	5.48
SEM	5.99	9.20	5.09	5.40	0.21	6.41	1.37	1.83

[0144] Among all nine samples tested, high frequencies (>70%) of EpCAM$^+$ cells had a HER2$^+$, Survivin$^+$ or RANBP2$^+$ phenotype (Table 5). Lower but detectable levels of mesothelin and IL-13Rα2 were observed, although mesothelin expression was highly variable among specimens tested with some cells expressing no detectable levels of expression. GP100 levels when detectable were low. AIM2 was not detected in any primary ovarian cancer cell, but expressed at low levels in A375 control cells. CD133$^+$ EpCAM+ cells (putative cancer stem cells) were detected at frequencies >1% in six of nine samples tested.

[0145] Table 6 provides the values for mean fluorescence intensity (MFI) in comparison to their matched isotype antibody control. Antigens that were expressed on the greatest frequency of EpCAM$^+$ tumor cells, such as HER2, survivin, and RANBP2, were also expressed at the highest level, as shown by analysis of MFI.

TABLE 6

Expression of tumor antigens in human ovarian tumor samples (MFI)

Tumor	Mesothelin (Iso)	Her-2 (Iso)	IL-13Rα2 (Iso)	Survivin (Iso)	AIM2 (Iso)	RANBP2 (Iso)	gp100 (Iso)
1796 TuTcE	178	300	142	596	155	6327	229
	(117)	(43.8)	(43.8)	(54.5)	(218)	(54.5)	(54.5)
1797 TuTcE	99.6	5340	118	852	197	3852	339
	(76.2)	(30.7)	(30.7)	(41.4)	(222)	(41.4)	(41.4)
1807 TuTcE	104	467	187	2301	202	2672	339
	(71.3)	(55.3)	(55.3)	(55.8)	(228)	(55.8)	(55.8)
1836 TuTcE	861	815	159	1113	137	1588	185
	(149)	(50.5)	(50.5)	(48.9)	(201)	(48.9)	(48.9)
1884 TuTcE	159	156	99.2	330	96.5	810	141
	(133)	(49)	(49)	(48.8)	(101)	(48.8)	(48.8)
1913 TuTcE	326	473	148	1038	168	1487	216
	(150)	(44.6)	(44.6)	(57.3)	(179)	(57.3)	(57.3)
1922 Bellco	300	338	78.6	612	191	1198	278
	(150)	(24.1)	(24.1)	(47.3)	(227)	(47.3)	(47.3)
1934 TuTcE	164	139	99.6	269	103	792	130
	(129)	(58)	(58)	(52.6)	(115)	(52.6)	(52.6)
1938 Bellco	151	687	207	2359	472	1.36E+04	654
	(122)	(37)	(37)	(192)	(484)	(192)	(192)
average	260.29	968.33	137.60	1052.22	191.28	3591.78	279.00
	(121.28)	(43.67)	(43.67)	(66.51)	(219.44)	(66.51)	(66.51)
SD	238.55	1654.63	42.76	778.47	112.10	4158.01	159.83
	(29.27)	(11.25)	(11.25)	(47.31)	(110.14)	(47.31)	(47.31)
SEM	79.52	551.54	14.25	259.49	37.37	1386.00	53.28
	(9.76)	(3.75)	(3.75)	(15.77)	(36.71)	(15.77)	(15.77)

Conclusions:

[0146] The above results suggest an opportunity for immune-based therapy for advanced ovarian cancer. In particular, expression levels of HER2, survivin, and RANBP2 suggest that these molecules may allow for near universal therapy among ovarian cancer patients. Mesothelin and IL-13Rα2 also represent reasonable targets, though their expression level is moderate, and in some patients' cancer cells, completely lacking.

[0147] In sum, these data provide a rationale for the creation of immunotherapy targeting a broad array of antigens including HER2, mesothelin, IL-13Rα, survivin, and RANBP2 for women with ovarian cancer.

Example 5

Quantitative Real-Time PCR-Based Analysis of Gene Expression in Human Ovarian Cancer Cells, Cancer Stem Cells, and Ovarian Cancer Daughter Cells

Objective:

[0148] To compare the gene expression of antigens in human ovarian cancer cells, cancer stem cells, and ovarian cancer daughter cells using real-time PCR (RT-PCR).

Materials & Methods:

1. Antigens:

[0149] Her-2, IL-13Rα2, mesothelin, survivin, CD133, gp100, EGFR, AIM2

2. PCR TaqMan® Gene Expression Probes and Reagents:

[0150] MSLN (Mesothelin) gene expression assay, Life Technologies, Part# Hs00245879_m1;

[0151] HER2 gene expression assay, Life Technologies, Part# Hs01001580_m1;

[0152] IL-13Rα2 gene expression assay, Life Technologies, Part# Hs00152924_m1;

[0153] BIRC5 (Survivin) gene expression assay, Life technologies, Part# Hs03043576_m1;

[0154] PROM1 (CD133) gene expression assay, Life Technologies, Part# Hs01009250_m1

[0155] PMEL (gp100) gene expression assay, Life Technologies, Part#Hs00173854_m1

[0156] AIM2 (Custom TaqMan® Gene Expression Assay), Life Technologies, Cat#4331348

[0157] GAPDH gene expression assay, Life Technologies, Part# Hs02758991_g1

[0158] EGFR gene expression assay, Life Technologies, Part# Hs01076078_m1

[0159] TaqMan gene expression master mix; Life technologies, part#4369016

[0160] Rneasy Mini Kit RNA isolation (cat#74104, Qiagen)

[0161] High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (cat#4374966, Life Technologies)

3. Cell Lines:

[0162] human ovarian cancer cells (AC) 882AC and 1031AC, cancer stem cells (CSC) 882CSC and 1031 CSC, ovarian cancer daughter cells (ADC) 882 ADC and 1031 ADC

4. Human Ovarian Cancer Cells (AC) Culture

[0163] Ovarian cancer cell lines 882AC and 1031AC were cultured in McCoy's 5A medium (Mediatech, Herndon, Va.) supplied with 10% fetal bovine serum (Omega Scientific, Inc.) and Pen Strep Glutamine (100×) (Invitrogen). All cells were cultured in 5% CO_2 and at 37° C. in a cell incubator (Form a Scientific, Inc.).

5. Human Ovarian Cancer Stem Cells (CSC) Culture

[0164] Human ovarian cancers cells (882AC, 1031AC) were grown in Dulbecco's modified Eagle's medium DMEM/F12 medium (Invitrogen) containing 10% fetal bovine serum (FBS) as growth medium and plated at a density of 1×10^6 cells per 75 cm^2 cell culture flask (Corning Inc.). The cells attached and grew as a monolayer in flasks. The monolayers were then switched into DMEM/F12 medium supplemented with B-27 (Invitrogen, Carlsbad, Calif.), 20 ng/ml of basic fibroblast growth factor, and 20 ng/ml of endothelial-derived growth factor (Peprotech, Rocky Hill, N.J.).

6. Human Ovarian Cancer Daughter Cells (ADC) Culture

[0165] Human ovarian cancer stem cells (882CSC, 1031 CSC) were grown in Dulbecco's modified Eagle's medium DMEM/F12 medium (Invitrogen) containing 10% fetal bovine serum (FBS) as growth medium and plated at a density of 1×10^6 cells per 75 cm^2 cell culture flask (Corning Inc.). The cells attached and grew as a monolayer in flasks in about 2-3 weeks.

7. RNA Extraction, cDNA Synthesis, and qPCR

[0166] Total RNA was extracted from cell lines 882AC, 882CSC, 882ADC, and 1031AC, 1031CSC, and 1031ADC using Rneasy Mini Kit (Qiagen) according to the manufacturer's instructions. The complementary DNA was synthesized using High-Capacity® cDNA Reverse Transcription (cat#4374966), Life Technologies, CA) following the manufacturer's protocol.

[0167] The real-time PCR reactions were performed according to the manufacturer's instructions. The reaction consisted of 8.0 μl cDNA (42 ng), 10 μl TaqMan PCR Master Mix, 1.0 μl nuclease-free water and the following 1.0 μl TaqMan PCR probes (20×) for these genes: Hs01001580_m1 (HER2), Hs00152924_m1 (IL-13Rα2), Hs00245879_m1 (mesothelin), Part# Hs03043576 ml (BIRC5, Survivin), Part# Hs01009250 ml (PROM1,CD133), Part#Hs00173854_m1 (PMEL,GP100), Cat#4331348 (AIM2 Custom probe), Part# Hs01076078_m1 (EGFR), as well as internal control Hs02758991_g1 (GAPDH).

[0168] The reactions were performed on Bio-Rad iQ5 Real Time PCR system with the following thermal cycles: one cycle of 50° C. for 2 minutes and 95° C. for 10 minutes, followed by 40 cycles with a denaturation at 95° C. for 15 seconds and an annealing/extension at 56° C. for 60 seconds, extension at 72° C. for 30 seconds and a final extension step at 72° C. for 5 min. A melting curve was determined at the end of each reaction to verify the specificity of the PCR reaction. Ct Data analysis was performed using the Bio-Rad software supplied with the IQ5 Cycler system.

8. Data Analysis Using [2^−Δ(ΔCt)] Method

[0169] Relative quantities for each antigen gene were calculated using the comparative [2^−Δ(ΔCt)] method. The Ct value represents the cycle number at which the fluorescence passes the defined threshold. Delta Ct values (delta $Ct=Ct_{test\ gene}-Ct_{mean\ of\ control\ genes}$) were used to compare the difference of gene expression. Ct values of antigens gene expression levels were normalized to GAPDH and comparative Ct method [2^−Δ(ΔCt)] was used to evaluate the gene expression.

