Title: COMBINATION VACCINE DEVICES AND METHODS OF KILLING CANCER CELLS

Abstract: The present invention comprises compositions, methods, and devices for enhancing an endogenous immune response against a cancer. Devices and methods provide therapeutic immunity to subjects against cancer.

FIG. 2

A

B

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COMBINATION VACCINE DEVICES AND METHODS OF KILLING CANCER CELLS

RELATED APPLICATIONS
[01] This application claims the benefit of priority under 35 U.S.C. § 119(e) to U.S.
Provisional Application No: 61/986,600, filed April 30, 2014, which is incorporated herein by reference in its entirety.

GOVERNMENT SUPPORT
[02] The invention made with Government support awarded by the National Institutes of Health Grant No. R01 EB015498. The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION
[03] Many cancers are recalcitrant to treatment because they co-opt the host immune system and evade the endogenous anti-tumor immune response. One such mechanism by which cancer cells evade the immune system is by upregulating immune-inhibitory proteins. Thus, agents that block these immune-inhibitory proteins have been explored as potential therapies that re-enable the endogenous anti-tumor immune response. However, these agents when used alone are ineffective in killing poorly immunogenic tumors. Thus, there is a need for compositions and methods to prevent or treat cancer by promoting the endogenous anti-tumor immune response, in particular in poorly immunogenic tumors. This invention addresses this need.

SUMMARY OF THE INVENTION
[04] The invention features material-based cancer vaccines (e.g., cancer vaccine devices) in combination with immune checkpoint antibodies to boost T cell activity and anti-tumor immune responses.

[05] A device of the invention comprises an inhibitor of an immune-inhibitory protein; a scaffold composition; a cell recruitment composition; and a bioactive composition, where the bioactive composition is incorporated into or coated onto the scaffold composition, and where the bioactive composition causes modification of cells in or recruited to the device.

[06] For example, an immune-inhibitory protein is a protein that decreases and/or inhibits the activity of an immune cell. For example, an immune-inhibitory protein decreases and/or
inhibits the activity of a T cell, B cell, NK cell, or dendritic cell. For example, a decrease in activity or inhibition of a T cell, B cell, NK cell, or dendritic cell decreases an endogenous immune response against an antigen (e.g., a cancer cell antigen). For example, the immune-inhibitory protein decreases and/or inhibits a T cell effector activity and/or an NK cell killing activity. In some cases, an immune-inhibitory protein reduces or inhibits the activity of a cytotoxic T-lymphocyte (CTL). For example an immune-inhibitory protein reduces or inhibits CTL-mediated lysis of a target cell. For example, an immune-inhibitory protein is an immune checkpoint protein (e.g., CTLA4 or PD1). For example, an immune-inhibitory protein (e.g., CTLA4) competes with CD28 for binding to CD80 and/or CD86, thereby interfering with T cell activation. See, e.g., Pardoll et al. Nat. Reviews Cancer. (2012) 12:252-264, incorporated herein by reference. For example, an immune inhibitory protein (e.g., PD1, PDL1, or PDL2) inhibits a kinase or phosphatase (e.g., SHP2) involved in T cell activation. For example, an immune inhibitory protein modulates the duration of T cell–antigen presenting cell (APC) contact or T cell-target cell contact. For example, an immune-inhibitory protein (e.g., CTLA4 or PD1) enhances the immunosuppressive function of a Treg cell.

[07] In some embodiments, the immune-inhibitory protein is cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), programmed cell death protein 1 (PD1), programmed cell death protein 1 ligand (PDL1), lymphocyte activation gene 3 (LAG3), B7-H3, B7-H4, or T cell membrane protein 3 (TIM3). For example, the immune-inhibitory protein is CTLA4. In other examples, the immune-inhibitory protein is PD1.

[08] In some cases, the device comprises an inhibitor of CTLA4 and an inhibitor of PD1. For example, the inhibitor comprises a protein, peptide, or nucleic acid, e.g., an antibody or fragment thereof. In some examples, the antibody or fragment thereof binds to CTLA4. Exemplary anti-CTLA4 antibodies or fragments thereof include Ipilimumab, Tremelimumab, or a fragment thereof. In other examples, the inhibitor binds to PD1, and the inhibitor is a protein, e.g., MDX-1106, MK3475, CT-011, AMP-224, or a fragment thereof. In some cases, the inhibitor is a PDL2-immunoglobulin (Ig) fusion protein.

[09] In some embodiments, the inhibitor is a protein, and the inhibitor, e.g., MDX-1 105, binds to PDL1.

[10] Other exemplary inhibitors are proteins that bind to LAG3, e.g., a LAG3-Ig fusion protein, such as IMP321; or proteins that bind to B7-H3, e.g., MGA271.
The cell recruitment composition of the device recruits an immune cell. The immune cell comprises an antigen presenting cell, e.g., a dendritic cell, a macrophage, a T cell, a B cell, or a natural killer (NK) cell.

The device contains a scaffold that comprises open, interconnected macro pores. The device further comprises a deployment signal capable of inducing or promoting migration of cells, where in some examples, the deployment signal comprises a protein, peptide, or nucleic acid. For example, the deployment signal comprises i) one or more factors that induces migration of cells and has or is capable of forming a gradient; ii) a nucleic acid molecule encoding a protein that induces migration of cells out of the device; or iii) depletion or diffusion of the cell recruitment composition.

Exemplary cell recruitment compositions comprise a cytokine, chemokine, or growth factor. For example, the cell recruitment composition comprises GM-CSF, Flt3L, or CCL20.

In some cases, the bioactive composition of the device comprises a target antigen composition.

In some embodiments, the cell recruitment composition recruits an immune cell to the device, where the immune cells encounters the target antigen, and where the immune cell resides until a deployment signal induces egress of the immune cell to a lymph node tissue outside of the device.

In some examples, the level of immune activation of the immune cell at egress is greater than that prior to entering the device.

For example, the immune cell is antigen-primed at egress compared to the level of priming prior to entering the device. In some cases, the immune cells recruited to the device remain resident in the device for 2 hours to 4 weeks, e.g., 2 hours to 24 hours, 2 to 6 days, or 1 to 4 weeks.

In some cases, the target antigen composition of the device comprises a cancer antigen or a cancer derived antigen. The cancer antigen, cancer derived antigen, or cancer cell is, e.g., derived from a melanoma, a central nervous system (CNS) cancer, a CNS germ cell tumor, a lung cancer, leukemia, multiple myeloma, a renal cancer, a malignant glioma, a medulloblatoma, a breast cancer, an ovarian cancer, a prostate cancer, a bladder cancer, a fibrosarcoma, a pancreatic cancer, a gastric cancer, a head and neck cancer, or a colorectal cancer. For example, a cancer cell is derived from a solid cancer or hematological cancer. The hematological cancer is, e.g., a leukemia or a lymphoma. A leukemia is acute lymphoblastic leukemia (ALL), acute mylogenous leukemia (AML), chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), chronic mylogenous leukemia
(CML), or acute monocytic leukemia (AMoL). A lymphoma is follicular lymphoma, Hodgkin's lymphoma (e.g., Nodular sclerosing subtype, mixed-cellularity subtype, lymphocyte-rich subtype, or lymphocyte depleted subtype), or Non-Hodgkin's lymphoma. Exemplary solid cancers include but are not limited to melanoma (e.g., unresectable, metastatic melanoma), renal cancer (e.g., renal cell carcinoma), prostate cancer (e.g., metastatic castration resistant prostate cancer), ovarian cancer (e.g., epithelial ovarian cancer, such as metastatic epithelial ovarian cancer), breast cancer (e.g., triple negative breast cancer), and lung cancer (e.g., non-small cell lung cancer).

[019] The device is administered to a subject in need thereof. For example, the subject is a mammal, e.g., a human. The subject was previously treated with a cancer therapy (e.g., for NSCLC, metastatic melanoma, or RCC) prior to administration with a device/vaccine and/or inhibitor of the invention. For example, the subject was previously treated with an inhibitor of the invention. For example, the subject was previously treated with one or more inhibitors of the invention. For example, the subject was previously treated with an inhibitor of the invention in the absence of co-administration with a cancer vaccine (e.g., a cancer vaccine device of the invention). The mammal is any mammal, e.g., a human, a primate, a mouse, a rat, a dog, a cat, a horse, as well as livestock or animals grown for food consumption, e.g., cattle, sheep, pigs, chickens, and goats. In a preferred embodiment, the mammal is a human.

[020] In some cases, the device contains a cancer-derived antigen. Exemplary cancer-derived antigens are described herein. For example, a cancer-derived antigen/tumor antigen comprises an antigen that is unique to tumor cells and/or arising from a mutation, e.g., an antigen shown in Table 1. In another example, a cancer-derived antigen comprises a shared antigen, e.g., a tumor specific antigen, a differentiation antigen, and/or an overexpressed antigen, e.g., as shown in Tables 2-4. In some examples, a cancer-derived antigen comprises a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 35-434.

[021] For example, a cancer-derived antigen is selected from the group consisting of MAGE series of antigens, MART-1/melanA, Tyrosinase, ganglioside, gpIOO, GD-2, O-acetylated GD-3, GM-2, MUC-1, Sos1, Protein kinase C-binding protein, Reverse transcriptase protein, AKAP protein, VRK1, KIAA1735, T7-1, T1I-3, T1I-9, Homo Sapiens telomerase ferment (hTRT), Cytokeratin-19 (CYFRA21-1), SQUAMOUS CELL CARCINOMA ANTIGEN 1 (SCCA-1), (PROTEIN T4-A), SQUAMOUS CELL CARCINOMA ANTIGEN 2 (SCCA-2), Ovarian carcinoma antigen CA125 (1A1-3B) (KIAA0049), MUCIN 1 (TUMOR-ASSOCIATED MUCIN), (CARCINOMA-ASSOCIATED MUCIN), (POLYMORPHIC
EPITHELIAL MUCIN), (PEM), (PEMT), (EPISIALIN), (TUMOR-ASSOCIATED EPITHELIAL MEMBRANE ANTIGEN), (EMA), (H23AG), (PEANUT-REACTIVE URINARY MUCIN), (PUM), (BREAST CARCINOMA-ASSOCIATED ANTIGEN DF3), CTCL tumor antigen sel-1, CTCL tumor antigen sel4-3, CTCL tumor antigen se20-4, CTCL tumor antigen se20-9, CTCL tumor antigen se33-1, CTCL tumor antigen se37-2, CTCL tumor antigen se57-1, CTCL tumor antigen se89-1, Prostate-specific membrane antigen, 5T4 oncofetal trophoblast glycoprotein, Orf73 Kaposi’s sarcoma-associated herpesvirus, MAGE-C1 (cancer/testis antigen CT7), MAGE-B1 ANTIGEN (MAGE-XP ANTIGEN) (DAM10), MAGE-B2 ANTIGEN (DAM6), MAGE-2 ANTIGEN, MAGE-4a antigen, MAGE-4b antigen, Colon cancer antigen NY-CO-45, Lung cancer antigen NY-LU-12 variant A, Cancer associated surface antigen, Adenocarcinoma antigen ART1, Paraneoplastic associated brain-testis-cancer antigen (onconeuronal antigen MA2; paraneoplastic neuronal antigen), Neuro-oncological ventral antigen 2 (NOVA2), Hepatocellular carcinoma antigen gene 520, TUMOR-ASSOCIATED ANTIGEN CO-029, Tumor-associated antigen MAGE-X2, Synovial sarcoma, X breakpoint 2, Squamous cell carcinoma antigen recognized by T cell, Serologically defined colon cancer antigen 1, Serologically defined breast cancer antigen NY-BR-15, Serologically defined breast cancer antigen NY-BR-16, Chromogranin A, parathyroid secretory protein 1, DUPAN-2, CA 19-9, CA 72-4, CA 195, and Carcinoembryonic antigen (CEA).

[022] In other cases, the bioactive composition of the device comprises a tumor lysate, e.g., comprising lysate derived from a melanoma tumor. In other cases, the bioactive composition comprises irradiated tumor cells, e.g., comprising a melanoma cell (e.g., a B16-F10 cell).

[023] The bioactive composition can also comprise a cancer cell surface antigen, or a viral or bacterial antigen.

[024] In some embodiments, the device further comprises an adjuvant, e.g., a CpG rich oligonucleotide, such as a condensed CpG oligonucleotide. Exemplary condensed CpG oligonucleotides include PEI-CpG.