Results:

[0170] The gene expression of HER2, mesothelin, survivin, gp100, EGFR, AIM2, CD133, IL-13Rα2 was evaluated in human ovarian cancer cells (1031AC), cancer stem cells (1031CSC), and ovarian cancer daughter cells (1031ADC). As shown in FIG. **1**, the relative gene expression of HER2, mesothelin, survivin, gp100, and EGFR in 1031AC were 0.8312, 0.0015, 7.6, 0.637, and 0.385, respectively. These results suggest that the gene expression of survivin was higher (7.6 fold change) in 1031AC relative to control cell, whereas the gene expression of HER2, mesothelin, gp100 and EGFR in 1031AC was lower than that on the control cell. The relative gene expression of HER2, mesothelin, survivin, gp100, and EGFR in 1031CSC were 0.8467, 0.0027, 4.59, 0.4579, and 0.0518, respectively. These results suggest that the relative gene expression of survivin was higher (4.59 fold change) in 1031CSC relative to the control cell, whereas the relative gene expression of HER2, mesothelin, gp100, and EGFR in 1031CSC were lower expression than that in control cell. The relative gene expression of HER2, mesothelin, survivin, gp100, and EGFR in 1031ADC were 1.02, 0.00372, 22.94, 0.305, and 0.475, respectively. These results suggest that the relative gene expression of survivin was higher (22.94 fold) in 1031ADC relative to control cell, whereas the relative gene expression of HER2, mesothelin, gp100, and EGFR in 1031ADC was lower expression than that in control cell. The gene expression of AIM2 is only detectable in 1031AC, 1031CSC and 1031ADC, and is undetectable in the control cell. The gene expression of CD133 is only detectable in

1031CSC, and is undetectable in both 1031AC and 1031ADC. The gene expression of IL-13Rα2 is undetectable in 1031AC, 1031CSC and 1031ADC, and at low levels in the control cell.

[0171] The gene expression of HER2, mesothelin, survivin, gp100, EGFR, AIM2, CD133, and IL-13Rα2 was compared amongst human ovarian cancer cells (1031AC), cancer stem cells (1031CSC), and ovarian cancer daughter cells (1031ADC). As shown in FIG. **2**, the relative gene expression of HER2, mesothelin, survivin, gp100, EGFR, and AIM2 was 1.02, 1.81, 0.6057, 0.717, 0.1346, and 1.04 fold in 1031CSC relative to 1031AC. These results suggest that the gene expression of HER2, mesothelin and AIM2 in 1031CSC were a little higher than that in 1031AC, whereas the relative gene expression of survivin, gp100, and EGFR in 1031CSC were a little lower level than that in 1031AC. The relative gene expression of HER2, mesothelin, survivin, gp100, EGFR, AIM2, CD133, and IL-13Rα2 were 1.203, 1.37, 4.98, 0.6659, 9.37, 1.37 fold in 1031ADC relative to 1031CSC. These results suggest that the gene expression of HER2, mesothelin, and AIM2 in 1031ADC were a little higher than in 1031CSC, whereas survivin and EGFR were over-expressed in 1031ADC compared to 1031CSC. The gene expression of gp100 in 1031ADC was lower than that in 1031CSC. The gene expression of CD133 was only detectable in 1031CSC, and was undetectable either in 1031AC or 1031ADC. The gene expression of IL-13Rα2 was undetectable in 1031AC, 1031CSC, and 1031ADC, and at a lower level in control cells.

Conclusion:

[0172] 1. Based on the Ct value of q-PCR, HER2, mesothelin, survivin, gp100, EGFR, and AIM2 were expressed in ovarian cancer cells (1031AC), ovarian cancer stem cells (1031CSC) and ovarian cancer daughter cells (1031ADC). The Ct of CD133 was only detectable in 1031CSC and was undetectable in 1031AC and 1031ADC under the experimental conditions. The Ct of IL-13Rα2 was undetectable in 1031AC, 1031CSC, and 1031ADC under the experimental conditions used herein.

2. The relative gene expression of survivin was 7.6, 4.59, and 22.94 fold in 1031AC, 1031CSC, and 1031ADC, respectively, suggesting that survivin has a higher level of expression in 1031AC, 1031CSC, and 1031ADC relative to control cells. The relative gene expression of HER2 in 1031ADC was 1.02 fold, suggesting that the expression in 1031ADC was a little higher than that in control cell, whereas the expression of HER2 in 1031AC and 1031CSC was lower level relative to control cell.

3. The gene expression of HER2, mesothelin, AIM2, and survivin in 1031CSC and 1031ADC were 1.02, 1.203; 1.81, 1.37; 1.04, 1.37; and 0.6057, 4.98 fold relative to 1031AC and 1031CSC, respectively, indicating that the genes expression of HER2, mesothelin, AIM2, and survivin in 1031ADC were higher than that in 1031CSC, and the gene expression of HER2, mesothelin, AIM2 in 1031CSC were higher than that in 1031AC. The gene expression of survivin in 1031CSC was lower level than that in 1031AC.

4. CD133 showed lower level expression in 1031CSC and undetectable expression in either 1031AC or 1031ADC. IL-13Rα2 was undetectable in 1031AC, 1031CSC, and 1031ADC under the experimental conditions used, suggesting that these genes are expressed at a lower level in these cells.

[0173] Taken together, identification of unique genes expression molecular signatures of HER2, mesothelin, survivin, gp100, EGFR, AIM2, CD133, and IL-13Rα2 provide a framework for the rational design of immunotherapy target for human ovarian cancer cell, cancer stem cell and ovarian cancer daughter cell.

Example 6

Analysis of the Expression of Tumor Antigens in Human Ovarian Tumor Cancer Cells, Cancer Stem Cells, and Ovarian Cancer Daughter Cells Based on Flow Cytometric Assay

Objective:

[0174] To utilize flow cytometry-based analysis of antigen expression profiles in primary human ovarian cancer cells, cancer stem cells, and ovarian cancer daughter cells for potential immunotherapeutic targeting.

Materials & Methods:

1. Reagents

[0175] DMEM/F12: Invitrogen, Cat#11330-057 (Lot#1184632, Lot#1109388, Lot#891768);

[0176] McCoy's 5A, 1×: Mediatech, Inc, cat#10-050-CV (Lot#10050090, Lot#10050088);

[0177] B-27 supplement (50×): Invitrogen, cat#12587-010 (Lot#1192265, Lot#1153924, Lot#1079052);

[0178] Fetal Bovine Serum: Omega Scientific, Inc. Cat# FB-11 (Lot#170108, Lot#110300);

[0179] Pen Strep Glutamine: Invitrogen, cat#10378-016 (Lot#1030595);

[0180] Human FGF-basic: PeproTech, cat#100-18B (Lot#041208-1, Lot#051108);

[0181] Human EGF: cat# AF-100-15 (Lot#0212AFC05, Lot#0711AFC05, Lot#0211AFC05-1, Lot#0911AFC05-1);

[0182] BD Cytofix/cytoperm, Fixation and permeabilization kit. Cat#51-6896KC (Lot#81617); and

[0183] The antibodies used for the flow cytometric assay were as follows: PE-labeled antibodies against human survivin were from R&D Systems (Minneapolis, Minn.); PE-labeled antibodies against human HER-2/neu, IL-13Rα2, and EGFR were from Biolegend (San Diego, Calif.); PE-labeled antibody against human CD133 was from Miltenyi Biotec (San Diego, Calif.); antibody against human gp100 was from AbCam (Cambridge, Mass.); and antibody against human mesothelin was from Santa Cruz Biotechnology (Dallas, Tex.).

2. Cell Lines

[0184] Primary human ovarian cancer cells (AC): 882AC, 1031AC, 1078AC, 1082AC, 1077AC, 1105AC, and 1064AC;

[0185] Human ovarian cancer stem cells (CSC): 882CSC, 1031CSC, 1078CSC, and 1082CSC;

[0186] Human ovarian cancer daughter cells (ADC): 882ADC, 1031ADC, and 1078ADC; and SKOV3 human ovarian cancer cell (American Type Culture Collection).

3. Human Ovarian Cancer Cells (AC) Culture

[0187] Human ovarian cancer cell lines (AC) (882AC, 1031AC, 1078AC, 1082AC, 1077AC, 1105AC, 1064AC, and SKOV3) were cultured in McCoy's 5A medium (Mediatech,

Herndon, Va.) supplied with 10% fetal bovine serum (Omega Scientific, Inc.) and Pen Strep Glutamine (100×) (Invitrogen). All cells were cultured in 5% CO_2 and 37° C. in a cell incubator (Form a Scientific, Inc).

4. Human Ovarian Cancer Stem Cells (CSC) Culture

[0188] Human ovarian cancers cells (AC) (882AC, 1031AC, 1078AC, 1082AC) were grown in Dulbecco's modified Eagle's medium DMEM/F12 medium (Invitrogen) containing 10% fetal bovine serum (FBS) as growth medium and plated at a density of 1×10^6 cells per 75 cm^2 cell culture flask (Corning Inc.). The cells attached and grew as a monolayer in flasks. Then, these monolayer cells were switched into DMEM/F12 medium supplemented with B-27 (Invitrogen, Carlsbad, Calif.), 20 ng/ml of basic fibroblast growth factor, and 20 ng/ml of endothelial-derived growth factor (Peprotech, Rocky Hill, N.J.).

5. Human Ovarian Cancer Daughter Cells (ADC) Culture

[0189] Human ovarian cancer stem cells (ADC) (882ADC, 1031ADC, 1078ADC) were grown in Dulbecco's modified Eagle's medium DMEM/F12 medium (Invitrogen) containing 10% fetal bovine serum (FBS) as growth medium and plated at a density of 1×10^6 cells per 75 cm^2 cell culture flask (Corning Inc.). The cells attached and grew as a monolayer in flasks in about 2-3 weeks.

6. Flow Cytometric Analysis

[0190] The human ovarian cancer cells, cancer stem cells, and ovarian cancer daughter cells (0.5×10^6 or 1×10^6) were resuspended in 1% FBS-PBS and stained with the following specific PE labeled antibodies: PE-labeled antibodies against human survivin from R&D Systems (Minneapolis, Minn.); PE-labeled antibodies against human HER-2/neu, IL-13Rα2, and EGFR from Biolegend (San Diego, Calif.); PE-labeled antibody against human CD133 from Miltenyi Biotec (San Diego, Calif.); antibody against human gp100 from AbCam (Cambridge, Mass.); and antibody against human mesothelin from Santa Cruz Biotechnology (Dallas, Tex.).

[0191] For intracellular antigens (gp100) staining, cells were permeabilized using Cytofix/Cytoperm kit (BD Biosciences) and stained with PE-conjugated 2nd antibody.

[0192] Flow cytometric analysis was performed using a CyAn™ flow cytometer (Beckman Coulter) and the data was analyzed using Summit (Dako, Carpinteria, Calif.) software.

Results:

[0193] In this study, expression of several antigens was tested using a FACS assay in seven primary human ovarian cancer cells, four human ovarian cancer stem cells, and three human ovarian cancer daughter cells. The expression results are listed in Tables 7-9.