[025] In some examples, the scaffold further comprises an RGD-modified alginate. In other cases, the device further comprises a toll-like receptor (TLR) agonist, e.g., a TLR agonist that preferentially binds to TLR3. For example, the TLR agonist comprises a TLR3 agonist, e.g., polyinosine-polycytidylic acid (poly I:C) or PEI-poly (I:C).

[026] In some cases, the scaffold comprises a hydrogel or porous polymer, said scaffold comprising a polymer or co-polymer of polylactic acid, polyglycolic acid, PLGA, alginate, gelatin, collagen, agarose, poly(llysine), polyhydroxybutyrate, poly-epsilon-caprolactone,
polyphosphazines, poly(vinyl alcohol), poly(alkylene oxide), poly(ethylene oxide),
poly(allylamine), poly(acrylate), poly(4-aminomethylstyrene), pluronic polyl, polyoxamer,
poly(uronic acid), poly(allylamine) or poly(vinylpyrrolidone). For example, a preferred
polymer is PLG or alginate.
[027] For example, the porous polymer is produced by gas-foaming.
[028] In some cases, the device is in the form of a bead, pellet, sheet, or disc.
[029] The invention also features a method of killing a cancer cell in a subject in need
thereof comprising administering the device described herein.
[030] In addition, the invention provides a method of killing a cancer cell in a subject in
need thereof comprising administering: a) an inhibitor of an immune-inhibitory protein; and
b) a device comprising i) a scaffold composition, ii) a cell recruitment composition, and
[031] iii) a bioactive composition, where the bioactive composition is incorporated into or
coated onto the scaffold composition, and wherein the bioactive composition causes
modification of cells in or recruited to the device.
[032] For example, the scaffold comprises open, interconnected macropores, and wherein
migration of the modified cells to another site in the body is promoted by the open,
interconnected macropores and by the deployment signal.
[033] In some cases, the other site in the body is a nearby or remote tissue target.
[034] For example, the inhibitor is present in or on the device. Alternatively, or in addition,
[035] the inhibitor is coated in or on the scaffold composition.
[036] In some cases, the inhibitor is not present in or on the device, e.g., the inhibitor is not
coated in or on the scaffold composition. For example, the inhibitor and the device are
formulated separately.
[037] In other cases, the inhibitor and the device are formulated together.
[038] In some example, the inhibitor and the device are administered to the subject
simultaneously. Alternatively, the inhibitor and the device are administered to the subject
sequentially.
[039] In some embodiments, the device is implanted subcutaneously into the subject. For
example, the inhibitor is administered intravenously, intraperitoneally, subcutaneously,
orally, intradermally, by inhalation, transmucosally, or rectally. For example, the inhibitor is
administered by injection, infusion, or inhalation.
[040] In some cases, the inhibitor is administered at a dosage of 0.01-10 mg/kg (e.g., 0.01,
0.05, 0.1, 0.5, 1, 5, or 10 mg/kg) bodyweight. For example, the inhibitor is administered in
an amount of 0.01-30 mg (e.g., 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20, or 30 mg) per dose.
In some methods of the invention, the subject comprises a cancer cell, where the cancer cell is poorly immunogenic. For example, the cancer cell is resistant to cytotoxic T-lymphocyte (CTL)-mediated lysis and/or is resistant to natural killer (NK) cell mediated killing. In other examples, the subject does not comprise an autoantibody. For example, an autoantibody is an antibody produced by the immune system that is directed against one or more of an individual’s own protein, e.g., regardless of whether the individual is immunized with a tumor lysate or a specific purified antigen. For example, the autoantibody is directed against a cancer cell. In some cases, the subject does not comprise an autoantibody against a cancer cell.

In some cases, the invention provides methods utilizing a combination of a cancer vaccine device with an inhibitor of an immune-inhibitory protein. For example, the inhibitor of an immune-inhibitory protein comprises an inhibitor of CTLA4 and an inhibitor of PD1. In some cases, the inhibitor of CTLA4 comprises an anti-CTLA-4 antibody and the inhibitor of PD1 comprises an anti-PD1 antibody. Preferably, intratumoral cytotoxic T cells are enhanced relative to immunosuppressive Treg cells after administration of the vaccine and antibodies. That is, the utilization of a combination of a cancer vaccine device along with an inhibitor of an immune-inhibitory protein results in the preferential generation and expansion of intratumoral effector T cells (e.g., cytotoxic T cells) as compared to immunosuppressive cells, e.g., Treg cells. For example, the methods described herein result in an intratumoral ratio of CD8(+) effector T cells to Treg cells that is at least doubled as compared to vaccination alone. In other examples, the cancer vaccine device and inhibitor of an immune-inhibitory protein results in at least a 2-fold increase in the intratumoral ratio of CD8(+) effector T cells to Treg cells, e.g., at least a 3-fold increase; at least a 4-fold increase; at least a 5-fold increase; at least a 6-fold increase; at least a 7-fold increase; at least an 8-fold increase; at least a 9-fold increase; at least a 10-fold increase; at least an 11-fold increase; at least a 12-fold increase; at least a 13-fold increase; at least a 14-fold increase; at least a 15-fold increase; at least a 16-fold increase; at least a 17-fold increase; at least an 18-fold increase; at least a 19-fold increase; or at least a 20-fold increase in the intratumoral ratio of CD8(+) effector T cells to Treg cells.

In some cases, the inhibitor of an immune-inhibitory protein is administered prior to, concurrently with, and/or subsequent to administration of the cancer vaccine device to maintain efficacy and tumor inhibition effects. Preferably, antibody treatment continues after the cancer vaccine device is administered. For example, antibody treatment continues for at least one day, e.g., for two days, for three days, for four days, for five days, for six days, for
seven days, for two weeks, for three weeks, for four weeks, for two months, for six months, for seven months, for eight months, for nine months, for ten months, for eleven months, for twelve months, for two years, for three years, for four years, or for five years or more after the cancer vaccine device is administered.

[044] In some cases, the scaffold comprises a hydrogel or porous polymer, e.g., a polymer or co-polymer of poly (D,L-lactide-co-glycolide) (PLG).

[045] The composition and methods of making a vaccine of the invention are described in US Application No. 13/741,271, Publication No. 2013-0202707, and US Patent No. 8,067,237, the contents of which are incorporated herein in their entireties.

[046] The invention provides a device and method for stimulating immune cells, such as dendritic cells, in situ. For example, presentation of Toll-like receptor (TLR) agonists in the context of the device is used for cancer vaccination. Incorporation and presentation of the TLR agonists embedded in structural polymeric devices specifically stimulates CD8(+) dendritic cells (DCs) (corresponding to CD141+ DCs in humans) and plasmacytoid DCs, which are subsets of DCs that are critical for cancer vaccination.

[047] Accordingly, the invention provides a device comprising a porous polymeric structure composition, a tumor antigen, and a toll-like receptor (TLR) agonist. For example, the device comprises a polymeric structure composition, a tumor antigen, and a combination of toll-like receptor (TLR) agonists, where the TLR agonist is selected from the group consisting of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13. For example, the polymeric structure comprises poly (D,L-lactide-co-glycolide) (PLG). Exemplary TLR agonists include pathogen associated molecular patterns (PAMPs), e.g., an infection-mimicking composition such as a bacterially-derived immunomodulator. TLR agonists include nucleic acid or lipid compositions (e.g., monophosphoryl lipid A (MPLA)).

[048] Certain nucleic acids function as TLR agonists, e.g., TLR1 agonists, TLR2 agonists, TLR3 agonists, TLR4 agonists, TLR5 agonists, TLR6 agonists, TLR7 agonists, TLR8 agonists, TLR9 agonists, TLR10 agonists, TLR11 agonists, TLR12 agonists, or TLR13 agonists. In one example, the TLR agonist comprises a TLR9 agonist such as a cytosine-guanosine oligonucleotide (CpG-ODN), a poly(ethylenimine) (PEI)-condensed oligonucleotide (ODN) such as PEI-CpG-ODN, or double stranded deoxyribonucleic acid (DNA). TLR9 agonists are useful to stimulate plasmacytoid DCs. For example, the device comprises 5 µg, 10 µg, 25 µg, 50 µg, 100 µg, 250µg, or 500 µg of CpG-ODN.
[049] In another example, the TLR agonist comprises a TLR3 agonist such as polyinosine-polycytidylic acid (poly I:C), PEI-poly (I:C), polyadenyl-copolyuridylic acid (poly (A:U)), PEI-poly (A:U), or double stranded ribonucleic acid (RNA).

[050] TLR3 agonists are useful to stimulate CD8+ DCs in mice and CD141+ DCs in humans. A plurality of TLR agonists, e.g., a TLR3 agonist such as poly I:C and a TLR9 agonist such as CpG act in synergy to activate an anti-tumor immune response. For example, the device comprises a TLR3 agonist such as poly (I:C) and the TLR9 agonist (CpG-ODN) or a PEI-CpG-ODN. Preferably, the TLR agonist comprises the TLR3 agonist, poly (I:C) and the TLR9 agonist, CpG-ODN. The combination of poly (I:C) and CpG-ODN act synergistically as compared to the vaccines incorporating CpG-ODN or P(I:C) alone.

[051] In some cases, the TLR agonist comprises a TLR4 agonist selected from the group consisting of lipopolysaccharide (LPS), monophosphoryl lipid A (MPLA), a heat shock protein, fibrinogen, heparin sulfate or a fragment thereof, hyaluronic acid or a fragment thereof, nickel, an opoid, a l-acid glycoprotein (AGP), RC-529, murine β-defensin 2, and complete Freund’s adjuvant (CFA). In other cases, the TLR agonist comprises a TLR5 agonist, wherein the TLR5 agonist is flagellin. Other suitable TLR agonists include TRL7 agonists selected from the group consisting of single-stranded RNA, guanosine anologs, imidazoquinolines, and loxorbine.

[052] Preferably, the TLR agonist is present at a concentration effective to induce the local production of interleukin-12 (IL-12) by dendritic cells.

[053] In some embodiments, the device contains an immunogenic factor/infection-mimicking composition, e.g., a toll-like receptor ligand, a CpG-ODN sequence or derivative thereof, a tumor antigen, a growth factor, a heat-shock protein, a product of cell death, or a cytokine.

[054] The invention also provides a device comprising a porous polymeric structure composition, a disease-associated antigen, and a toll-like receptor (TLR) agonist, wherein the TLR agonist preferentially binds to TLR3. In some cases, the polymeric structure composition comprises poly-lactide-co-glycolide (PLG). The TLR3 agonist is present in an amount to preferentially stimulate CD8+ dendritic cells or CD141+ dendritic cells.

[055] Preferably, the TLR agonist comprises a TLR3 agonist. In some cases, the TLR3 agonist comprises polyinosine-polycytidylic acid (poly I:C) or PEI-poly (I:C). For example, the TLR agonist comprises a nucleic acid. In other cases, the TLR agonist further comprises a TLR9 agonist. For example, the TLR9 agonist comprises a cytosine-guanosine oligonucleotide (CpG-ODN) or a PEI-CpG-ODN. Optionally, the device comprises a
combination of TLR agonists, the combination comprising a TLR3 agonist and a TLR9 agonist. For example, the TLR3 agonist comprises poly (I:C) and the TLR9 agonist comprises CpG-ODN.

[056] Alternatively, the device comprises a combination of TLR agonists, the combination comprising a TLR3 agonist and a TLR4 agonist. For example, the TLR3 agonist comprises poly (I:C) and the TLR4 agonist comprises MPLA.

[057] Optionally, the device further comprises a recruitment composition. Exemplary recruitment compositions include granulocyte macrophage colony stimulating factor (GM-CSF), Flt3L, and CCL20. For example, the recruitment composition comprises encapsulated GM-CSF.

[058] In some cases, the disease-associated antigen comprises a tumor antigen. For example, the tumor antigen comprises a tumor lysate, purified protein tumor antigen, or synthesized tumor antigen.

[059] Optionally, the TLR agonist further comprises pathogen associated molecular patterns (PAMPs). For example, the PAMP comprises a monophosphoryl lipid A (MPLA).

[060] Also provided is a device comprising a polymeric structure composition, a tumor antigen, and a combination of TLR agonists, wherein the TLR agonist is selected from the group consisting of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13.

[061] A method for eliciting an anti-tumor immune response is carried out by contacting or implanting into a subject a device comprising a polymeric structure composition, a tumor antigen, and a TLR agonist, wherein the TLR agonist preferentially binds to TLR3. For example, the TLR agonist comprises a TLR3 agonist. Alternatively, the TLR agonist comprises a TLR3 agonist and a TLR9 agonist.

[062] Preferably, the anti-tumor immune response comprises activation of a CD8+ dendritic cell or a CD141+ dendritic cell. In some cases, the anti-tumor immune response comprises activation of a plasmacytoid dendritic cell or a CD141+ dendritic cell. Alternatively, the anti-tumor immune response comprises a reduction in tumor burden.

[063] Preferably, the TLR agonist is present at a concentration effective to induce production of interleukin-12 (IL-12) by dendritic cells.

[064] Optionally, the device further comprises granulocyte macrophage colony stimulating factor (GM-CSF). In some examples, the GM-CSF is encapsulated. Another optional recruitment composition is a cytokine. For example, the device comprises 1 µg, 3 µg, 5 µg, 10 µg, 25 µg, or 50 µg of GM-CSF.
[065] The device also contains a tumor antigen, e.g., in the form of a tumor lysate (cultured cells or patient-derived primary cells) or purified tumor antigen such as a chemically synthesized/synthetic protein, a recombinant (e.g., biochemically-purified) protein/antigen (e.g., purified from a tumor cell). In some cases, the recombinant protein/antigen is made in a prokaryotic cell. In other cases, the recombinant protein/antigen is made in a eukaryotic (e.g., mammalian) cell.

[066] Also with in the invention is a method for eliciting an anti-tumor immune response by contacting a subject, e.g., implanting into a subject, a device comprising a porous polymeric structure composition, a tumor antigen, and a TLR agonist. For example, the TLR agonist is selected from the group consisting of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13. The device described above is associated with advantages over earlier vaccines. The most significant advantage is its ability to stimulate critical subsets of DCs that mediate potent anti-tumor activity. The method involves administering to a subject a device that contains a TLR3 agonist and/or a TLR9 agonist, which leads to elicitation of an anti-tumor immune response characterized by activation of plasmacytoid DCs and/or CD141+ DCs in the subject to which the vaccine was administered. The vaccine is useful for prophylaxis as well as therapy.

[067] The device is administered, e.g., topically applied or implanted, and is present over a period of time, e.g., in-dwelling, while constantly recruiting, educating, and dispersing or sending cells forth to lymph nodes or sites of disease or infection in the body. Improvements over existing devices include long term, ongoing activation of cells that enter the device and concomitant long term, ongoing egress of immunologically activated, e.g., antigen primed cells. The device includes a scaffold composition, a recruitment composition, and a deployment composition. The deployment composition that mediates prolonged and continuous egress of primed cells is an infection-mimicking composition such as a bacterially-derived immunomodulator. In preferred embodiments, the bacterially-derived immunomodulator is a nucleic acid such as a cytosine-guanosine oligonucleotide (CpG-ODN).

[068] The methods are used to treat a wide variety of diseases and to develop vaccines against a wide variety of antigens. In a preferred embodiment, the invention is used to develop a cancer vaccine. Another preferred embodiment of the invention comprises an infection-mimicking microenvironment with means to activate the host immune system and subsequently induce an immune response. The use of a synthetic cytosine-guanosine oligodeoxynucleotide (CpG-ODN) sequence with exogenous granulocyte macrophage colony
stimulating factor (GM-CSF) provides a method for controlling dendritic cell migration and modulating antigen-specific immune responses. The approach of using of this synthetic cytosine-guanosine oligonucleotide (CpG-ODN) sequence and/or poly (I:C), e.g., condensed oligonucleotides (e.g., PEI-CpG-ODN, or PEI-poly (I:C)), demonstrates significant improvements over earlier immune therapies. See, e.g., US 2012-0100182, e.g., at page 14, [0106]-page 15, [0110]; and page 24, [0176], incorporated herein by reference.

Devices perform three primary functions, e.g. attracting cells to the device, presenting an immunogenic factor, and inducing cell migration away from the device, e.g., to drain lymph nodes where activated immune cells exert their anti-tumor actions. Each of these primary functions are performed by the scaffold and/or biological composition(s). Various combinations of either the scaffold or biological composition achieve at least one primary function in exemplary devices. For example, the scaffold composition performs each of the three primary functions in some devices. In an alternative example, the scaffold composition performs one primary function, e.g. attracts cells to the device (preferably, dendritic cells), whereas the biological composition performs two primary functions, e.g. presents an immunogenic factor and induces cells (preferably, dendritic cells) to migrate away from the device, while some devices, for instance, are the inverse combination. Exemplary secondary functions of the scaffold and/or biological compositions include, but are not limited to, targeting the device to a particular cell or tissue type, adhering/releasing the device to/from the surface of one or more cells or tissues, and modulating the stability/degradation of the device.

The invention comprises a device comprising a scaffold composition and bioactive composition, the bioactive composition being incorporated into or conjugated onto the scaffold composition, wherein the scaffold composition attracts a dendritic cell, introduces a immunogenic factor into the dendritic cell thereby activating the dendritic cell, and induces the dendritic cell to migrate away from the scaffold composition. Alternatively the bioactive composition incorporated into or coated onto the scaffold composition attracts a dendritic cell, introduces an immunogenic factor into the dendritic cell thereby activating the dendritic cell, and induces the dendritic cell to migrate away from the scaffold composition. In other preferred embodiments, the scaffold composition or bioactive composition separately attract a dendritic cell to the device, introduce an immunogenic factor into the dendritic cell, and induce the dendritic cell to migrate away from the device.

DCs include conventional DCs as well as specific subsets of DCs. The TLR agonists, e.g., TLR3 agonists, preferentially attract and stimulate CD141+ DCs in the human (CD8+
DCs in the mouse). The TLR9 agonist, e.g., CpG, preferentially attract and stimulate plasmacytoid DCs.

[072] In preferred embodiments, the recruitment composition is GM-CSF, e.g., encapsulated GM-CSF. The device temporally controls local GM-CSF concentration, thereby controlling recruitment, residence, and subsequent dispersement/deployment of immune cells to lymph nodes or tissue sites distant from location of the device, e.g., sites of infection or tumor location. The concentration of GM-CSF determines whether if functions as a recruitment element or a deployment element. Accordingly, a method of programming dendritic cells in situ is carried out by introducing to a subject a device comprising scaffold composition and encapsulated recruitment composition. A pulse of recruitment composition is released from the device within 1-7 days of introduction of the device, leaving a residual amount of the recruitment composition in or on the device. The pulse is followed by slow release of the residual amount over several weeks. The local concentration of the recruitment composition and the temporal pattern of release mediates recruitment, retention, and subsequent release of dendritic cells from the device. For example, the pulse comprises at least 50, 60, 75, 90, or 95% of the amount of the recruitment composition associated with the device. An exemplary temporal release profile comprises a pulse characterized by release of at least 60% of the amount of the recruitment composition associated with the device in 1-5 days following the introduction of the device to a subject. Following the pulse, the residual amount is slowly released over an extended period of time (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 days or 2, 3, 4, 5 or more weeks) following the pulse period. Other recruitment compositions include Flt3L and/or CCL20. The recruitment compounds are used individually or in combination.

[073] The method of making a scaffold is carried out by providing a scaffold composition, incorporating into or coating onto the scaffold composition a first bioactive composition comprising polypeptides with means for attracting or repelling a dendritic cell, and contacting the scaffold composition with a second bioactive composition, wherein the second bioactive composition is covalently or non-covalently associated with the scaffold composition wherein the second bioactive composition comprises a immunogenic factor. In an alternate embodiment of this method, the linking and contacting steps are repeated to yield a plurality of layers, wherein the second bioactive composition comprises a combination of compounds with means to activate a dendritic cell.

[074] Methods comprise continuous in situ dendritic cell programming, comprising administering to a subject, a device comprising a scaffold composition and bioactive
composition, the bioactive composition being incorporated into or conjugated onto the scaffold composition, wherein the scaffold composition attracts a dendritic cell, introduces an immunogenic factor into the dendritic cell thereby activating the dendritic cell, and induces the dendritic cell to migrate away from the scaffold composition. The devices recruit and stimulate a heterogeneous population of dendritic cells. Each subset is specialized and contributes significantly to the generation of an immune response. For example, the device mediates CpG-ODN presentation and enrichment of a subset of dendritic cells, plasmacytoid DC (pDC), or CD141+ DCs, which are particularly important in development of anti-tumor immunity.

[075] Methods comprise increasing vaccine efficacy, comprising administering to a subject, a device comprising a scaffold composition and bioactive composition, the bioactive composition being incorporated into or conjugated onto the scaffold composition, wherein the scaffold composition attracts a dendritic cell, introduces an immunogenic factor into the dendritic cell thereby activating the dendritic cell, and induces the dendritic cell to migrate away from the scaffold composition, thereby increasing the effectiveness of a vaccination procedure.

[076] Methods comprise vaccinating a subject against cancer, comprising administering to a subject, a device comprising a scaffold composition and bioactive composition, the bioactive composition being incorporated into or conjugated onto the scaffold composition, wherein the scaffold composition attracts a dendritic cell, introduces an immunogenic factor into the dendritic cell thereby activating the dendritic cell, and induces the dendritic cell to migrate away from the scaffold composition, thereby conferring upon a subject anti-tumor immunity, e.g., IL-12 production, and reduced tumor burden. In the case of a localized or solid tumor, the device is administered or implanted at or near the tumor site or site from which the tumor was excised or surgically removed. For example, the device is implanted at a distance of 1, 3, 5, 10, 15, 20, 25, 40 mm from a tumor site or site of excision, e.g., the PLG vaccine device is administered 16-21 mm away from a tumor mass.

[077] Immunogenic factors include TLR ligands. For example, the immunogenic factor used is a modified TLR-9 ligand sequence, PEI-CpG-ODN. Preferably, the TLR ligand is a TLR3 agonist such as poly (I:C) or condensed PEI-poly (I:C).

[078] Scaffold compositions comprise a non-biodegradable material. Exemplary non-biodegradable materials include, but are not limited to, metal, plastic polymer, or silk polymer. Moreover, scaffold compositions are composed of a biocompatible material. This biocompatible material is non-toxic or non-immunogenic.
Bioactive compositions are covalently or non-covalently linked to the scaffold composition. Bioactive compositions comprise an element, either covalently or non-covalently bonded to the surface of the scaffold composition, with means to attract a dendritic cell. Alternatively, or in addition, bioactive compositions comprise an element, either covalently or non-covalently bonded to the surface of the scaffold composition, with means to introduce an immunogenic factor into a dendritic cell. Alternatively, or further in addition, bioactive compositions comprises an element, either covalently or non-covalently bonded to the surface of the scaffold composition, with means to induce a dendritic cell to migrate away from the scaffold composition.

The element of the bioactive composition with means to manipulate a dendritic cell is a secreted or membrane-bound amino acid, peptide, polypeptide, protein, nucleotide, dinucleotide, oligonucleotide, polynucleotide, polymer, small molecule or compound. In a preferred embodiment, this element is granulocyte macrophage colony stimulating factor (GM-CSF), because this element attracts dendritic cells to the scaffold composition. In another preferred embodiment, this element is a PEI-CpG-ODN sequence because this element has means to introduce CpG-ODN sequences into a dendritic cell thereby activating the cell. In some embodiments, this element is a polynucleotide or polypeptide encoding for CCR7, a chemokine receptor that mediates dendritic cell migration towards lymph nodes and away from the scaffold composition. The CCR7 element is introduced into a dendritic cell simultaneously or sequentially with PEI-CpG-ODN sequences to enhance dendritic cell migration away from the scaffold composition.

Scaffold compositions of the present invention contain an external surface. Scaffold compositions of the present invention alternatively, or in addition, contain an internal surface. External or internal surfaces of the scaffold compositions are solid or porous. Pore size is less than about 10 nm, in the range of about 100 nm-20 µm in diameter, or greater than about 20 µm, e.g., up to and including 1000 µm. In preferred embodiments, the size of the pores allows the migration into and subsequent exit of cells such as DCs from the device. For example, the pores are nanoporous, microporous, or macroporous. For example, the diameter of nanopores are less than about 10 nm; micropore are in the range of about 100 µm-20 µm in diameter; and, macropores are greater than about 20 µm (preferably greater than about 100 µm and even more preferably greater than about 400 µm, e.g., greater than 600 µm or greater than 800 µm). In one example, the scaffold is macroporous with open, interconnected pores of about 100-500 µm in diameter, e.g., 100-200, 200-400, or 400-500 µm. The size of the pores and the interconnected architecture allows the cells to enter, traverse within the
volume of the device via the interconnected pores, and then leave the device via the pores to
go to locations in the body outside of the device, e.g. to a tumor site, where an immune
response is mounted against tumor cells. The activated DCs migrate away from the device
and mount an immune response to solid tumors at discrete locations or throughout the body
in the case of metastatic tumor cells or blood tumors such as leukemias.