TABLE 7

Expression of tumor antigens in human ovarian tumor samples (%)

Tumor ID	Mesothelin	HER2	IL13Rα2	Survivin	CD133	EGFR	gp100
882-CSC	2.24	84.33	15.67	29.58	2.17	65.87	10.2
882-AC	1.57	95.75	18.39	7.05	0.88	88.98	4.76
882-ADC	2.27	97.78	5.4	8.19	4.02	92.82	0.62
1031-CSC	2.12	49.93	9.67	25.3	1.45	46.24	
1031-AC	1.36	97.57	5.03	6.78	1.42	93.98	
1031-ADC	2.49	98.59	5.06	10.97	0.38	96.99	
1078-CSC	2.58	83.94	31.47	36.43	10.77	17.78	0.2
1078-AC	1.55	99.16	58.81	6.31	1.37	91.61	23.59
1078-ADC	2.89	96.15	93.79	17.02	3.58	88.73	
1085AC	1.65	86.87	31.55	11.79	3.12	59.37	
Average	2.072	89.007	27.484	15.942	2.916	74.237	7.874
SD	0.514	14.98	28.76	10.81	3.01	26.34	9.66
SEM	0.163	4.73	9.09	3.419	0.948	8.34	4.32

[0194] Table 7 is a summary of the expression of antigens of interest in four primary human ovarian cancer cells, three human ovarian cancer stem cells, and three human ovarian daughter cells. The results indicate that the average antigen expression of mesothelin, HER2, IL13Rα2, survivin, CD133, EGFR, and gp100 were 2.072%, 89.07%, 27.49%, 15.94%, 2.92%, 74.24% and 7.87%, respectively. The expression levels of mesothelin and CD133 were lower compared to the other antigens in ovarian cancer cells, cancer stem cells and ovarian cancer daughter cells. HER2 and EGFR were highly expressed in ovarian cancer cells, cancer stem cells, and ovarian cancer daughter cells.

[0195] Table 8 provides the values of mean fluorescence intensity (MFI) in comparison to their matched isotype antibody control (Iso). The MFI results indicated that the MFI of isotype Abs are lower than that of the MFI of antigen Abs.

TABLE 8

Expression of tumor antigens in human ovarian tumor samples (MFI)

Tumor ID	Meso	Meso (Iso)	HER2	HER2 (Iso)	IL13Rα2	IL13Rα2 (Iso)	Survivin	Survivin (Iso)	CD133	CD133 (Iso)
882-CSC	10.6	6.48	21.86	6.48	19.13	6.48	23.67	6.48	15.57	6.48
882-AC	8.33	10.28	22.69	10.28	18.9	10.28	34.13	10.28	16.59	10.28

TABLE 8-continued

Expression of tumor antigens in human ovarian tumor samples (MFI)

Tumor ID	Meso	Meso (Iso)	HER2	HER2 (Iso)	IL13Rα2	IL13Rα2 (Iso)	Survivin	Survivin (Iso)	CD133	CD133 (Iso)
882-ADC	102.36	12.85	89.58	16.96	32.75	14.78	71.32	26.03	50.02	21.16
1031-CSC	24.66	47.21	6.5	47.21	10.59	47.21	20.99	47.21	24.89	47.21
1031-AC	42.83	7.35	48.02	6.75	16.3	6.75	39.52	6.63	35.26	9.96
1031-ADC	28.75	2.86	29.52	3	10.85	4.41	8.36	2.87	18.55	2.16
1078-CSC	17.73	29.82	81.21	29.82	95.99	29.82	43.53	29.82	86.23	29.52
1078-AC	25.42	19.97	48.15	19.97	42.58	19.97	39.61	19.97	30.39	19.97
1078-ADC	15.49	9.05	50.9	9.05	53.6	9.05	47.95	9.05	22.37	9.05
Skov3	79.95	58.12	500	58.12	121.8	58.12	17.88	58.12	55.4	58.12
Avg.	35.61	20.40	89.84	20.76	42.25	20.69	34.70	21.65	35.53	21.39
SD	31.34	18.83	146.45	18.72	38.26	18.64	18.04	18.76	22.44	18.51
SEM	9.91	5.95	46.17	5.91	12	5.89	5.7	5.93	7.09	5.85

[0196] As shown in Table 9, the HER2 and IL13Rα2 antigens were highly expressed in 1082AC, 1082CSC, 1077AC, 1105AC, and 1064AC, their expression levels being 82.03% and 44.97%, respectively. Mesothelin, CD133, and gp100 were expressed at lower levels. HER2 was also expressed at a high level in the SKOV3 human ovarian cancer cell. IL-13Rα2 and mesothelin were also expressed at a high level in A375 and Hela-229 cells, their expression levels being 82.87% and 55.9%, respectively.

TABLE 9

Expression of tumor antigens in human ovarian tumor samples (%)

Tumor ID	Meso	HER2	IL13Rα2	Survivin	CD133	EGFR	gp100
1082-CSC	3.96	87.64	53.15	45.8	7.62	0.6	
1082-AC	5.22	98.25	7.25	9.25	2.68	1.65	7.8
1077-AC	1.55	83.63	88.73	7.84	0.72	37.55	
1105-AC	3.28	78.59	4.5	80.73	7.89		
1064-AC	1.27	62.05	71.22	4.61	0.12	75.87	
Avg.	3.06	82.03	44.97	29.65	3.81	28.92	
SD	1.66	13.31	37.85	33.12	3.73	35.7	
SEM	0.74	5.94	16.92	14.82	1.66	17.85	
SKOV3	1.23	99.5	0.51	1.91	0.63	1.55	14.07
Avg.	1.23	99.5	0.51	1.91	0.63	1.55	
SD	0.94	0.63	0.19		0.15		
SEM	0.54	0.36	0.11		0.11		
A375			82.87				
Hela-229	55.9						

Conclusion:

[0197] The above results demonstrated that ovarian cancer cells, ovarian cancer stem cells, and ovarian cancer daughter cell express the tested antigens. Of these, HER2 and EGFR showed the highest expression, and the expression of IL13Rα2, survivin was at a moderate level. gp100, mesothelin, and CD 133 were expressed at a lower level; however, in view of their RNA expression levels based on the q-PCR assay, they are still good candidates for immunotherapy targets.

[0198] Taken together, a vaccine based on the antigens of HER2, mesothelin, survivin, gp100, EGFR, AIM2, CD133, and IL-13Rα2 can target human ovarian cancer cells, cancer stem cells, as well as ovarian cancer daughter cells. Moreover, the antigens up-regulated expressions in ovarian cancer stem cells compared to ovarian cancer cells and daughter cells based on FACS data in Table 7 provide a new target cell for immunotherapy specifically targeting ovarian cancer stem cells.

Example 7

IFN-γ ELISPOT Assay of Antigen-Specific T Cell Response

Objective:

[0199] To conduct an IFN-γ ELISPOT assay to check the antigen-specific T cell response to the CD133 HLA-A2 peptides: CD133p405, CD133p753, and CD133p804.

[0200] In order to develop new generation of immunotherapy targets for ovarian cancer cell and ovarian cancer stem cells, we proposed the above HLA-A2 peptides as potential targets. We hypothesized that CD133 HLA-A2 A2 peptides could induce an antigen-specific immune response.

[0201] To test this hypothesis, effector $CD8^+$ T cells were isolated and co-cultured with HLA-A2+ DC pulsed with CD133 peptides to induce antigen-specific CTLs. Antigens-specific T cell responses were evaluated by an IFN-γ ELISPOT assay.

Materials & Methods:

Generation of Human Dendritic Cells

[0202] Human monocyte-derived DC was generated using previously described methods. Briefly, monocytes were isolated from PBMC by magnetic immunoselection using EasySep human monocyte enrichment kit (Stem Cell Technologies) in accordance with the manufacturer's instructions and

then cultured at 5×10^7/ml in 20 ml of GMP CellGenix DC serum-free medium (Cat#20801-0500, Cellgenix) supplemented with 1000 unit/ml of recombinant human GM-CSF (Cat#AF-300-03, Peprotech, Inc) and recombinant human IL-4(Cat# AF-200-04, Peprotech, Inc). Cells were harvested after 3 or 6 days of culture. The DCs were washed and plated in 6-well plates at a concentration of 5×10^6 cells/well IFN-γ (1000 unit/ml) and monophosphoryl lipid A (MPLA, 20-50 μg/ml) was added into the wells to mature the DC for 24 hr or 48 hrs. Prior to some assays, DC was frozen and stored into liquid nitrogen.

CTL-Induction and Detection of Mart1-Specific $CD8^+$ by HLA-A*0201/Mart1 Tetramers

[0203] In order to evaluate antigen-specific immune responses, $CD8^+$ T cells were isolated from fresh or frozen apheresis by positive selection using Dynabeads® CD8 Positive Isolation Kit (Life Technologies, Grand Island, N.Y.) and co-cultured with autologous mDC for four weeks. DCs were added weekly. Briefly, mDC was pulsed with synthetic peptides (10 μg/μl) for 6-8 hours at 37° C., and then treated with 20 μg/ml Mitomycin C (Sigma-Aldrich, St. Louis, Mo.) for 25 min at 37° C. and 5% CO2. The mDCs (5×10^4 cells/well) were co-cultured with autologous $CD8^+$ T cells (5×10^5 cells/well) in a 96-well plate at 37° C., 5% CO_2 in a final volume of 200 μl CTL medium (IMDM with 0.24 mM Asparagine, 0.55 mM L-Arginine, 1.5 mM L-Glutamine and 10% heat inactivated human AB serum). Half of the medium was replaced every other day by fresh culture medium containing 40 IU/ml IL-2 and 20 ng/ml IL-7, and in the 3rd and 4th week 40 IU/ml of IL-2 was replaced with 25 ng/ml of IL-15. Peptides also could be added to the culture well at a final concentration of 1-2 μg/ml.

IFN-γ ELISPOT Assay

[0204] Antigen-specific immune responses were evaluated by the IFN-γ Elispot kit (BD Biosciences) following previously described methods. Briefly, 1×10^5 CTL cells were co-cultured with 7.5×10^4 T2 cells pulsed with or without 10 μg/ml of peptides and seeded into 96-well plates for 20 hours. CTL cells without T2 cells and CTL plus 5 μg/ml PHA were set as negative and positive controls, respectively. The colored spots, representing cytokine-producing cells, were counted under a dissecting microscope. The results were evaluated by an automated ELISPOT reader system using KS ELISPOT 4.3 software.