[082] Scaffold compositions of the present invention comprise one or more compartments.

[083] Devices of the present invention are administered or implanted orally, systemically,
sub- or trans-cunataneously, as an arterial stent, or surgically.

[084] The devices and methods of the invention provide a solution to several problems
associated with protocols for continuous cell programming in situ. In situ cell programming
systems that stimulate immune responses of the cells and induce their outward migration to
populate infected or diseased bodily tissues enhance the success of recovery, e.g., the specific
elimination of diseased tissue. Such a device that controls cell function and/or behavior, e.g.,
locomotion, contains a scaffold composition and one or more bioactive compositions. The
bioactive composition is incorporated into or coated onto the scaffold composition. The
scaffold composition and/or bioactive composition temporally and spatially (directionally)
controls dendritic cell attraction, programming, and migration.

[085] The devices mediate active recruitment, modification, and release of host cells from
the material in vivo, thereby improving the function of cells that have contacted the scaffold.
For example, the device attracts or recruits cells already resident in the body to the scaffold
material, and programs or reprograms the resident cells to a desired fate (e.g., immune
activation).

[086] This device includes a scaffold composition which incorporates or is coated with a
bioactive composition; the device regulates attraction, activation, and migration of dendritic
cells. Depending on the application for which the device is designed, the device regulates
attraction, activation, and/or migration of dendritic cells through the physical or chemical
characteristics of the scaffold itself. For example, the scaffold composition is differentially
permeable, allowing cell migration only in certain physical areas of the scaffold. The
permeability of the scaffold composition is regulated, for example, by selecting or
engineering a material for greater or smaller pore size, density, polymer cross-linking,
stiffness, toughness, ductility, or viscoelasticity. The scaffold composition contains physical
channels or paths through which cells can move more easily towards a targeted area of egress
of the device or of a compartment within the device. The scaffold composition is optionally
organized into compartments or layers, each with a different permeability, so that the time
required for a cell to move through the device is precisely and predictably controlled.
Migration is also regulated by the degradation, de- or re-hydration, oxygenation, chemical or pH alteration, or ongoing self-assembly of the scaffold composition.

[087] Attraction, activation, and/or migration are regulated by a bioactive composition. The device controls and directs the activation and migration of cells through its structure. Chemical affinities are used to channel cells towards a specific area of egress. For example, cytokines are used to attract or retard the migration of cells. By varying the density and mixture of those bioactive substances, the device controls the timing of the migration. The density and mixture of these bioactive substances is controlled by initial doping levels or concentration gradient of the substance, by embedding the bioactive substances in scaffold material with a known leaching rate, by release as the scaffold material degrades, by diffusion from an area of concentration, by interaction of precursor chemicals diffusing into an area, or by production/excretion of compositions by resident support cells. The physical or chemical structure of the scaffold also regulates the diffusion of bioactive agents through the device.

[088] The bioactive composition includes one or more compounds that regulate cell function and/or behavior. The bioactive composition is covalently linked to the scaffold composition or non-covalently associated with the scaffold.

[089] Signal transduction events that participate in the process of cell migration are initiated in response to immune mediators. Thus, the device optionally contains a second bioactive composition that comprises GM-CSF, a CpG-ODN or poly (I:C) sequence, a cancer antigen, and/or an immunomodulator.

[090] In some cases, the second bioactive composition is covalently linked to the scaffold composition, keeping the composition relatively immobilized in or on the scaffold composition. In other cases, the second bioactive composition is noncovalently associated with the scaffold. Noncovalent bonds are generally one to three orders of magnitude weaker than covalent bonds permitting diffusion of the factor out of the scaffold and into surrounding tissues. Noncovalent bonds include electrostatic, hydrogen, van der Waals, π aromatic, and hydrophobic.

[091] The scaffold composition is biocompatible. The composition is biodegradable/erodable or resistant to breakdown in the body. Relatively permanent (degradation resistant) scaffold compositions include metals and some polymers such as silk. Preferably, the scaffold composition degrades at a predetermined rate based on a physical parameter selected from the group consisting of temperature, pH, hydration status, and porosity, the cross-link density, type, and chemistry or the susceptibility of main chain
linkages to degradation or it degrades at a predetermined rate based on a ratio of chemical polymers. For example, a high molecular weight polymer comprised of solely lactide degrades over a period of years, e.g., 1-2 years, while a low molecular weight polymer comprised of a 50:50 mixture of lactide and glycolide degrades in a matter of weeks, e.g., 1, 2, 3, 4, 6, 10 weeks. A calcium cross-linked gels composed of high molecular weight, high guluronic acid alginate degrade over several months (1, 2, 4, 6, 8, 10, 12 months) to years (1, 2, 5 years) in vivo, while a gel comprised of low molecular weight alginate, and/or alginate that has been partially oxidized, will degrade in a matter of weeks.

Exemplary scaffold compositions include polyactic acid, polyglycolic acid, PLGA polymers, alginates and alginate derivatives, gelatin, collagen, fibrin, hyaluronic acid, laminin rich gels, agarose, natural and synthetic polysaccharides, polyamino acids, polypeptides, polyesters, polyanhydrides, polyphosphazenes, poly(vinyl alcohols), poly(alkylene oxides), poly(allylamines)(PAM), poly(acrylates), modified styrene polymers, pluronic polyols, polyoxamers, poly(urea acid), poly(vinylpyrrolidone) and copolymers or graft copolymers of any of the above. One preferred scaffold composition includes an RGD-modified alginate. In other examples, the scaffold composition includes crosslinked polymers, e.g., crosslinked alginites, gelatins, or derivatives thereof, such as those that are methacrylated.

Another preferred scaffold composition a macroporous poly-lactide-co-glycolide (PLG). For example, the PLG matrix includes GM-CSF, danger signals, and a target antigen, e.g., a cancer antigen and serves as a residence for recruited DCs as they are programmed. The recruitment element, GM-CSF, is encapsulated into the PLG scaffolds. PLG matrices that comprise the encapsulated GM-CSF provide a pulse of the dendritic cell recruitment composition and then a gradual slower rate of release. The pulse comprises at least 40, 50, 60, 75, 80% or more of the initial amount of bioactive composition with the remaining percent being released gradually over then next days or weeks after administration to the site in or on the subject to be treated. For example, release is approximately 60% of bioactive GM-CSF load within the first 5 days, followed by slow and sustained release of bioactive GM-CSF over the next 10 days. This release profile mediates a rate of diffusion of the factor through the surrounding tissue to effectively recruit resident DCs.

Porosity of the scaffold composition influences migration of the cells through the device. Pores are nanoporous, microporous, or macroporous. For example, the diameter of nanopores are less than about 10 nm; micropore are in the range of about 100 nm-20 μm in diameter; and, macropores are greater than about 20 μm (preferably greater than about 100 μm and even more preferably greater than about 400 μm). In other examples, the pore size
ranges from less than 10 nm to about 1000 μm. In some cases, the average pore size ranges from about 250 μm to about 500 μm. In some cases, the porous architecture is random or aligned. In one example, the scaffold is macroporous with aligned pores of about 400-500 μm in diameter.

[095] A method of making a scaffold is carried out by providing a scaffold composition and covalently linking or noncovalently associating the scaffold composition with a first bioactive composition. Exemplary devices and methods of making them are described in US 2012/0100182, PCT/US2010/057630, and PCT/US2012/35505, each of which is hereby incorporated by reference. The first bioactive composition preferably contains granulocyte macrophage colony stimulating factor. The scaffold composition is also contacted with a second bioactive composition, preferably one or more cytosine-guanosine oligonucleotide (CpG-ODN) sequences. The second bioactive composition is associated with the scaffold composition to yield a doped scaffold, i.e., a scaffold composition that includes one or more bioactive substances. The contacting steps are optionally repeated to yield a plurality of doped scaffolds, e.g., each of the contacting steps is characterized by a different amount of the second bioactive composition to yield a gradient of the second bioactive composition in the scaffold device. Rather than altering the amount of composition, subsequent contacting steps involve a different bioactive composition, i.e., a third, fourth, fifth, sixth..., composition or mixture of compositions, that is distinguished from the prior compositions or mixtures of prior doping steps by the structure or chemical formula of the factor(s). The method optionally involves adhering individual niches, layers, or components to one another and/or insertion of semi-permeable, permeable, or nonpermeable membranes within or at one or more boundaries of the device to further control/regulate locomotion of cells or bioactive compositions.

[096] Therapeutic applications of the device include the instruction of immune cells. For example, the method includes the steps of providing a device that includes scaffold composition with a bioactive composition incorporated therein or thereon and a mammalian cell bound to the scaffold and contacting a mammalian tissue with the device, e.g., by implanting or affixing the device into or onto a mammalian tissue. At the time of administering or implanting the device, exemplary relative amounts of each component, recruiting composition (e.g., GM-CSF, Flt3L, or CCL20), danger signal (e.g., CpG-ODN), and antigen (e.g., purified tumor antigen or tumor cell lysate) are as follows: GM-CSF: 0.5 μg - 500 μg; CpG-ODN: 50 μg - 3,000 μg; and Tumor antigen/lysate: 100 μg - 10,000 μg.
[097] A method of modulating an activity of a cell, e.g., a host cell, is carried out by administering to a mammal a device containing a scaffold composition and a recruitment composition incorporated therein or thereon, and then contacting the cell with a deployment signal. The cells leave the device after encountering antigen (and other factors) and thus being activated to seek out tumor cells in the body to which an immune response is mounted. The activity of the cell at egress differs from that prior to entering the device. Cells are recruited into the device and remain resident in the device for a period of time, e.g., minutes; 0.2, 0.5, 1, 2, 4, 6, 12, 24 hours; 2, 4, 6, days; weeks (1-4), months (2, 4, 6, 8, 10, 12) or years, during which the cells are exposed to structural elements and bioactive compositions that lead to a change in the activity or level of activity of the cells. Encountering the antigen and other compounds in the device induces egress of the altered (re-educated or reprogrammed) cells, and the cells migrate out of the device and into surrounding tissues or remote target locations to seek out and mediate immunity against diseased cells such as tumor cells.

[098] The deployment signal is a composition such as protein, peptide, or nucleic acid or a state of activation of the cell. For example, having ingested antigen, DCs become activated and migrate to lymph nodes, the spleen, and other anatomical locations, where they meet up with T cells to further propagate an antigen-specific immune response, e.g., anti-cancer response. For example, cells migrating into the device only encounter the deployment signal once they have entered the device. In some cases, the deployment signal is a nucleic acid molecule, e.g., a plasmid containing sequence encoding a protein that induces migration of the cell out of the device and into surrounding tissues. The deployment signal occurs when the cell encounters the plasmid in the device, the DNA becomes internalized in the cell (i.e., the cell is transfected), and the cell manufactures the gene product encoded by the DNA. In some cases, the molecule that signals deployment is an element of the device and is released from the device in delayed manner (e.g., temporally or spatially) relative to exposure of the cell to the recruitment composition. Alternatively, the deployment signal is a reduction in or absence of the recruitment composition. For example, a recruitment composition induces migration of cells into the device, and a reduction in the concentration or depletion, dissipation, or diffusion of the recruitment composition from the device results in egress of cells out of the device. In this manner, immune cells such as T cells, B cells, or dendritic cells (DCs) of an individual are recruited into the device, primed and activated to mount an immune response against an antigen-specific target. Optionally, an antigen corresponding to a target to which an immune response is desired is incorporated into or onto the scaffold.
structure. Cytokines, such as granulocyte macrophage colony stimulating factor (GM-CSF) are also a component of the device to amplify immune activation and/or induce migration of the primed cells to lymph nodes. Other cell specific recruitment compositions are described below.