Results:

[0205] As shown in FIG. **3**, CTLs produce more IFN-γ against T2 cell loaded with the peptides compared with T2 control (no peptides). The results of IFN-γ ELISPOT assay indicated that CD133 peptides of CD133p405, CD133p753, and CD133p804-specific CTLs can efficiently recognize T2 pulsed with these antigens and boost the T cell immune response.

Conclusion:

[0206] The IFN-γ ELISPOT assay demonstrated that CD133 peptides of CD133p405, CD133p753, and CD133p804-specific CTLs can efficiently recognize these antigens containing epitopes and induce T2 cell immune response. This result forms the basis to further develop immunotherapy target for human ovarian cancer cells and ovarian cancer stem cells as well as ovarian cancer daughter cells.

Example 8

Microarray Dataset Analyses Genes Expression Profiles and the Correlation Between RNA Expression and Overall Survival (OS)

Objective:

[0207] To compare gene expression of genes of interest in human ovarian cancer and normal tissue from the TCGA microarray dataset and to determine whether the gene expression is associated with poor overall survival (OS) in patients with high-grade serous ovarian cancer.

Background:

[0208] The goal of gene expression profiling studies is to identify gene expression signatures between tumor and normal tissue and to identify the correlation between gene expression and clinical outcome such as overall survival (OS) in order to discover potential biomarkers for treatment (e.g., for use as an immunotherapy target).

Methods:

[0209] The Cancer Genome Atlas (TCGA) project has analyzed mRNA expression, microRNA expression, promoter methylation, and DNA copy number in 586 high-grade serous ovarian cystadenocarcinoma that were profiled on the Affymetrix U133A platform and preprocessed with dChip (version Dec. 5, 2011) software as described in the manual (Nature, 2011:609; Proc Natl Acad Sci USA 2001; 9:31).

[0210] GSE9891 contains the expression data and clinical data of 285 ovarian cancer samples and has been deposited in the Gene Expression Omnibus (GEO) (GSE9891) (Clin Cancer Res 2008; 14:5198).

[0211] The microarray dataset was analyzed for the RNA expression of genes of interest in human ovarian cancer samples. In addition, this example compared the correlation between RNA expression and overall survival (OS) of ovarian cancer patients.

[0212] Gene expression analysis tools at tcga-data.nci.nih.gov/tcga/, cancergenome.nih.gov, and oncomine.org were used to examine the RNA expression of ICT140 genes in 586 human serous ovarian cancer samples in TCGA dataset.

[0213] The Kaplan-Meier method was used to estimate the correlation between RNA expression and overall survival (OS) and the log-rank test was employed to compare OS across group. All analyses were performed using the web-based Kaplan-Meier plotter tool (kmplot.com). The overall survival curves and the number-at-risk were indicated below the main plot. Hazard ratio (HR; and 95% confidence intervals) and log-rank P values were also calculated.

Results:

[0214] As shown in FIG. **4**, the mRNA expression value of HER2, survivin, gp100, and IL-13Rα2 were 1.025, 11.29, 1.06, and 1.463, respectively, in the TCGA ovarian cancer microarray dataset, indicating that the expression of these genes in ovarian cancer tissue were higher than that in normal tissue. In contrast, the expression value of mesothelin (MSLN), EGFR, and CD133 were −1.464, −2.552, and

−4.331 indicating that the expression of these genes in ovarian cancer tissue is lower than that in normal tissue.

[0215] Correlation between RNA expression of these genes and the overall survival (OS) in ovarian cancer patients in TCGA microarray dataset was evaluated by comparing survival in patient groups with "high" and 'low" RNA expression of these genes. For the TCGA dataset, the Kaplan-Meier results of overall survival (OS) for the patients in the "high" and "low" expression groups are depicted in FIG. **5**A and FIG. **5**B. The results in FIG. **5**A and FIG. **5**B showed that the patient group with "high" RNA expression of the genes HER2, MSLN, survivin, gp100, EGFR, and CD133 had poor overall survival (OS) with statistical significance ($p<0.05$), whereas there were no significant differences between overall survival (OS) and the RNA expression of IL-13Rα2 gene.

[0216] In order to validate the correlation between overall survival (OS) and RNA expression of IL-13Rα2, the GSE9891 dataset was analyzed and it was found that the patient group with "high" RNA expression of IL-13Rα2 had poor overall survival (OS) (FIG. **6**).

Conclusion:

[0217] These findings demonstrate that the proposed genes of HER2, MSLN, survivin, gp100, EGFR, CD133, and IL-13Rα2 are associated with poor overall survival (OS) in patients with high-grade ovarian cancer based on the TCGA and GSE9891 datasets. These results provide the basis for the rational design of novel treatment strategies including immunotherapy.

Other Embodiments

[0218] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the disclosure. Other aspects, advantages, and modifications are within the scope of the following claims.

SEQUENCE LISTING

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            20                  25                  30

Pro Ser Arg Thr Leu Ala Gly Glu Thr Gly Gln Glu Ala Ala Pro Leu
        35                  40                  45

Asp Gly Val Leu Ala Asn Pro Pro Asn Ile Ser Ser Leu Ser Pro Arg
    50                  55                  60

Gln Leu Leu Gly Phe Pro Cys Ala Glu Val Ser Gly Leu Ser Thr Glu
65                  70                  75                  80

Arg Val Arg Glu Leu Ala Val Ala Leu Ala Gln Lys Asn Val Lys Leu
                85                  90                  95

Ser Thr Glu Gln Leu Arg Cys Leu Ala His Arg Leu Ser Glu Pro Pro
            100                 105                 110

Glu Asp Leu Asp Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu Asn Pro
        115                 120                 125

Asp Ala Phe Ser Gly Pro Gln Ala Cys Thr Arg Phe Phe Ser Arg Ile
    130                 135                 140

Thr Lys Ala Asn Val Asp Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln
145                 150                 155                 160

Arg Leu Leu Pro Ala Ala Leu Ala Cys Trp Gly Val Arg Gly Ser Leu
                165                 170                 175

Leu Ser Glu Ala Asp Val Arg Ala Leu Gly Gly Leu Ala Cys Asp Leu
            180                 185                 190

Pro Gly Arg Phe Val Ala Glu Ser Ala Glu Val Leu Leu Pro Arg Leu
        195                 200                 205

Val Ser Cys Pro Gly Pro Leu Asp Gln Asp Gln Gln Glu Ala Ala Arg
    210                 215                 220
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Ser Val Ser Thr Met Asp Ala Leu Arg Gly Leu Leu Pro Val Leu Gly
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Gln Pro Ile Ile Arg Ser Ile Pro Gln Gly Ile Val Ala Ala Trp Arg
            260                 265                 270

Gln Arg Ser Ser Arg Asp Pro Ser Trp Arg Gln Pro Glu Arg Thr Ile
        275                 280                 285

Leu Arg Pro Arg Phe Arg Arg Glu Val Glu Lys Thr Ala Cys Pro Ser
    290                 295                 300

Gly Lys Lys Ala Arg Glu Ile Asp Glu Ser Leu Ile Phe Tyr Lys Lys
305                 310                 315                 320

Trp Glu Leu Glu Ala Cys Val Asp Ala Ala Leu Leu Ala Thr Gln Met
                325                 330                 335

Asp Arg Val Asn Ala Ile Pro Phe Thr Tyr Glu Gln Leu Asp Val Leu
            340                 345                 350

Lys His Lys Leu Asp Glu Leu Tyr Pro Gln Gly Tyr Pro Glu Ser Val
        355                 360                 365

Ile Gln His Leu Gly Tyr Leu Phe Leu Lys Met Ser Pro Glu Asp Ile
    370                 375                 380

Arg Lys Trp Asn Val Thr Ser Leu Glu Thr Leu Lys Ala Leu Leu Glu
385                 390                 395                 400

Val Asn Lys Gly His Glu Met Ser Pro Gln Val Ala Thr Leu Ile Asp
                405                 410                 415

Arg Phe Val Lys Gly Arg Gly Gln Leu Asp Lys Asp Thr Leu Asp Thr
            420                 425                 430

Leu Thr Ala Phe Tyr Pro Gly Tyr Leu Cys Ser Leu Ser Pro Glu Glu
        435                 440                 445

Leu Ser Ser Val Pro Pro Ser Ser Ile Trp Ala Val Arg Pro Gln Asp
    450                 455                 460

Leu Asp Thr Cys Asp Pro Arg Gln Leu Asp Val Leu Tyr Pro Lys Ala
465                 470                 475                 480

Arg Leu Ala Phe Gln Asn Met Asn Gly Ser Glu Tyr Phe Val Lys Ile
                485                 490                 495

Gln Ser Phe Leu Gly Gly Ala Pro Thr Glu Asp Leu Lys Ala Leu Ser
            500                 505                 510

Gln Gln Asn Val Ser Met Asp Leu Ala Thr Phe Met Lys Leu Arg Thr
        515                 520                 525

Asp Ala Val Leu Pro Leu Thr Val Ala Glu Val Gln Lys Leu Leu Gly
    530                 535                 540

Pro His Val Glu Gly Leu Lys Ala Glu Glu Arg His Arg Pro Val Arg
545                 550                 555                 560

Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp Leu Asp Thr Leu Gly Leu
                565                 570                 575

Gly Leu Gln Gly Gly Ile Pro Asn Gly Tyr Leu Val Leu Asp Leu Ser
            580                 585                 590

Met Gln Glu Ala Leu Ser Gly Thr Pro Cys Leu Leu Gly Pro Gly Pro
        595                 600                 605

Val Leu Thr Val Leu Ala Leu Leu Leu Ala Ser Thr Leu Ala
    610                 615                 620
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<210> SEQ ID NO 2
<211> LENGTH: 1255
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

Met Glu Leu Ala Ala Leu Cys Arg Trp Gly Leu Leu Leu Ala Leu Leu
1 5 10 15

Pro Pro Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr Asp Met Lys
20 25 30

Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met Leu Arg His
35 40 45

Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr
50 55 60

Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
65 70 75 80

Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu
85 90 95

Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
100 105 110

Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro
115 120 125

Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
130 135 140

Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
145 150 155 160

Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn
165 170 175

Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys
180 185 190

His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser
195 200 205

Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
210 215 220

Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
225 230 235 240

Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu
245 250 255

His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
260 265 270

Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
275 280 285

Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu
290 295 300

Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln
305 310 315 320

Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys
325 330 335

Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu
340 345 350

Val Arg Ala Val Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys
355 360 365

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Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
    370                 375                 380

Pro Ala Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe
385                 390                 395                 400

Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
                405                 410                 415

Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
            420                 425                 430

Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
        435                 440                 445

Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly
    450                 455                 460

Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val
465                 470                 475                 480

Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Thr
                485                 490                 495

Ala Asn Arg Pro Glu Asp Glu Cys Val Gly Glu Gly Leu Ala Cys His
            500                 505                 510

Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro Thr Gln Cys
        515                 520                 525

Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys Val Glu Glu Cys
    530                 535                 540

Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Asn Ala Arg His Cys
545                 550                 555                 560

Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys
                565                 570                 575

Phe Gly Pro Glu Ala Asp Gln Cys Val Ala Cys Ala His Tyr Lys Asp
            580                 585                 590

Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro Asp Leu
        595                 600                 605

Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu Gly Ala Cys Gln
    610                 615                 620

Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys
625                 630                 635                 640

Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser Ile Ile Ser
                645                 650                 655

Ala Val Val Gly Ile Leu Leu Val Val Val Leu Gly Val Val Phe Gly
            660                 665                 670

Ile Leu Ile Lys Arg Arg Gln Gln Lys Ile Arg Lys Tyr Thr Met Arg
        675                 680                 685

Arg Leu Leu Gln Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly
    690                 695                 700

Ala Met Pro Asn Gln Ala Gln Met Arg Ile Leu Lys Glu Thr Glu Leu
705                 710                 715                 720

Arg Lys Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys
                725                 730                 735

Gly Ile Trp Ile Pro Asp Gly Glu Asn Val Lys Ile Pro Val Ala Ile
            740                 745                 750

Lys Val Leu Arg Glu Asn Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu
        755                 760                 765
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Asp Glu Ala Tyr Val Met Ala Gly Val Gly Ser Pro Tyr Val Ser Arg
    770                 775                 780

Leu Leu Gly Ile Cys Leu Thr Ser Thr Val Gln Leu Val Thr Gln Leu
785                 790                 795                 800

Met Pro Tyr Gly Cys Leu Leu Asp His Val Arg Glu Asn Arg Gly Arg
                805                 810                 815

Leu Gly Ser Gln Asp Leu Leu Asn Trp Cys Met Gln Ile Ala Lys Gly
            820                 825                 830

Met Ser Tyr Leu Glu Asp Val Arg Leu Val His Arg Asp Leu Ala Ala
        835                 840                 845

Arg Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe
    850                 855                 860

Gly Leu Ala Arg Leu Leu Asp Ile Asp Glu Thr Glu Tyr His Ala Asp
865                 870                 875                 880

Gly Gly Lys Val Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Leu Arg
                885                 890                 895

Arg Arg Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val
            900                 905                 910

Trp Glu Leu Met Thr Phe Gly Ala Lys Pro Tyr Asp Gly Ile Pro Ala
        915                 920                 925

Arg Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro
    930                 935                 940

Pro Ile Cys Thr Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp Met
945                 950                 955                 960

Ile Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ser Glu Phe
                965                 970                 975

Ser Arg Met Ala Arg Asp Pro Gln Arg Phe Val Val Ile Gln Asn Glu
            980                 985                 990

Asp Leu Gly Pro Ala Ser Pro Leu  Asp Ser Thr Phe Tyr  Arg Ser Leu
        995                 1000                1005

Leu Glu  Asp Asp Asp Met Gly  Asp Leu Val Asp Ala  Glu Glu Tyr
    1010                1015                1020

Leu Val  Pro Gln Gln Gly Phe  Phe Cys Pro Asp Pro  Ala Pro Gly
    1025                1030                1035

Ala Gly  Gly Met Val His His  Arg His Arg Ser Ser  Ser Thr Arg
    1040                1045                1050

Ser Gly  Gly Gly Asp Leu Thr  Leu Gly Leu Glu Pro  Ser Glu Glu
    1055                1060                1065

Glu Ala  Pro Arg Ser Pro Leu  Ala Pro Ser Glu Gly  Ala Gly Ser
    1070                1075                1080

Asp Val  Phe Asp Gly Asp Leu  Gly Met Gly Ala Ala  Lys Gly Leu
    1085                1090                1095

Gln Ser  Leu Pro Thr His Asp  Pro Ser Pro Leu Gln  Arg Tyr Ser
    1100                1105                1110

Glu Asp  Pro Thr Val Pro Leu  Pro Ser Glu Thr Asp  Gly Tyr Val
    1115                1120                1125

Ala Pro  Leu Thr Cys Ser Pro  Gln Pro Glu Tyr Val  Asn Gln Pro
    1130                1135                1140

Asp Val  Arg Pro Gln Pro Pro  Ser Pro Arg Glu Gly  Pro Leu Pro
    1145                1150                1155

Ala Ala  Arg Pro Ala Gly Ala  Thr Leu Glu Arg Pro  Lys Thr Leu
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    1160                1165                1170

Ser Pro  Gly Lys Asn Gly Val  Val Lys Asp Val Phe  Ala Phe Gly
    1175                1180                1185

Gly Ala  Val Glu Asn Pro Glu  Tyr Leu Thr Pro Gln  Gly Gly Ala
    1190                1195                1200

Ala Pro  Gln Pro His Pro Pro  Pro Ala Phe Ser Pro  Ala Phe Asp
    1205                1210                1215

Asn Leu  Tyr Tyr Trp Asp Gln  Asp Pro Pro Glu Arg  Gly Ala Pro
    1220                1225                1230

Pro Ser  Thr Phe Lys Gly Thr  Pro Thr Ala Glu Asn  Pro Glu Tyr
    1235                1240                1245

Leu Gly  Leu Asp Val Pro Val
    1250                1255


<210> SEQ ID NO 3
<211> LENGTH: 380
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Met Ala Phe Val Cys Leu Ala Ile Gly Cys Leu Tyr Thr Phe Leu Ile
1               5                   10                  15

Ser Thr Thr Phe Gly Cys Thr Ser Ser Ser Asp Thr Glu Ile Lys Val
            20                  25                  30

Asn Pro Pro Gln Asp Phe Glu Ile Val Asp Pro Gly Tyr Leu Gly Tyr
        35                  40                  45

Leu Tyr Leu Gln Trp Gln Pro Pro Leu Ser Leu Asp His Phe Lys Glu
    50                  55                  60

Cys Thr Val Glu Tyr Glu Leu Lys Tyr Arg Asn Ile Gly Ser Glu Thr
65                  70                  75                  80

Trp Lys Thr Ile Ile Thr Lys Asn Leu His Tyr Lys Asp Gly Phe Asp
                85                  90                  95

Leu Asn Lys Gly Ile Glu Ala Lys Ile His Thr Leu Leu Pro Trp Gln
            100                 105                 110

Cys Thr Asn Gly Ser Glu Val Gln Ser Ser Trp Ala Glu Thr Thr Tyr
        115                 120                 125

Trp Ile Ser Pro Gln Gly Ile Pro Glu Thr Lys Val Gln Asp Met Asp
    130                 135                 140

Cys Val Tyr Tyr Asn Trp Gln Tyr Leu Leu Cys Ser Trp Lys Pro Gly
145                 150                 155                 160

Ile Gly Val Leu Leu Asp Thr Asn Tyr Asn Leu Phe Tyr Trp Tyr Glu
                165                 170                 175

Gly Leu Asp His Ala Leu Gln Cys Val Asp Tyr Ile Lys Ala Asp Gly
            180                 185                 190

Gln Asn Ile Gly Cys Arg Phe Pro Tyr Leu Glu Ala Ser Asp Tyr Lys
        195                 200                 205

Asp Phe Tyr Ile Cys Val Asn Gly Ser Ser Glu Asn Lys Pro Ile Arg
    210                 215                 220

Ser Ser Tyr Phe Thr Phe Gln Leu Gln Asn Ile Val Lys Pro Leu Pro
225                 230                 235                 240

Pro Val Tyr Leu Thr Phe Thr Arg Glu Ser Ser Cys Glu Ile Lys Leu
                245                 250                 255
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Lys Trp Ser Ile Pro Leu Gly Pro Ile Pro Ala Arg Cys Phe Asp Tyr
            260                 265                 270

Glu Ile Glu Ile Arg Glu Asp Asp Thr Thr Leu Val Thr Ala Thr Val
        275                 280                 285

Glu Asn Glu Thr Tyr Thr Leu Lys Thr Thr Asn Glu Thr Arg Gln Leu
    290                 295                 300

Cys Phe Val Val Arg Ser Lys Val Asn Ile Tyr Cys Ser Asp Asp Gly
305                 310                 315                 320

Ile Trp Ser Glu Trp Ser Asp Lys Gln Cys Trp Glu Gly Glu Asp Leu
                325                 330                 335

Ser Lys Lys Thr Leu Leu Arg Phe Trp Leu Pro Phe Gly Phe Ile Leu
            340                 345                 350

Ile Leu Val Ile Phe Val Thr Gly Leu Leu Leu Arg Lys Pro Asn Thr
        355                 360                 365

Tyr Pro Lys Met Ile Pro Glu Phe Phe Cys Asp Thr
    370                 375                 380


<210> SEQ ID NO 4
<211> LENGTH: 137
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Met Gly Ala Pro Thr Leu Pro Pro Ala Trp Gln Pro Phe Leu Lys Asp
1               5                   10                  15

His Arg Ile Ser Thr Phe Lys Asn Trp Pro Phe Leu Glu Gly Cys Ala
            20                  25                  30

Cys Thr Pro Glu Arg Met Ala Glu Ala Gly Phe Ile His Cys Pro Thr
        35                  40                  45

Glu Asn Glu Pro Asp Leu Ala Gln Cys Phe Phe Cys Phe Lys Glu Leu
    50                  55                  60

Glu Gly Trp Glu Pro Asp Asp Asp Pro Met Gln Arg Lys Pro Thr Ile
65                  70                  75                  80

Arg Arg Lys Asn Leu Arg Lys Leu Arg Arg Lys Cys Ala Val Pro Ser
                85                  90                  95

Ser Ser Trp Leu Pro Trp Ile Glu Ala Ser Gly Arg Ser Cys Leu Val
            100                 105                 110

Pro Glu Trp Leu His His Phe Gln Gly Leu Phe Pro Gly Ala Thr Ser
        115                 120                 125

Leu Pro Val Gly Pro Leu Ala Met Ser
    130                 135


<210> SEQ ID NO 5
<211> LENGTH: 856
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

Met Ala Leu Val Leu Gly Ser Leu Leu Leu Leu Gly Leu Cys Gly Asn
1               5                   10                  15

Ser Phe Ser Gly Gly Gln Pro Ser Ser Thr Asp Ala Pro Lys Ala Trp
            20                  25                  30

Asn Tyr Glu Leu Pro Ala Thr Asn Tyr Glu Thr Gln Asp Ser His Lys
        35                  40                  45
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Ala Gly Pro Ile Gly Ile Leu Phe Glu Leu Val His Ile Phe Leu Tyr
    50                  55                  60

Val Val Gln Pro Arg Asp Phe Pro Glu Asp Thr Leu Arg Lys Phe Leu
65                  70                  75                  80

Gln Lys Ala Tyr Glu Ser Lys Ile Asp Tyr Asp Lys Ile Val Tyr Tyr
                85                  90                  95

Glu Ala Gly Ile Ile Leu Cys Cys Val Leu Gly Leu Leu Phe Ile Ile
            100                 105                 110

Leu Met Pro Leu Val Gly Tyr Phe Phe Cys Met Cys Arg Cys Cys Asn
        115                 120                 125

Lys Cys Gly Gly Glu Met His Gln Arg Gln Lys Glu Asn Gly Pro Phe
    130                 135                 140

Leu Arg Lys Cys Phe Ala Ile Ser Leu Leu Val Ile Cys Ile Ile Ile
145                 150                 155                 160

Ser Ile Gly Ile Phe Tyr Gly Phe Val Ala Asn His Gln Val Arg Thr
                165                 170                 175

Arg Ile Lys Arg Ser Arg Lys Leu Ala Asp Ser Asn Phe Lys Asp Leu
            180                 185                 190

Arg Thr Leu Leu Asn Glu Thr Pro Glu Gln Ile Lys Tyr Ile Leu Ala
        195                 200                 205

Gln Tyr Asn Thr Thr Lys Asp Lys Ala Phe Thr Asp Leu Asn Ser Ile
    210                 215                 220

Asn Ser Val Leu Gly Gly Gly Ile Leu Asp Arg Leu Arg Pro Asn Ile
225                 230                 235                 240

Ile Pro Val Leu Asp Glu Ile Lys Ser Met Ala Thr Ala Ile Lys Glu
                245                 250                 255

Thr Lys Glu Ala Leu Glu Asn Met Asn Ser Thr Leu Lys Ser Leu His
            260                 265                 270

Gln Gln Ser Thr Gln Leu Ser Ser Ser Leu Thr Ser Val Lys Thr Ser
        275                 280                 285

Leu Arg Ser Ser Leu Asn Asp Pro Leu Cys Leu Val His Pro Ser Ser
    290                 295                 300

Glu Thr Cys Asn Ser Ile Arg Leu Ser Leu Ser Gln Leu Asn Ser Asn
305                 310                 315                 320

Pro Glu Leu Arg Gln Leu Pro Pro Val Asp Ala Glu Leu Asp Asn Val
                325                 330                 335

Asn Asn Val Leu Arg Thr Asp Leu Asp Gly Leu Val Gln Gln Gly Tyr
            340                 345                 350

Gln Ser Leu Asn Asp Ile Pro Asp Arg Val Gln Arg Gln Thr Thr Thr
        355                 360                 365

Val Val Ala Gly Ile Lys Arg Val Leu Asn Ser Ile Gly Ser Asp Ile
    370                 375                 380

Asp Asn Val Thr Gln Arg Leu Pro Ile Gln Asp Ile Leu Ser Ala Phe
385                 390                 395                 400

Ser Val Tyr Val Asn Asn Thr Glu Ser Tyr Ile His Arg Asn Leu Pro
                405                 410                 415

Thr Leu Glu Glu Tyr Asp Ser Tyr Trp Trp Leu Gly Gly Leu Val Ile
            420                 425                 430

Cys Ser Leu Leu Thr Leu Ile Val Ile Phe Tyr Tyr Leu Gly Leu Leu
        435                 440                 445

Cys Gly Val Cys Gly Tyr Asp Arg His Ala Thr Pro Thr Thr Arg Gly
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    450                 455                 460

Cys Val Ser Asn Thr Gly Gly Val Phe Leu Met Val Gly Val Gly Leu
465                 470                 475                 480

Ser Phe Leu Phe Cys Trp Ile Leu Met Ile Ile Val Val Leu Thr Phe
                485                 490                 495

Val Phe Gly Ala Asn Val Glu Lys Leu Ile Cys Glu Pro Tyr Thr Ser
            500                 505                 510

Lys Glu Leu Phe Arg Val Leu Asp Thr Pro Tyr Leu Leu Asn Glu Asp
        515                 520                 525

Trp Glu Tyr Tyr Leu Ser Gly Lys Leu Phe Asn Lys Ser Lys Met Lys
    530                 535                 540

Leu Thr Phe Glu Gln Val Tyr Ser Asp Cys Lys Lys Asn Arg Gly Thr
545                 550                 555                 560

Tyr Gly Thr Leu His Leu Gln Asn Ser Phe Asn Ile Ser Glu His Leu
                565                 570                 575

Asn Ile Asn Glu His Thr Gly Ser Ile Ser Ser Glu Leu Glu Ser Leu
            580                 585                 590

Lys Val Asn Leu Asn Ile Phe Leu Leu Gly Ala Ala Gly Arg Lys Asn
        595                 600                 605

Leu Gln Asp Phe Ala Ala Cys Gly Ile Asp Arg Met Asn Tyr Asp Ser
    610                 615                 620

Tyr Leu Ala Gln Thr Gly Lys Ser Pro Ala Gly Val Asn Leu Leu Ser
625                 630                 635                 640

Phe Ala Tyr Asp Leu Glu Ala Lys Ala Asn Ser Leu Pro Pro Gly Asn
                645                 650                 655

Leu Arg Asn Ser Leu Lys Arg Asp Ala Gln Thr Ile Lys Thr Ile His
            660                 665                 670

Gln Gln Arg Val Leu Pro Ile Glu Gln Ser Leu Ser Thr Leu Tyr Gln
        675                 680                 685

Ser Val Lys Ile Leu Gln Arg Thr Gly Asn Gly Leu Leu Glu Arg Val
    690                 695                 700

Thr Arg Ile Leu Ala Ser Leu Asp Phe Ala Gln Asn Phe Ile Thr Asn
705                 710                 715                 720

Asn Thr Ser Ser Val Ile Ile Glu Glu Thr Lys Lys Tyr Gly Arg Thr
                725                 730                 735

Ile Ile Gly Tyr Phe Glu His Tyr Leu Gln Trp Ile Glu Phe Ser Ile
            740                 745                 750

Ser Glu Lys Val Ala Ser Cys Lys Pro Val Ala Thr Ala Leu Asp Thr
        755                 760                 765

Ala Val Asp Val Phe Leu Cys Ser Tyr Ile Ile Asp Pro Leu Asn Leu
    770                 775                 780

Phe Trp Phe Gly Ile Gly Lys Ala Thr Val Phe Leu Leu Pro Ala Leu
785                 790                 795                 800

Ile Phe Ala Val Lys Leu Ala Lys Tyr Tyr Arg Arg Met Asp Ser Glu
                805                 810                 815

Asp Val Tyr Asp Asp Val Glu Thr Ile Pro Met Lys Asn Met Glu Asn
            820                 825                 830

Gly Asn Asn Gly Tyr His Lys Asp His Val Tyr Gly Ile His Asn Pro
        835                 840                 845

Val Met Thr Ser Pro Ser Gln His
    850                 855
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<210> SEQ ID NO 6
<211> LENGTH: 661
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Met Asp Leu Val Leu Lys Arg Cys Leu Leu His Leu Ala Val Ile Gly
1 5 10 15

Ala Leu Leu Ala Val Gly Ala Thr Lys Val Pro Arg Asn Gln Asp Trp
20 25 30

Leu Gly Val Ser Arg Gln Leu Arg Thr Lys Ala Trp Asn Arg Gln Leu
35 40 45

Tyr Pro Glu Trp Thr Glu Ala Gln Arg Leu Asp Cys Trp Arg Gly Gly
50 55 60

Gln Val Ser Leu Lys Val Ser Asn Asp Gly Pro Thr Leu Ile Gly Ala
65 70 75 80

Asn Ala Ser Phe Ser Ile Ala Leu Asn Phe Pro Gly Ser Gln Lys Val
85 90 95

Leu Pro Asp Gly Gln Val Ile Trp Val Asn Asn Thr Ile Ile Asn Gly
100 105 110

Ser Gln Val Trp Gly Gly Gln Pro Val Tyr Pro Gln Glu Thr Asp Asp
115 120 125

Ala Cys Ile Phe Pro Asp Gly Gly Pro Cys Pro Ser Gly Ser Trp Ser
130 135 140

Gln Lys Arg Ser Phe Val Tyr Val Trp Lys Thr Trp Gly Gln Tyr Trp
145 150 155 160

Gln Val Leu Gly Gly Pro Val Ser Gly Leu Ser Ile Gly Thr Gly Arg
165 170 175

Ala Met Leu Gly Thr His Thr Met Glu Val Thr Val Tyr His Arg Arg
180 185 190

Gly Ser Arg Ser Tyr Val Pro Leu Ala His Ser Ser Ser Ala Phe Thr
195 200 205

Ile Thr Asp Gln Val Pro Phe Ser Val Ser Val Ser Gln Leu Arg Ala
210 215 220

Leu Asp Gly Gly Asn Lys His Phe Leu Arg Asn Gln Pro Leu Thr Phe
225 230 235 240

Ala Leu Gln Leu His Asp Pro Ser Gly Tyr Leu Ala Glu Ala Asp Leu
245 250 255

Ser Tyr Thr Trp Asp Phe Gly Asp Ser Ser Gly Thr Leu Ile Ser Arg
260 265 270

Ala Leu Val Val Thr His Thr Tyr Leu Glu Pro Gly Pro Val Thr Ala
275 280 285

Gln Val Val Leu Gln Ala Ala Ile Pro Leu Thr Ser Cys Gly Ser Ser
290 295 300

Pro Val Pro Gly Thr Thr Asp Gly His Arg Pro Thr Ala Glu Ala Pro
305 310 315 320

Asn Thr Thr Ala Gly Gln Val Pro Thr Thr Glu Val Val Gly Thr Thr
325 330 335

Pro Gly Gln Ala Pro Thr Ala Glu Pro Ser Gly Thr Thr Ser Val Gln
340 345 350

Val Pro Thr Thr Glu Val Ile Ser Thr Ala Pro Val Gln Met Pro Thr

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        355                 360                 365