[0099] The device recruit cells in vivo, modifies these cells, and then promotes their migration to another site in the body. This approach is exemplified herein in the context of dendritic cells and cancer vaccine development but is also useful to other vaccines such as those against microbial pathogens as well as cell therapies in general. Cells educated using the devices described herein promote regeneration of a tissue or organ immediately adjacent to the material, or at some distant site. Alternatively, the cells are educated to promote destruction of a tissue (locally or at a distant site). The methods are also useful for disease prevention, e.g., to promote cell-based maintenance of tissue structure and function to stop or retard disease progression or age-related tissue changes. The education of cells within the device, "programming" and "reprogramming" permits modification of the function or activity of any cell in the body to become a multipotent stem cell again and exert therapeutic effects.

[0100] The inability of traditional and ex vivo DC-based vaccination strategies to coordinate and sustain an immune response mediated by the heterogeneous DC network in cancer patients has led to limited clinical effectiveness of these approaches. The devices and methods described herein have distinct advantages, because preferential recruitment and expansion of pDCs dramatically improves immune responses to cancer antigens and reduces tumor progression compared to previous vaccine approaches.

[0101] Described herein is a material-based (e.g., PLG) vaccine which has been optimized, e.g., to control the presentation of GM-CSF and adjuvants, relative to other vaccine formulations in order to enhance T effector activity and downregulate Treg cells and other immunosuppressive mechanisms that may be induced by some adjuvants. The material-based vaccine represents a significant advantage over previous vaccine systems in that it creates a tumor and vaccine microenvironment that responds to an immune-inhibitory protein, e.g., anti-CTLA-4, by preferentially enhancing effector T cell generation and expansion over Treg cells.

[0102] Polynucleotides, polypeptides, or other agents are purified and/or isolated. Specifically, as used herein, an "isolated" or "purified" nucleic acid molecule, polynucleotide, polypeptide, or protein, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized. Purified compounds are at least 60% by weight (dry weight) the compound of
interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. For example, a purified compound is one that is at least 90%, 91%, 92%, 93%, 94%, 95%, 98%, 99%, or 100% (w/w) of the desired compound by weight. Purity is measured by any appropriate standard method, for example, by column chromatography, thin layer chromatography, or high-performance liquid chromatography (HPLC) analysis. A purified or isolated polynucleotide (ribonucleic acid (RNA) or deoxyribonucleic acid (DNA)) is free of the genes or sequences that flank it in its naturally-occurring state. A purified or isolated polypeptide is free of the amino acids or sequences that flank it in its naturally-occurring state. Purified also defines a degree of sterility that is safe for administration to a human subject, e.g., lacking infectious or toxic agents.

[0103] Similarly, by "substantially pure" is meant a nucleotide or polypeptide that has been separated from the components that naturally accompany it. Typically, the nucleotides and polypeptides are substantially pure when they are at least 60%, 70%, 80%, 90%, 95%, or even 99%, by weight, free from the proteins and naturally-occurring organic molecules with they are naturally associated.

[0104] By "isolated nucleic acid" is meant a nucleic acid that is free of the genes which flank it in the naturally-occurring genome of the organism from which the nucleic acid is derived. The term covers, for example: (a) a DNA which is part of a naturally occurring genomic DNA molecule, but is not flanked by both of the nucleic acid sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner, such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a synthetic cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Isolated nucleic acid molecules according to the present invention further include molecules produced synthetically, as well as any nucleic acids that have been altered chemically and/or that have modified backbones. For example, the isolated nucleic acid is a purified cDNA or RNA polynucleotide. Isolated nucleic acid molecules also include messenger ribonucleic acid (mRNA) molecules.

[0105] "Conservatively modified variations" of a particular polynucleotide sequence refers to those polynucleotides that encode identical or essentially identical amino acid sequences, or where the polynucleotide does not encode an amino acid sequence, to essentially identical
sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For instance, the codons CGU, CGC, CGA, CGG, AGA, and AGG all encode the amino acid arginine. Thus, at every position where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent substitutions" or "silent variations," which are one species of "conservatively modified variations." Every polynucleotide sequence described herein which encodes a polypeptide also describes every possible silent variation, except where otherwise noted. Thus, silent substitutions are an implied feature of every nucleic acid sequence which encodes an amino acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule by standard techniques.

[0106] Similarly, "conservative amino acid substitutions," in one or a few amino acids in an amino acid sequence are substituted with different amino acids with highly similar properties are also readily identified as being highly similar to a particular amino acid sequence, or to a particular nucleic acid sequence which encodes an amino acid. Such conservatively substituted variations of any particular sequence are a feature of the present invention. Individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are "conservatively modified variations" where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. See, e.g., Creighton (1984) Proteins, W.H. Freeman and Company, incorporated herein by reference.

[0107] By the terms "effective amount" and "therapeutically effective amount" of a formulation or formulation component is meant a sufficient amount of the formulation or component, alone or in a combination, to provide the desired effect. For example, by "an effective amount" is meant an amount of a compound, alone or in a combination, required to reduce or prevent cancer in a mammal. Ultimately, the attending physician or veterinarian decides the appropriate amount and dosage regimen.

[0108] The terms "treating" and "treatment" as used herein refer to the administration of an agent or formulation to a clinically symptomatic individual afflicted with an adverse condition, disorder, or disease, so as to effect a reduction in severity and/or frequency of
symptoms, eliminate the symptoms and/or their underlying cause, and/or facilitate improvement or remediation of damage.

[0109] The terms "preventing" and "prevention" refer to the administration of an agent or composition to a clinically asymptomatic individual who is susceptible or predisposed to a particular adverse condition, disorder, or disease, and thus relates to the prevention of the occurrence of symptoms and/or their underlying cause.

[0110] The transitional term "comprising," which is synonymous with "including," "containing," or "characterized by," is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. By contrast, the transitional phrase "consisting of" excludes any element, step, or ingredient not specified in the claim. The transitional phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention.

[0111] Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. 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 by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

[0112] Figure 1A is a graph of tumor size in melanoma tumor-bearing mice after several days with or without treatment with anti-PDL or anti-CTLA4 antibodies. Values represent means and standard deviations (n=10).

[0113] Figure 1B is a Kaplan Meier survival curve showing the survival time of melanoma tumor-bearing mice with or without treatment with anti-PDL or anti-CTLA4 antibodies.

[0114] Figure 2A is a graph of tumor area in melanoma tumor bearing mice after several days with or without vaccine and/or anti-PDL or anti-CTLA4 antibodies. Values (n=10) represent mean and standard deviation. * P<0.05 as compared to all other experimental conditions unless otherwise noted.
Figure 2B is a Kaplan Meier survival curve showing the survival time of melanoma tumor bearing mice after treatment with or without vaccine and/or anti-PD1 or anti-CTLA4 antibodies. Values (n=10) represent mean and standard deviation.

Figure 3A is a bar graph of the number of CD3+CD8+ tumor infiltrating T cells present in B16 tumors extracted from melanoma tumor bearing mice after treatment with or without vaccine and/or anti-PD1 or anti-CTLA4 antibodies. Values (n=5) represent mean and standard deviation. * P<0.05 ** P<0.01 as compared to all other experimental conditions unless otherwise noted.

Figure 3B is a bar graph of the ratio of CD3+CD8+ T effector cells to CD3+ FoxP3+ T regulatory cells isolated from the B16 tumors of the mice with or without treatment with vaccine and/or anti-PD1 or anti-CTLA4 antibodies. Values (n=5) represent mean and standard deviation. * P<0.05 ** P<0.01 as compared to all other experimental conditions unless otherwise noted.

Figure 4A is a flow cytometric histogram of CD3+ T cell infiltrates isolated from mice treated with PLG vaccines alone (VAX) or in combination with anti-CTLA4 antibody (VAX+CTLA-4) for 14 days.

Figure 4B is a bar graph showing the total number of CD3+CD8+ effector T cells isolated from mice implanted with blank matrices (Con), PLG vaccines alone (VAX), or vaccines in combination with anti-PD1 (VAX+PD1) or anti-CTLA4 (VAX+CTLA) antibodies for 14 days. Values (n=5) represent mean and standard deviation. * P<0.05 ** P<0.01 as compared to all other experimental conditions unless otherwise noted.

Figure 5A is a set of flow cytometric scatterplots showing the percentage of the cell population isolated from the scaffolds that were positive for CD8 and CD107a in the four treatment groups.

Figure 5B is a bar graph showing the fold increase in number of activated CD8+, scaffold-infiltrating T cells that were positive for CD107a and IFNy in the four treatment groups. Values (n=5) represent mean and standard deviation. * P<0.05 ** P<0.01 as compared to all other experimental conditions unless otherwise noted.

Figure 6A is a panel of fluorescence activated cell sorting (FACS) scatterplots showing the proportion of T cell infiltrates isolated from PLG vaccine implants that express CD4, CD8, and/or FoxP3 in mice treated with vaccine only (VAX), vaccine plus anti-CTLA4 antibody (VAX+CTLA4), or vaccine plus anti-PD1 antibody (VAX+PD1).

Figure 6B is a bar graph showing the ratio of CD3+CD8+ effector T cells to CD4+FoxP3+ T cells at the vaccination site of mice treated with vaccine only (VAX), vaccine
plus anti-CTLA4 antibody (VAX+CTLA4), or vaccine plus anti-PDL antibody (VAX+PDL). Values represent mean and standard deviation (n=5). *P<0.05 **P<0.01 as compared to all other experimental conditions unless otherwise noted.

Figure 7A is a set of flow cytometry histograms showing the number of CD8+ T effector cells in the vaccine draining lymph nodes of mice treated with vaccine alone (VAX) or in combination with anti-CTLA4 (VAX+CTLA4) or anti-PDL (VAX+PDL) antibodies.

Figure 7B is a set of flow cytometry histograms showing the number of FoxP3+ Treg cells in the vaccine draining lymph nodes of mice treated with vaccine alone (VAX) or in combination with anti-CTLA4 (VAX+CTLA4) or anti-PDL (VAX+PDL) antibodies.

Figure 8A is a bar graph showing the percentage of T cells in the draining lymph nodes that are CD8+ or FoxP3+ from mice treated with vaccine alone (VAX) or in combination with anti-CTLA4 (+CTLA-4) or anti-PDL (+PD-1) antibodies. Values (n=5) represent mean and standard deviation (n=5). *P<0.05 **P<0.01 as compared to all other experimental conditions unless otherwise noted.

Figure 8B is a bar graph showing the ratio of CD8+ T cells to FoxP3+ T cells in the vaccine draining lymph nodes of mice treated with vaccine alone (VAX) or in combination with anti-CTLA4 (VAX+CTLA4) or anti-PDL (VAX+PDL) antibodies. Values (n=5) represent mean and standard deviation (n=5). *P<0.05 **P<0.01 as compared to all other experimental conditions unless otherwise noted.

Figure 9A-Figure 9D is a series of bar graphs illustrating the combination of multiple checkpoint blockades with PLG cancer vaccines. Figure 9A is a bar graph showing the size of B16 melanoma tumors in mice treated with vaccine alone (V), or vaccine in combination with anti-PDL (+P), anti-CTLA4 (+C) or both anti-PDL and anti-CTLA4 (+P+C) at 35 days after inoculation of 10^5 tumor cells. Figure 9B is a bar graph that shows the numbers of CD8(+) T cells and Figure 9C is a bar graph that shows the numbers of FoxP3(+) Tregs isolated from B16 tumors in mice treated with vaccine alone (V), or vaccine in combination with anti-PDL (+P), anti-CTLA4 (+C) or both anti-PDL and anti-CTLA4 (+P+C) at 35 days after inoculation of 10^5 tumor cells. Figure 9D is a bar graph that shows the CD8(+) T cell and FoxP3(+) Treg ratio in B16 tumors in mice treated with vaccine alone (V), or vaccine in combination with anti-PDL (+P), anti-CTLA4 (+C) or both anti-PDL and anti-CTLA4 (+P+C) at 35 days after inoculation of 10^5 tumor cells. Values in B (n=8), C & D (n=5) represent mean and standard deviation. *P<0.05 **P<0.01 as compared to other experimental conditions as noted.