Ala Glu Ser Thr Gly Met Thr Pro Glu Lys Val Pro Val Ser Glu Val
    370                 375                 380

Met Gly Thr Thr Leu Ala Glu Met Ser Thr Pro Glu Ala Thr Gly Met
385                 390                 395                 400

Thr Pro Ala Glu Val Ser Ile Val Val Leu Ser Gly Thr Thr Ala Ala
                405                 410                 415

Gln Val Thr Thr Thr Glu Trp Val Glu Thr Thr Ala Arg Glu Leu Pro
            420                 425                 430

Ile Pro Glu Pro Glu Gly Pro Asp Ala Ser Ser Ile Met Ser Thr Glu
        435                 440                 445

Ser Ile Thr Gly Ser Leu Gly Pro Leu Leu Asp Gly Thr Ala Thr Leu
    450                 455                 460

Arg Leu Val Lys Arg Gln Val Pro Leu Asp Cys Val Leu Tyr Arg Tyr
465                 470                 475                 480

Gly Ser Phe Ser Val Thr Leu Asp Ile Val Gln Gly Ile Glu Ser Ala
                485                 490                 495

Glu Ile Leu Gln Ala Val Pro Ser Gly Glu Gly Asp Ala Phe Glu Leu
            500                 505                 510

Thr Val Ser Cys Gln Gly Gly Leu Pro Lys Glu Ala Cys Met Glu Ile
        515                 520                 525

Ser Ser Pro Gly Cys Gln Pro Pro Ala Gln Arg Leu Cys Gln Pro Val
    530                 535                 540

Leu Pro Ser Pro Ala Cys Gln Leu Val Leu His Gln Ile Leu Lys Gly
545                 550                 555                 560

Gly Ser Gly Thr Tyr Cys Leu Asn Val Ser Leu Ala Asp Thr Asn Ser
                565                 570                 575

Leu Ala Val Val Ser Thr Gln Leu Ile Met Pro Gly Gln Glu Ala Gly
            580                 585                 590

Leu Gly Gln Val Pro Leu Ile Val Gly Ile Leu Leu Val Leu Met Ala
        595                 600                 605

Val Val Leu Ala Ser Leu Ile Tyr Arg Arg Arg Leu Met Lys Gln Asp
    610                 615                 620

Phe Ser Val Pro Gln Leu Pro His Ser Ser Ser His Trp Leu Arg Leu
625                 630                 635                 640

Pro Arg Ile Phe Cys Ser Cys Pro Ile Gly Glu Asn Ser Pro Leu Leu
                645                 650                 655

Ser Gly Gln Gln Val
            660


<210> SEQ ID NO 7
<211> LENGTH: 75
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

Met Val Val Leu Gly Met Gln Thr Glu Glu Gly His Cys Ile Met Leu
1               5                   10                  15

Arg Gly Leu Ala Pro Ser Leu Gly Gly Thr Gln Val Ile Cys Lys Val
            20                  25                  30

Val Gly Leu Pro Ser Ser Ile Gly Phe Asn Thr Ser Ser His Leu Leu
        35                  40                  45
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Phe Pro Ala Thr Leu Gln Gly Ala Pro Thr His Phe Pro Cys Arg Trp
    50                  55                  60

Arg Gln Gly Gly Ser Thr Asp Asn Pro Pro Ala
65                  70                  75


<210> SEQ ID NO 8
<211> LENGTH: 1210
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met Arg Pro Ser Gly Thr Ala Gly Ala Ala Leu Leu Ala Leu Leu Ala
1               5                   10                  15

Ala Leu Cys Pro Ala Ser Arg Ala Leu Glu Glu Lys Lys Val Cys Gln
            20                  25                  30

Gly Thr Ser Asn Lys Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe
        35                  40                  45

Leu Ser Leu Gln Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly Asn
    50                  55                  60

Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys
65                  70                  75                  80

Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val
                85                  90                  95

Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr
            100                 105                 110

Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala Asn
        115                 120                 125

Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu
    130                 135                 140

His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val Glu
145                 150                 155                 160

Ser Ile Gln Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn Met
                165                 170                 175

Ser Met Asp Phe Gln Asn His Leu Gly Ser Cys Gln Lys Cys Asp Pro
            180                 185                 190

Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys Gln
        195                 200                 205

Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser Gly Arg Cys Arg
    210                 215                 220

Gly Lys Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys
225                 230                 235                 240

Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Arg Lys Phe Arg Asp
                245                 250                 255

Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro
            260                 265                 270

Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly
        275                 280                 285

Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His
    290                 295                 300

Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr Glu Met Glu Glu
305                 310                 315                 320

Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys Val
                325                 330                 335
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Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asn
            340                 345                 350

Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp
        355                 360                 365

Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His Thr
    370                 375                 380

Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys Glu
385                 390                 395                 400

Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr Asp
                405                 410                 415

Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln
            420                 425                 430

His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu
        435                 440                 445

Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser
    450                 455                 460

Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu
465                 470                 475                 480

Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly Glu
                485                 490                 495

Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser Pro
            500                 505                 510

Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg Asn
        515                 520                 525

Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Glu Gly
    530                 535                 540

Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro
545                 550                 555                 560

Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro
                565                 570                 575

Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val
            580                 585                 590

Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val Trp
        595                 600                 605

Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn Cys
    610                 615                 620

Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn Gly
625                 630                 635                 640

Pro Lys Ile Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu Leu
                645                 650                 655

Leu Leu Val Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg Arg His
            660                 665                 670

Ile Val Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg Glu Leu
        675                 680                 685

Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala Leu Leu
    690                 695                 700

Arg Ile Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu Gly Ser
705                 710                 715                 720

Gly Ala Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu Gly Glu
                725                 730                 735
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Lys Val Lys Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala Thr Ser
            740                 745                 750

Pro Lys Ala Asn Lys Glu Ile Leu Asp Glu Ala Tyr Val Met Ala Ser
        755                 760                 765

Val Asp Asn Pro His Val Cys Arg Leu Leu Gly Ile Cys Leu Thr Ser
    770                 775                 780

Thr Val Gln Leu Ile Thr Gln Leu Met Pro Phe Gly Cys Leu Leu Asp
785                 790                 795                 800

Tyr Val Arg Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu Leu Asn
                805                 810                 815

Trp Cys Val Gln Ile Ala Lys Gly Met Asn Tyr Leu Glu Asp Arg Arg
            820                 825                 830

Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Thr Pro
        835                 840                 845

Gln His Val Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu Gly Ala
    850                 855                 860

Glu Glu Lys Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile Lys Trp
865                 870                 875                 880

Met Ala Leu Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln Ser Asp
                885                 890                 895

Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly Ser
            900                 905                 910

Lys Pro Tyr Asp Gly Ile Pro Ala Ser Glu Ile Ser Ser Ile Leu Glu
        915                 920                 925

Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr
    930                 935                 940

Met Ile Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys
945                 950                 955                 960

Phe Arg Glu Leu Ile Ile Glu Phe Ser Lys Met Ala Arg Asp Pro Gln
                965                 970                 975

Arg Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro Ser Pro
            980                 985                 990

Thr Asp Ser Asn Phe Tyr Arg Ala  Leu Met Asp Glu Glu  Asp Met Asp
        995                 1000                1005

Asp Val  Val Asp Ala Asp Glu  Tyr Leu Ile Pro Gln  Gln Gly Phe
    1010                1015                1020

Phe Ser  Ser Pro Ser Thr Ser  Arg Thr Pro Leu Leu  Ser Ser Leu
    1025                1030                1035

Ser Ala  Thr Ser Asn Asn Ser  Thr Val Ala Cys Ile  Asp Arg Asn
    1040                1045                1050

Gly Leu  Gln Ser Cys Pro Ile  Lys Glu Asp Ser Phe  Leu Gln Arg
    1055                1060                1065

Tyr Ser  Ser Asp Pro Thr Gly  Ala Leu Thr Glu Asp  Ser Ile Asp
    1070                1075                1080

Asp Thr  Phe Leu Pro Val Pro  Glu Tyr Ile Asn Gln  Ser Val Pro
    1085                1090                1095

Lys Arg  Pro Ala Gly Ser Val  Gln Asn Pro Val Tyr  His Asn Gln
    1100                1105                1110

Pro Leu  Asn Pro Ala Pro Ser  Arg Asp Pro His Tyr  Gln Asp Pro
    1115                1120                1125

His Ser  Thr Ala Val Gly Asn  Pro Glu Tyr Leu Asn  Thr Val Gln
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    1130                1135                1140

Pro Thr  Cys Val Asn Ser Thr  Phe Asp Ser Pro Ala  His Trp Ala
    1145                1150                1155

Gln Lys  Gly Ser His Gln Ile  Ser Leu Asp Asn Pro  Asp Tyr Gln
    1160                1165                1170

Gln Asp  Phe Phe Pro Lys Glu  Ala Lys Pro Asn Gly  Ile Phe Lys
    1175                1180                1185

Gly Ser  Thr Ala Glu Asn Ala  Glu Tyr Leu Arg Val  Ala Pro Gln
    1190                1195                1200

Ser Ser  Glu Phe Ile Gly Ala
    1205                1210


<210> SEQ ID NO 9
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

Ser Leu Leu Phe Leu Leu Phe Ser Leu
1               5


<210> SEQ ID NO 10
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Phe Leu Leu Phe Ser Leu Gly Trp Val
1               5


<210> SEQ ID NO 11
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

Val Leu Pro Leu Thr Val Ala Glu Val
1               5


<210> SEQ ID NO 12
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Lys Leu Leu Gly Pro His Val Glu Gly Leu
1               5                   10


<210> SEQ ID NO 13
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Lys Leu Leu Gly Pro His Val Leu Gly Leu
1               5                   10


<210> SEQ ID NO 14
<211> LENGTH: 10
<212> TYPE: PRT
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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

Lys Leu Leu Gly Pro His Val Leu Gly Val
1 5 10

<210> SEQ ID NO 15
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

Lys Met Leu Gly Pro His Val Leu Gly Val
1 5 10

<210> SEQ ID NO 16
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

Lys Met Leu Gly Pro His Val Leu Gly Leu
1 5 10

<210> SEQ ID NO 17
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Lys Ile Leu Gly Pro His Val Leu Gly Leu
1 5 10

<210> SEQ ID NO 18
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

Tyr Leu Leu Gly Pro His Val Leu Gly Val
1 5 10

<210> SEQ ID NO 19
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

Tyr Leu Leu Gly Pro His Val Leu Gly Leu
1 5 10

<210> SEQ ID NO 20
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

Ala Leu Cys Arg Trp Gly Leu Leu Leu
1 5

<210> SEQ ID NO 21
<211> LENGTH: 9

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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