Figure 10A-Figure 10E is a series of bar charts and a dot plot showing that PLG
vaccine in combination with blockade antibodies enhances intratumoral T effector cell activity. Figure 10A is a bar chart that shows the total number of CD3(+)CD8(+) T cells isolated from the B16 tumors of untreated mice (Control) and mice treated with PLG vaccines alone (Vax) or in combination with anti-PD-1 (+PD-1) and anti-CTLA-4 (+CTLA4) antibodies. Figure 10B is a bar chart that shows the total number of CD3(+)FoxP3(+) T regulatory cells isolated from the B16 tumors of untreated mice (Control) and mice treated with PLG vaccines alone (Vax) or in combination with anti-PD-1 (+PD-1) and anti-CTLA-4 (+CTLA4) antibodies. Figure 10C is a bar chart that shows the ratio of CD3(+)CD8(+) T cells to CD3(+)FoxP3(+) T regulatory cells isolated from the B16 tumors of untreated mice (Control) and mice treated with PLG vaccines alone (Vax) or in combination with anti-PD-1 (+PD-1) and anti-CTLA-4 (+CTLA4) antibodies. Figure 10D is a series of FACS plots representing tumor infiltrating leukocytes in tumors of untreated mice (Control) and mice treated with PLG vaccines alone (Vax) or in combination with anti-PD-1 (+PD-1) and anti-CTLA-4 (+CTLA4) antibodies. Single cell suspensions were prepared from tumors at Day 18 and stained for activated, cytotoxic T cell markers, CD8(+) and CD107a. Numbers in FACS plots indicate the percentage of the cell population positive for both markers. Figure 10E is a bar chart showing the numbers of CD8(+) tumor-infiltrating T cells positive for either IFNy or CD107a in blank matrices (Control), PLG vaccines alone or vaccines in combination with anti-PD-1 and anti-CTLA-4. Values in A, B, C &D (n=5) represent mean and standard deviation. * P<0.05 ** P<0.01 as compared to the vaccine alone (V vs V+P; V vs V+C) unless otherwise noted.

[0130] Figure 11 is a bar chart showing the ratio of CD3(+)CD8(+) T cells to CD3(+)FoxP3(+) T regulatory cells isolated from the B16 tumors of untreated mice (Control) and mice treated with PLG vaccines alone (Vax) or in combination with anti-PD-1 (+PD-1) and anti-CTLA-4 (+CTLA4) antibodies. Values represent mean and standard deviation (n=5). ** P<0.01 as calculated comparing V+C versus the other experimental conditions.

[0131] Figure 12 is a dot plot showing the effects of stopping antibody treatment after PLG vaccination on tumor growth. A comparison of tumor area in mice bearing established melanoma tumors (inoculated with 5x10^5 B16-F10 cells) and treated with vaccines alone (V) or with vaccines in combination with i.p. injections of anti-PD1 (V+P) or anti-CTLA-4 (V+C) antibodies. Each data point represents one animal (n=8) and lines in the dot plot represent the average tumor area.
DETAILED DESCRIPTION OF THE INVENTION

Prior to the invention, cancer vaccines typically depended on cumbersome and expensive manipulation of cells in the laboratory, and subsequent cell transplantation resulted in poor lymph node homing and limited efficacy. In terms of cancer treatment, many existing therapies become ineffective because cancers can co-opt immune checkpoint pathways to evade the endogenous immune response. Although agents have been identified and are used to prevent or minimize this ability of cancer cells to evade the immune system, these agents lack efficacy in poorly immunogenic tumors. The invention solves these problems by using materials for cancer vaccination that mimic key aspects of bacterial infection to directly control immune cell trafficking and activation in the body. The invention further combines these cancer vaccines with inhibitors of immune-inhibitory proteins (e.g., immune checkpoint proteins), thereby enabling an endogenous immune response strong enough to eliminate tumors or minimize their progression. Also, the cancer vaccines work synergistically with the inhibitors of the immune-inhibitory proteins to lower the dosage of inhibitor required for efficacy in treating cancer compared to the dosage required when the inhibitor is used as a single agent.

The results described herein demonstrate that poly(lactide-co-glicolide) (PLG) cancer vaccines produce significant numbers of antigen specific T cells in melanoma models. In summary, to test the effects of vaccine and antibody (e.g., anti-CTLA4 and/or anti-PDL antibody) treatments in combination, an aggressive, therapeutic B16 melanoma model was utilized. In mice bearing B16 melanoma tumors, treatment with anti-CTLA4 and anti-PDL antibodies alone had no effect on tumor size and survival outcomes in these animals (Figs. 1A-B). PLG vaccination modestly suppressed tumor progression but did not affect long-term survival in any mice bearing B16 melanoma tumors. Surprisingly, the administration of CTLA-4 and PD-1 antibodies combined with PLG vaccines was able to promote long-term survival rates of 75% and 40%, respectively, in mice that would otherwise die when treated with each agent alone. These treatments synergize to promote significant T cell activity at tumor sites and locally within vaccines (Figs. 3A-6B). The response is significantly skewed toward cytotoxic T cell activity relative to suppressive regulatory T cell activity, and these responses can be maintained for extended times when anti-CTLA4 antibodies are combined with vaccination (Fig. 6B).

Immune checkpoint pathways and cancer

In healthy subjects, immune checkpoint pathways (also known as immune-inhibitory pathways) are important for maintaining self-tolerance and preventing autoimmunity.
However, immune checkpoint pathways in cancer cells are often dysregulated, leading to the ability of tumors to evade the body's endogenous anti-tumor immune response. Cancers co-opt the immune checkpoint pathways by a number of ways, such as upregulating the expression of immune checkpoint proteins that normally serve immune-inhibitory roles. For example, inhibitory ligands and receptors that regulate T cell effector activity are often overexpressed in cancer cells.

[0135] An exemplary inhibitory receptor is cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), also called CD152, which reduces the level of T cell activation. Another exemplary inhibitory receptor is programmed cell death protein 1 (PD1), also called CD279, which limits T effector cell activity. For example, cancer cells upregulate ligands for PD1 (e.g., programmed cell death protein ligand 1 (PDL1)), thereby blocking anti-tumor immune responses.

[0136] The blockade of immune checkpoints in cancer immunotherapy has emerged as a promising approach to combat this mechanism by which cancer cells evade the anti-tumor immune response. For example, antibodies directed against immune-inhibitory proteins, such as immune checkpoint proteins (also referred to as blockade antibodies herein) are being explored as potential anti-cancer therapeutics. See, e.g., Pardoll. Nat. Reviews Cancer. (2012) 12:252-264.

Immune-inhibitory proteins and their inhibitors

[0137] Immune checkpoint proteins include the B7/CD28 receptor super family. CTLA-4 belongs to the immunoglobulin superfamily of receptors, which also includes programmed cell death protein 1 (PD-1), B and T lymphocyte attenuator (BTLA), T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), and V-domain immunoglobulin suppressor of T cell activation (VISTA). Other immune regulatory checkpoint proteins include proteins in the TNF family (e.g., OX40 (also known as CD134) and 4-1BB ligand).

[0138] The amino acid sequence of Mus musculus VISTA, provided by Genbank Accession No. AEO22039.1, is shown below (SEQ ID NO: 1).

```
1  mgvpavpneas  sprwgtillla  iflaasrglv  aafkvttyps  lyvcpegqna  tltcrligpv
61  skghdvtiyik  twyissrgev  qmckehrpir  nftlqghqhh  gshlkanash  dpqkkhglel
121  aasdhnfnfal  tirmvtpdrds  glycclviel  knhhpeqrfy  gsmelqvgqag  kgagstcmas
181  neqdsdnita  aalatgaciv  gilcipliill  lvykqrqvas  hrraqelvrm  dsntqqgien
241  pgfettppfqq  gmpeaktrppl  lasvaqrqgqs  esgryllsdp  stplspcppgp  dffpsidpv
301  pdspnseal  (SEQ ID NO: 1)
```

[0139] The mRNA sequence encoding Mus musculus VISTA, provided by Genbank Accession No. JN602184.1, is shown below (SEQ ID NO: 2), with the start and stop codons in bold.
[0140] The amino acid sequence of human OX40 ligand, provided by Genbank Accession No. NP_003318.1, is shown below (SEQ ID NO: 3), with the signal peptide shown in underlined font and the mature peptide shown in italicized font.

1 mcvgarrlgr gcgaallllg lglstvtgll cvgdtypesnd rccheerpgn gmvsvcrersq
61 ntvcpcscp kpfwdsnwr sgserkqlt atqtvcvcccc agtggtgcgg
121 pgvdacpccc pphsppqdcna ckpwntct la gktltgqsm sdsaiedrd ptatqgqetq
181 gppspitvq pteawprtsq gspztpvevp ggrz.ssilg lgllvlgigp laillalyil
241 rrrqrlpdpd hkkpppggsfr tpgiigeqada hstlaki (SEQ ID NO: 3)

[0141] The mRNA sequence encoding human OX40 ligand, provided by Genbank Accession No. NM_003327.3, is shown below (SEQ ID NO: 4), with the start and stop codons in bold.

1 ccgcaagaa aacccaagact ctgcccagac ccagacagcc gtagctgctg gggtctgccc
61 ggtctggcccg gctggcccgtc cccccctgctg ctcctgctgg cctggggttc agccacggta
121 cggggctcca cctaggtttt gacccagtgca gcctggccga cccgccagcc gcctggccga
181 gaccaacgca aagcagtcgc tggagaggcc gcctgctgctg gctggaggtc accacaggca
241 cctgccctcc agggacactt ctccagacgg acaacaggca ctgoaagccgg tggaccacact
291 gcctgctcct ccctgctctcc ggtgagcact ggtgtgtctg ggtgtgtctg ggtgtgtctg
351 gggtggagtt cagcagccag cccagcagcag cggcggcc
411 gatccctttt gcgggtctag gcgtggaccc ggtggtcggt gcgcctggcc gcgcctggcc
471 ggcgtggttc gcgggtctag gcgtggaccc ggtggtcggt gcgcctggcc gcgcctggcc
531 gatccgtctgt gcgggtctag gcgtggaccc ggtggtcggt gcgcctggcc gcgcctggcc
591 gatccgtctgt gcgggtctag gcgtggaccc ggtggtcggt gcgcctggcc gcgcctggcc
651 gatccgtctgt gcgggtctag gcgtggaccc ggtggtcggt gcgcctggcc gcgcctggcc
711 gatccgtctgt gcgggtctag gcgtggaccc ggtggtcggt gcgcctggcc gcgcctggcc
771 gatccgtctgt gcgggtctag gcgtggaccc ggtggtcggt gcgcctggcc gcgcctggcc
831 gatccgtctgt gcgggtctag gcgtggaccc ggtggtcggt gcgcctggcc gcgcctggcc
891 gatccgtctgt gcgggtctag gcgtggaccc ggtggtcggt gcgcctggcc gcgcctggcc
951 gatccgtctgt gcgggtctag gcgtggaccc ggtggtcggt gcgcctggcc gcgcctggcc
1011 gatccgtctgt gcgggtctag gcgtggaccc ggtggtcggt gcgcctggcc gcgcctggcc
1071 gatccgtctgt gcgggtctag gcgtggaccc ggtggtcggt gcgcctggcc gcgcctggcc

[0142] The amino acid sequence of human 4-IBB, provided by Genbank Accession No.
NP_001552.2, is shown below (SEQ ID NO: 5), with the signal peptide in underlined font and the mature peptide in italicized font.

The mRNA sequence of human 4-IBB, provided by Genbank Accession No. NM-001561.5, is shown below (SEQ ID NO: 6), with the start and stop codons in bold.
CTLA4 is a receptor expressed only on activated T cells and it reduces the level of T cell activation by interfering with the activity of T cell co-stimulatory receptor, CD28. CTLA4 is a target of cancer immunotherapies (e.g., by antibodies that bind to and block CTLA4).

Ipilimumab (manufactured by Bristol-Myers Squibb) is a fully humanized anti-CTLA4
antibody that has been approved by the Food and Drug Administration (FDA) for the treatment of melanoma (in particular, unresectable or metastatic melanoma) and is undergoing clinical trials for use in other cancers. Tremelimumab (manufactured by Pfizer; CAS number 745013-59-6) is a fully humanized IgG2 monoclonal anti-CTLA4 antibody that is undergoing clinical trials for treatment of melanoma.

The amino acid sequence of human CTLA4, provided by Genbank Accession No. P16410.3, is shown below (SEQ ID NO: 7).

\[
\begin{align*}
1 & \text{maclgfrhk aqlnlstrtw pctlfflllf ipvfckamhv aqpavlass rgiasvfcey} \\
61 & \text{aspgaterv vtvrlgaqas vtevaatym mgneltfldd sictgtsgan qvnlitgqir} \\
121 & \text{amtdglyick velmyppppp ilgigngtqly videpcpmps dfllwilaa vsslffysfl} \\
181 & \text{ltavslskml kkrspilttgv yvkmpteppe cekqfqpsyf pin} \quad \text{(SEQ ID NO: 7)}
\end{align*}
\]

Amino acid residues 36-223 of SEQ ID NO: 7 corresponds to the mature sequence of CTLA4.

The mRNA sequence of human CTLA4, provided by Genbank Accession No. AF414120.1, is shown below (SEQ ID NO: 8).

\[
\begin{align*}
1 & \text{cccccttgtgt gtcacatgtg taatacatat cttggtatca aacgtctatc ataaagtctt} \\
61 & \text{ttgctgctgaa aacatagcac tggctacatt cttcaacag ggtgatgtgt gtaaatgccc ccaacagac} \\
121 & \text{agaaatgact gaaacagtct gcgcgggttg ggtttttcttc tcttcatctg tctttcatct} \\
181 & \text{atgcaagatg cccaatgcgt gttgtgtca aacccctcat cttgctgtgt tctttgctct} \\
241 & \text{ttttttcttc ttttctctgt tttgttgttg aacgtgaggg gtaaggagttt ttgagctctg ggaacccag} \\
301 & \text{tttattttctt attgtatctg ttttctctcg ttctctctgt ttttctgtgt ttttctctct} \\
361 & \text{ttctttccttt tttattttct ttttcttttt ttttcttttt ttttcttttt ttttcttttt} \\
421 & \text{ttttttcttt ttttttttt ttttttttt ttttttttt ttttttttt tttttttttt} \\
481 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
541 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
601 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
661 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
721 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
781 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
841 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
901 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
961 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1021 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1081 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1141 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1201 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1261 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1321 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1381 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1441 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1501 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1561 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1621 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1681 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1741 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1801 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1861 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1921 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \\
1981 & \text{tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt} \quad \text{(SEQ ID NO: 8)}
\end{align*}
\]
The atg start codon and the stop codon are bolded and underlined.

Blockade of CTLA4 enables a pre-existing endogenous anti-tumor immune response to destroy tumors. Thus, in the presence of an endogenous anti-tumor immune response in a subject, inhibition of CTLA4 lifts the resistance to the immune response and allows the body’s immune cells to destroy the cancer cells. However, in poorly immunogenic tumors, the endogenous immune response is either does not exist or is too weak to kill the cancer cells, and inhibition of CTLA4 alone has minimal efficacy.

The role of PD1 is to limit T cell activity in peripheral tissues during an immune response to infection and to minimize autoimmunity. PD1 is expressed on activated lymphocytes, including activated T effector cells, B cells, and natural killer (NK) cells. Ligands for PD1 include PD1 ligand 1 (PD-L1, also called B7-H1 and CD274), and PD-L2 (also called B7-DC and CD273). PD1 inhibits lymphocyte function when bound to its ligands. In subjects with cancer, PD1 is often expressed on a large percentage of tumor-infiltrating lymphocytes. Also, PD-L1 is overexpressed in cancers such as melanoma, ovarian, renal, and lung cancer. PD-L2 is overexpressed in cells from lymphomas, such as B cell lymphoma (e.g., primary mediastinal B cell lymphoma, follicular cell B cell lymphoma, and Hodgkin’s disease). Thus, PD1 and its ligands are main players in immune inhibition in the tumor microenvironment, and are therefore targets for cancer immunotherapy.

The mRNA sequence encoding human PD1, provided by Genbank Accession No. NM_005018.2, is shown below (SEQ ID NO: 9).

```
1 aagttccctt ccgctcaccct ccgcctgagc cagttggagaag gcggcagctc tggggcttg
61 ctccagcat gcagatcctca caggcgcctt ggcgactctt ctggggcttg atcaactgg
121 aaggtcagaa gagctcctgg ctgtggtggg cagggcagga aacccctcca cctttacaca
181 cccacagtgc gctctgtgttg acgagagggg acacgcagcc ctccacgcag ctccactcct
241 acacatgag ggcgtcttgct ctaaatgtgt acctgatag cccacagacg cagacgcaga
301 aagtgccgccc ctctccggag cagccagaccc agccgcagcc gagtcgtggct ttccgttgtc
361 ccacactgcc caacggcgcgg gttctccaca tcggctgtggt cagggcgcgg gcgaaagcaca
421 ccgcagcactta ccctgtgtggt gcctatctcc tggccacgcc cagggcagtc atggagagcc
481 aaccggcagctgctgagttg acagagagaa gcggcagagtc gcgccacggc cagccacaggc
541 ccctacccag gcagccgcggc cagctttcaca ccctgttgtg tgtgtgtgtgt gcgccgtgtc
601 ttggggccagt ggtctgtcata tctgtgggccc tgggctctcat tctgcctccc ggcgacagag
661 ggacataggg aggccacgccc acggcagcgc ccctgaagga ggaccgccta gcgctgtctg
721 tgttgtcttgt ggcctatggt gcctgtgcatt tccatggtgc agagagacgc cggccgaccc
781 cccgtgtccct cgtctgtgag cagacgaggt atggccacct ctggctttct acggcaagttg
841 gcacccctcc cccgcacgcc aggggctctag cttcacgcgc ctcgagctcc cagccacccgta
901 ggctgtagga tggacacatcg tctgtgggccc ttcgtacgcgt accagtcgtc
961 tgcagacactct ccacccatagc cccgggtcag ccacatcttc cagggagagc agggcaggttg
1021 cagggctatg gcgcgcctc ccggtgtcag gcgggcagtc ccagagcgcgg cccggctgtc
1081 cactgtcacgc ggcacaagcg cccacacagg atctctgtct atcgccccagc atctcgagcca
1141 gggacacagtt gcctcaccct cttcacaggg gcggcagagc cttacgacgc ttcgctcgctt
1201 gcgcagcacaag gcgcgtgctgc gttcaggtcc tctgatctt ctgctctgtg cttcgctctgc
1261 tgtggtgctgac tgggtgacgg gcggcgcgag gcggctgccg tcggctgcgg cgggccggcgt
1321 cgtcgccgag atggggggtct cttgtggagat cgcggctgcgc gccggcggcgt gcggcggcgt
1381 tgtggcatccc cggacaagcct gcacggcaggg cccacaactgc cggacacaggg tgggaggtc
1441 attcggcttgg gcctcagaccc aggtttacttc gctccctcgta gcggcagagc aggtttcagga
1501 aaggtcagaa gcagctctctcg cttgtgtgtgg cagggcagga aaccctctcca ccctttacaca
```
The amino acid sequence of human PDL, provided by Genbank Accession No. NP_005009.2, is shown below (SEQ ID NO: 10).

```
1 mgipqapwvp vwavllqlgw pgwfdspdr pwnpptfsa llvvetgda tftcsgsnts
61 esfvinwyrm spsqntdkla aipfdrsqgp qdfcrvtq1 ppgrdfhms vrarrnsgt
121 ylcaiaalap kaiqkesla elrvtterrae vptahpssp ppaqgftlv vggvqlggs
181 llllvwvlav icsraargti garrtpqiplk edpsapvpfs vdygelfqfw rktpeppyv
241 cvpeqteyay ifvspsmgts sparrsgadg prsaqplrpe dhccswpl (SEQ ID NO: 10)
```

Residues 1-20 of SEQ ID NO: 10 correspond to the signal peptide sequence, and residues 21-288 of SEQ ID NO: 10 correspond to the mature peptide sequence.

Q9NZ7.1, incorporated herein by reference, and is shown below (SEQ ID NO: 11).

```
1 mrifavfiffm tywhilnaft vtvpkdlvyv eygsntieec kfpveqldl aalivyweme
61 dkniiqfvhg eedlkvhss yqrarlikd qsligaalq itdvlqidxq vyrcmisygg
121 adykritzvkv napynkingr ilvdpvtae heltcqaeay pkaeiwiqts dqyvlqkttt
181 ttmskreekl fnvtstirin ttntiefyet fnrfdpeenh aelvielp lahpnerth
241 lvlgailleic lgvltf1f1 lrkgmmddv kcgldtsnk kqsdhleq (SEQ ID NO: 11)
```

The mRNA sequence encoding human PDL1 is provided by Genbank Accession No. AY291313.1, incorporated herein by reference, and is shown below (SEQ ID NO: 12), with the start and stop codons in bold.

```
1 atgaggata tttctgtcttt tatattctag acttacttgc attttgtgaa cgccccatac
61 acaaaaaatca accaacaagat tttggtttgtg gatcatgtca cctctcgaata tgaactgaca
121 tgcggggtgt agggctacc accagccac accaatctcc accaagagag cagagcttctt caagtgcacc
241 acagacactg aacacttccc caacacttccc acagctttct cactgttttgc ctaccion
301 gctgtcagq accaccatc cagctgaattgtgctacccg aacctctcct gacactctcct
361 ccaaaatgaa gagctccatt gttgtccttg gagagctctctt atatttccctt ctctgagca
421 tgcacacatc tggcctggttt aagaaagctt aagatgtgtg atgtgctga aatctgtggttt (SEQ ID NO: 12)
```

Q9BQ51.2, incorporated herein by reference, and is shown below (SEQ ID NO: 13).

```
1 mifllllmlsl elqlhqliaai ftvtpkqei liehgsnvtl ecnfdtghsv nigaitaslq
```
The mRNA sequence encoding human PDL2 is provided by Genbank Accession No. AF344424.1, incorporated herein by reference, and is shown below (SEQ ID NO: 14), with the start and stop codons in bold.

```
1 gcacaccccta agctgaatga acaactttttc ttctcttgaa tatactttaa gcacaaattt
61 tgagctgttt ttttgtacc tttcttcata ctgcccagctg gagaagatcc cggtttgag
121 ctactgtagt tttgtctttt ttttttctct tttggtcttg tattttggtt gcctatataa
181 gggaatctaa ccaacaacac aactgtttttg tttgttgtac atttttgcatct tatraattgq
241 agtgctgcca aacgtttctaa atcatacgaa acacaggttc cctctctctg aacggacgc
301 ttggaactgg aagcagcttg tatttacaca aagcagttgc gagaagtgtc aacggctgac
361 tcataaatag acaagatgga ccaagagttt tataagactgt tggatttggc ttagatagac
421 gtgaacagctt gcttcttggg ccttttcttg aagaggttgg ccaaagatgc atctctacca
481 ctgtagaaag gcccttggag ggcagctctag gttgctctag gttgacgggc gttccacata
541 cctcaactcca aagttgagga cgaagagcct tataatgata cttactatg gaagggcgcc
601 tgggactaca agtacctgca tctgaaagttt acaagttcttt aagggaggttta acacagtctc
661 atttcaaagg tctccagaga aagatgctttt tggctctgagct gcacagcttg aagttactttt
721 ctagcagga aacgctctgg gggagcttcg aagctttcttt gcacagctcag gcacagcagc
781 cttccatggg cctctctttct ttagctcttg actttttgct aagcgcctct ctcgcaacag
841 tttacaagctt cttctctgtt tctccttgaa aagaggtgtt aggacgtcct tggctctgac
901 ctgagactcg aagacacaccc ggcgcctcctt atgctctgtg aagcgcctctt ctcgcaacag
961 ttgatacactg ttttcttttt ctttggtttt gttgctctgtg aagcgcctctt ctcgcaacag
1021 aacgctgttt ctttccaaga ccaacacaaaa aagctttcttt cttcgctctg ggcacagcag
1081 aacagtctct aacaggttgg ggttctttgg gggacgtctt gctatgttag cttactattt
1141 aacgctctgg aagcgcctctt cttcgctctg ggcacagcag gggacgtctt cttactattt
1201 aaatgccttt ggatgacca ga (SEQ ID NO: 14)
```

[0150] MDX-1106 (also called BMS-936558; manufactured by Bristol Myers Squibb) is an anti-PDL1 human monoclonal antibody that is undergoing clinical trials for use in melanoma, renal, and lung cancers. See, e.g., Clinical Trials Identifier No. NCT00730639. MK-3475 (manufactured by Merck) is a monoclonal IgG4 antibody against PDL1 and is undergoing clinical trials for use in previously-treated patients with Non-Small Cell Lung Cancer (NSCLC). CT-011 (also called pidilizumab, produced by Cure Tech) is a humanized monoclonal antibody against PDL1 and is undergoing clinical trials for use in metastatic colorectal cancer, metastatic melanoma, and lymphoma. AMP-224 (developed by GlaxoSmithKline and Amplimmune) is an Fc fusion protein containing a ligand of PDL1. AMP-224 blocks the interaction between PDL1 and PDL2 or PDL1. AMP-224 is undergoing clinical trials for use in cancer. See, e.g., Clinical Trials Identifier No. NCT01352884. MDX1 105 (produced by Bristol-Myers Squibb) is a fully human monoclonal IgG4 anti-PDL1 antibody and clinical trials are undergoing for its use in cancer (e.g., relapsed/refractory renal cell carcinoma, NSCLC, colorectal adenocarcinoma, malignant melanoma, advanced/metastatic epithelial ovarian cancer, gastric cancer, pancreatic cancer, and breast cancer). See, e.g., Clinical Trial Identifier No. NCT00729664.
In addition to immune checkpoint receptors, B7 family immune-inhibitory ligands are also immune-inhibitory proteins that are candidate targets for cancer immunotherapy. For example, B7-H3 (also called CD276) and B7-H4 (also called B7-S1, B7x, or VCTN1) have been implicated in immune inhibition. In addition, B7-H3 and B7-H4 are overexpressed on cancer cells and on tumor infiltrating cells. MGA271 (produced by Macrogenics) is a humanized IgGl/kappa monoclonal antibody against B7-H3 and is currently undergoing clinical trials for use in refractory B7-H3-expressing neoplasms (e.g., prostate cancer and melanoma). See, e.g., Clinical Trial Identifier No. NCT01391143.