Arg Trp Gly Leu Leu Leu Ala Leu Leu
1 5

<210> SEQ ID NO 22
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

Thr Tyr Leu Pro Thr Asn Ala Ser Leu
1 5

<210> SEQ ID NO 23
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

Gln Leu Phe Glu Asp Asn Tyr Ala Leu
1 5

<210> SEQ ID NO 24
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

Lys Ile Phe Gly Ser Leu Ala Phe Leu
1 5

<210> SEQ ID NO 25
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 25

Ile Leu His Asn Gly Ala Tyr Ser Leu
1 5

<210> SEQ ID NO 26
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

Ile Ile Ser Ala Val Val Gly Ile Leu
1 5

<210> SEQ ID NO 27
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 27

Val Val Leu Gly Val Val Phe Gly Ile
1 5

<210> SEQ ID NO 28

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<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 28

Arg Leu Leu Gln Glu Thr Glu Leu Val
1 5

<210> SEQ ID NO 29
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 29

Val Leu Arg Glu Asn Thr Ser Pro Lys
1 5

<210> SEQ ID NO 30
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 30

Val Met Ala Gly Val Gly Ser Pro Tyr Val
1 5 10

<210> SEQ ID NO 31
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 31

Pro Tyr Val Ser Arg Leu Leu Gly Ile
1 5

<210> SEQ ID NO 32
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 32

Cys Leu Thr Ser Thr Val Gln Leu Val
1 5

<210> SEQ ID NO 33
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 33

Gln Leu Met Pro Tyr Gly Cys Leu Leu
1 5

<210> SEQ ID NO 34
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 34

Tyr Leu Glu Asp Val Arg Leu Val
1 5

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<210> SEQ ID NO 35
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 35

Val Leu Val Lys Ser Pro Asn His Val
1 5

<210> SEQ ID NO 36
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 36

Lys Val Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Leu Arg Arg Arg
1 5 10 15

Phe

<210> SEQ ID NO 37
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 37

Tyr Met Ile Met Val Lys Cys Trp Met Ile
1 5 10

<210> SEQ ID NO 38
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 38

Glu Leu Val Ser Glu Phe Ser Arg Met
1 5

<210> SEQ ID NO 39
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 39

Trp Leu Pro Phe Gly Phe Ile Leu Ile
1 5

<210> SEQ ID NO 40
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 40

Arg Ile Ser Thr Phe Lys Asn Trp Pro Phe Leu
1 5 10

<210> SEQ ID NO 41
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 41

Asp Leu Ala Gln Met Phe Phe Cys Phe Lys Glu Leu Glu Gly Trp

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1 5 10 15

<210> SEQ ID NO 42
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 42

Glu Leu Thr Leu Gly Glu Phe Leu Lys Leu
1 5 10

<210> SEQ ID NO 43
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 43

Leu Thr Leu Gly Glu Phe Leu Lys Leu
1 5

<210> SEQ ID NO 44
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 44

Leu Met Leu Gly Glu Phe Leu Lys Leu
1 5

<210> SEQ ID NO 45
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 45

Leu Leu Phe Ile Ile Leu Met Pro Leu Val
1 5 10

<210> SEQ ID NO 46
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 46

Ser Leu Asn Asp Pro Leu Cys Leu Val
1 5

<210> SEQ ID NO 47
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 47

Ile Leu Ser Ala Phe Ser Val Tyr Val
1 5

<210> SEQ ID NO 48
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 48

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Gly Leu Leu Glu Arg Val Thr Arg Ile
1 5

<210> SEQ ID NO 49
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 49

Phe Leu Leu Pro Ala Leu Ile Phe Ala Val
1 5 10

<210> SEQ ID NO 50
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 50

Ser Asn Asp Gly Pro Thr Leu Ile
1 5

<210> SEQ ID NO 51
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 51

Lys Thr Trp Gly Gln Tyr Trp Gln Val
1 5

<210> SEQ ID NO 52
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 52

Ile Thr Asp Gln Val Pro Phe Ser Val
1 5

<210> SEQ ID NO 53
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 53

Tyr Leu Glu Pro Gly Pro Val Thr Ala
1 5

<210> SEQ ID NO 54
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 54

Ser Leu Ile Tyr Arg Arg Arg Leu Met Lys
1 5 10

<210> SEQ ID NO 55
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 55

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Leu Ile Tyr Arg Arg Arg Leu Met Lys
1 5

<210> SEQ ID NO 56
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 56

Arg Leu Met Lys Gln Asp Phe Ser Val
1 5

<210> SEQ ID NO 57
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 57

Arg Leu Pro Arg Ile Phe Cys Ser Cys
1 5

<210> SEQ ID NO 58
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 58

Val Leu Tyr Arg Tyr Gly Ser Phe Ser Val
1 5 10

<210> SEQ ID NO 59
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 59

Arg Ser Asp Ser Gly Gln Gln Ala Arg Tyr
1 5 10

<210> SEQ ID NO 60
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 60

Ile Xaa Asp Phe Gly Leu Ala Lys Leu
1 5

<210> SEQ ID NO 61
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 61

His Leu Tyr Gln Gly Cys Gln Val Val
1 5

<210> SEQ ID NO 62

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<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 62

Tyr Leu Val Pro Gln Gln Gly Phe Phe Cys
1 5 10

<210> SEQ ID NO 63
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 63

Pro Leu Gln Pro Glu Gln Leu Gln Val
1 5

<210> SEQ ID NO 64
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 64

Thr Leu Glu Glu Ile Thr Gly Tyr Leu
1 5

<210> SEQ ID NO 65
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 65

Ala Leu Ile His His Asn Thr His Leu
1 5

<210> SEQ ID NO 66
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 66

Pro Leu Thr Ser Ile Ile Ser Ala Val
1 5

<210> SEQ ID NO 67
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 67

Ile Met Asp Gln Val Pro Phe Ser Val
1 5

<210> SEQ ID NO 68
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 68

Ala Met Leu Gly Thr His Thr Met Glu Val
1 5 10

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<210> SEQ ID NO 69
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 69

Leu Leu Asp Gly Thr Ala Thr Leu Arg Leu
1 5 10

<210> SEQ ID NO 70
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 70

Ser Leu Ala Asp Thr Asn Ser Leu Ala Val
1 5 10

<210> SEQ ID NO 71
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 71

Arg Leu Pro Arg Ile Phe Cys Ser Cys
1 5

<210> SEQ ID NO 72
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 72

Tyr Leu Gln Trp Ile Glu Phe Ser Ile
1 5

<210> SEQ ID NO 73
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 73

Arg Arg Lys Leu Leu Gly Pro His Val Glu Gly Leu
1 5 10

<210> SEQ ID NO 74
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 74

Lys Leu Leu Gly Pro His Val Glu Gly Leu Arg Arg
1 5 10

<210> SEQ ID NO 75
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

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<400> SEQUENCE: 75

Lys Lys Lys Leu Leu Gly Pro His Val Glu Gly Leu
1 5 10

<210> SEQ ID NO 76
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 76

Lys Leu Leu Gly Pro His Val Glu Gly Leu Lys Lys
1 5 10

<210> SEQ ID NO 77
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 77

Lys Arg Lys Leu Leu Gly Pro His Val Glu Gly Leu
1 5 10

<210> SEQ ID NO 78
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 78

Lys Leu Leu Gly Pro His Val Glu Gly Leu Lys Arg
1 5 10

<210> SEQ ID NO 79
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 79

Arg Lys Lys Leu Leu Gly Pro His Val Glu Gly Leu
1 5 10

<210> SEQ ID NO 80
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 80

Lys Leu Leu Gly Pro His Val Glu Gly Leu Arg Lys
1 5 10

1. A composition comprising a mixture of at least one major histocompatibility complex (MHC) class I epitope of at least five antigens selected from the group consisting of mesothelin, HER-2/neu, IL-13 receptor α2, survivin, CD133, gp100, AIM-2, and epidermal growth factor receptor (EGFR).

2. The composition of claim **1**, comprising a mixture of at least one major histocompatibility complex (MHC) class I epitope of at least six, at least seven, seven, or eight of the antigens.

3.-**5**. (canceled)

6. The composition of claim **1**, wherein the at least one MHC class I epitope is an HLA-A2 epitope.

7. The composition of claim **1**, wherein the at least one MHC class I epitope is synthetic.

8. The composition of claim **1**, further comprising at least one MHC class II epitope.

9. The composition of claim **1**, further comprising an adjuvant.

10. The composition of claim **1**, further comprising a pharmaceutically acceptable carrier.

11. A composition comprising isolated dendritic cells, wherein the dendritic cells present peptide sequences on their cell surface, wherein the peptide sequences comprise at least one major histocompatibility complex (MHC) class I epitope of at least five antigens selected from the group consisting of mesothelin, HER-2/neu, IL-13 receptor α2, survivin, CD133, gp100, AIM-2, and epidermal growth factor receptor (EGFR).

12. The composition of claim **11**, wherein the dendritic cells present peptide sequences comprising MHC class I epitopes of at least six, at least seven, seven, or eight of the antigens.

13.-**15**. (canceled)

16. The composition of claim **11**, wherein the at least one MHC class I epitope is an HLA-A2 epitope.

17. The composition of claim **11**, wherein the at least one MHC class I epitope is synthetic.

18. The composition of claim **11**, wherein the dendritic cells further present at least one MHC class II epitope.

19. The composition of claim **11**, further comprising an adjuvant.

20. The composition of claim **11**, further comprising a pharmaceutically acceptable carrier.

21. The composition of claim **11**, wherein the dendritic cells acquired the epitopes in vitro by exposure to synthetic peptides comprising the epitopes.

22. A method of treating an ovarian cancer, comprising administering to a subject in need thereof an effective amount of a composition of claim **1**.

23. A method of treating an ovarian cancer, comprising administering to a subject in need thereof an effective amount of a composition of claim **11**.

24. A method of killing ovarian cancer stem cells, comprising administering to a subject in need thereof an effective amount of a composition of claim **1**.

25. A method of killing ovarian cancer stem cells, comprising administering to a subject in need thereof an effective amount of a composition of claim **11**.

26. The method of claim **23**, further comprising administering a chemotherapeutic agent prior to administering the composition to the subject.

27.-**31**. (canceled)

* * * * *