The amino acid sequence of human B7-H3 is provided by Genbank Accession No. Q5ZPR3.1, incorporated herein by reference. The mRNA sequence encoding human B7-H3 is provided by Genbank Accession No. AJ583695.1, incorporated herein by reference.

The amino acid sequence of human B7-H4 is provided by Genbank Accession No. Q7Z7D3.1, incorporated herein by reference. The mRNA sequence encoding human B7-H4 is provided by Genbank Accession No. DQ103757.1, incorporated herein by reference.

A number of other proteins have been shown to be associated with inhibition of immune cell activity and are thus also potential targets for cancer immunotherapy. These proteins include lymphocyte activation gene 3 (LAG3, or CD223), 2B4 (CD244), B and T lymphocyte attenuator (BTLA, CD272), T membrane protein 3 (TIM3, HAVcr2), adenosine A2a receptor (A2aR), and killer inhibitory receptors. Killer inhibitory receptors include killer cell immunoglobulin-like receptors (KIRs) and C-type lectin receptors, both of which regulate the killing activity of NK cells. IMP321 (produced by Immutep) is a soluble LAG3-Ig fusion protein that targets LAG3 and is currently being studied for use in advanced renal cell adenocarcinoma and advanced pancreatic adenocarcinoma. See, e.g., Brignone et al. Clin. Cancer Res. (2009) 15:6225-6231 and Wang-Gillam et al. Invest. New Drugs (2013) 31:707-13.

The amino acid sequence of human BTLA is provided by Genbank Accession No. NP_861445.3, incorporated herein by reference, and is shown below (SEQ ID NO: 15), with the signal peptide shown in underlined font and the mature peptide shown in italicized font.

```
1 mktlpamlgt oklfwuffli yuiddiing kescdvglyi krqsehsila gdpfelecpv
61 kyca.nmph.vt wckingttcv kledrgt swk eeknlisffil hffepvlpndn gspcansnfq
12l anlieshtt lyvtdvkssas epksdemas rpwllysilp lgglplllitt cfcilfclrr
18l hqgkqnelds tagreinlvd abhksegtea strqnsqvl1 setgildndp dilfrmqegs
24l evsyonspclee nkpgivyasi nhsvigpnnr ia.rnveas.pt eyasicvrs (SEQ ID NO: 15)
```

The mRNA sequence encoding human BTLA is provided by Genbank Accession No.
NM_181780.3, incorporated herein by reference, and is shown below (SEQ ID NO: 16), with
the start and stop codons shown in bold.

```
1  gtctttctgt  tcaacttcttt  tcaacaactc  attcagagtc  ttctactctt  cctctccttac
6 1 ... herein by reference, and is shown below (SEQ ID NO: 17).
1  mfshlpfdcv  sseveyraev  gqnaylpcfy  tpaapgnlvp  vcwgkgacpv
```
The mRNA sequence encoding human TIM3 is provided by Genbank Accession No. AF450242. 1, incorporated herein by reference, and is shown below (SEQ ID NO: 18).

1 ggagaagttaa aacatgtcct aacacaggtt gtcctctgact tttcttcgcct aagctccatct
   61 ttcttccttc ttcctctggt taatgctcct gcctgcctgc tgaatggtta ctaaaggtcc
   121 tcagaagtgga aatacagagc ggaggtcgtg cagaatgctc atctggccct cttcttcacc
   181 ccagcgcgcc caggaacatt cgtgcgcgtcc tgcctgggca aagagacgct tcctgctttt
   241 gaattgtcgca acgttgtgct cagqactgat gaagaggtag tgaattttgg gacatccaga
   301 tctctggctaa atggggattt cgccaaagga gatggtcccc tcagccataa gaaatggtact
   361 ctgagcagacc gtcctgcctta cggctcgcct atcctaaccct cagcataaat gaaggatgaa
   421 aaatttaccg tgaagttggt catcaaaacc gcacaaagtt cccctgcccag gactctcagc
   481 agagacctca ctcagccttt tcccagagag cttcaccacc gggacacttg cccagcagag
   541 acacagacac tgggagaccc cctctgataa aatctcaacc aatactccac atggcacaat
   601 gaguattcggg aacactaggg gctccattcc ggcctagagt tcagcagact ctcagacaata
   661 gcagctctca ctcgagacag gatctgtgct gggctgctct tcggctctgat tctgcggcct
   721 ttaatccccg aattgatact caataaagag cagagaatag acaatattag cctcctctct
   781 ttggatcacc cccccccccc agatttggca aatgctagc aagagcggtc tgtcgcgtgaaa
   841 gaaaactacht atccacattga agagacagtaa aataagcactc gagaagctgg cttctctctct
   901 tctctgtgca gcagcagctg gcaactctctta ccaactgcttt ctggctctgct tgtcactgca
   961 tagataaccac cacctatttt ttcagctttg tgttctcggc tttttagaaca ctatgagctt
 1021 tgttaacctga tgtttttggag aactgctgtc cactgctatt gcacagagtt ttttcattttt
 1081 cagaagataaa tgactccatct gggcaattgaa ctggga (SEQ ID NO: 18)

Combination of inhibitors with vaccines

[0157] In the presence of an endogenous anti-tumor immune response in a subject, inhibition of an immune-inhibitory (e.g., immune checkpoint) protein described above lifts the resistance of cancer cells to the immune response and allows the body's immune cells to destroy the cancer. However, in poorly immunogenic tumors, the endogenous immune response either does not exist or is too weak to kill the cancer cells, and inhibition of immune-inhibitory proteins has minimal efficacy.

[0158] To address this problem, the invention provides a combination of a vaccine device with an inhibitor of an immune-inhibitory protein. As described in detail in the working examples, this combination surprisingly led to a greater decrease in tumor size and a longer survival time compared to administration of inhibitor alone. Described herein is a material-based (e.g., PLG) vaccine which has been optimized, e.g., to control the presentation of GM-CSF and adjuvants, relative to other vaccine formulations in order to enhance T effector activity and downregulate Treg cells and other immunosuppressive mechanisms that may be induced by some adjuvants. The material-based vaccine represents a significant advantage over previous vaccine systems in that it creates a tumor and vaccine microenvironment that responds to an immune-inhibitory protein, e.g., anti-CTLA-4, by
preferentially enhancing effector T cell generation and expansion over Treg cells.

[0159] The invention features a cancer vaccine device that comprises one or more (e.g., 1, 2, 3, 4, 5, 6, or more) inhibitors to an immune-inhibitory protein. For example, the inhibitor(s) is incorporated into or onto the cancer vaccine device, e.g., incorporated into or onto a scaffold composition within the device. Administration of a cancer vaccine device containing the inhibitor(s) allows for localized delivery of the inhibitor(s), e.g., at the same site as vaccine.

[0160] The inhibitor can be encapsulated in the vaccine device during fabrication of the device. Alternatively, the inhibitor is added to the vaccine device after it is fabricated. For example, the inhibitor is encapsulated in the PLG microspheres utilized to fabricate the vaccine, combined with the CpG and sucrose added to the PLG prior to foaming, or added to the vaccine device after fabrication, e.g., by adsorbing to the surface of the device, or by placing the inhibitor in a sustained release formulation that is subsequently combined with the vaccine device.

[0161] For example, the cancer vaccine device comprises an anti-CTLA4 antibody and/or an anti-PD1 antibody.

[0162] The invention also provides a method of killing a cancer cell, slowing cancer progression, reducing a tumor size, prolonging the survival time of a cancer patient, and/or treating cancer by administering a cancer vaccine in combination with an inhibitor of an immune-inhibitory protein.

[0163] For example, the vaccine and the inhibitor are formulated separately, i.e., the inhibitor is not included within the vaccine device. In some embodiments, the vaccine and one or more (e.g., 1, 2, 3, 4, 5, 6, or more) inhibitor(s) are administered simultaneously. In other cases, the vaccine and the inhibitor(s) are administered sequentially. For example, the inhibitor(s) is administered at least 6 hours (e.g., 6 h, 12 h, 24 h, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 1.5 weeks, 2 weeks, 3 weeks, 4 weeks, or more) prior to administration of the vaccine. In other cases, the vaccine is administered at least 6 hours (e.g., 6 h, 12 h, 24 h, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 1.5 weeks, 2 weeks, 3 weeks, 4 weeks, or more) prior to administration of the inhibitor(s). In other embodiments, the vaccine and the inhibitor are formulated together, e.g., the inhibitor is included within or coated onto the vaccine device.

[0164] For example, an anti-CTLA4 antibody and/or anti-PD1 antibody are administered in combination with the vaccine device (e.g., administered simultaneously or sequentially).
The combination of inhibitor(s) and vaccine in provides certain advantages. For example, the combination synergistically induces the activity of T effector cells that infiltrate tumors. Also, the combination enhances local T effector cell activity (e.g., T cells in close proximity to the implanted vaccine device, and/or T cells in the vaccine draining lymph nodes). Also, the combination of inhibitor(s) and vaccine in the same device provides advantages over non-device vaccines used in combination with the inhibitor(s). In particular, inclusion of the inhibitor(s) in the vaccine device allows for targeting of local and/or specific immune cells (such as those specifically recruited to the device). Unlike systemic administration of the inhibitor(s), this local administration of the inhibitor(s) in some cases leads to lower toxicity and a lower dosage needed for efficacy.

In some cases, the inhibitor is administered prior to the vaccine device. For example, after administration of the inhibitor (e.g., antibody), e.g., within a week, immune cells infiltrate into the tumor site. The infiltration can cause a transient increase in tumor size. After administration (e.g., implantation) of the vaccine device, regression in tumor size occurs. For example, regression in tumor size occurs at least 1 weeks (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60 weeks or more) after administration of the vaccine device. In some cases, the combination of the inhibitor and the vaccine device causes a reduction in tumor size (e.g., a reduction of at least 10%, e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more) compared to the tumor size prior to administration of the inhibitor and/or vaccine device. In some examples, the combination of the inhibitor and the vaccine device causes total eradication of the tumor.

Tumor size is determined by standard methods in the art. For example, tumor size is the weight of the tumor or the area of the tumor. Tumor size (area in mm²) is, e.g., the product of the two longest diameters of the tumor. Tumor diameters can be measured using standard methods (e.g., with calipers). In other examples, the weight of a tumor is a measure of its size.

Cancer vaccine device

In the cancer vaccine, presentation of toll like receptor (TLR) agonists for cancer vaccination leads to improved activation of immune cells. The vaccines and methods comprise incorporation and presentation of TLR agonists embedded in structural polymeric devices. CD8(+) Dendritic cells (DCs) and plasmacytoid DCs (as well as conventional DCs) play important roles in cancer vaccination; these cells are preferentially recruited and activated using the TLR-agonist containing structural polymeric device. The device is manufactured as a tiny bioengineered porous disc filled with tumor-specific antigens and
TLR agonists. The disc is implanted into the body, e.g., inserted under the skin, where it activates the immune system to destroy cancer cells. This approach reprograms cells that are already in the body.

[0169] In some examples, the device includes a recruitment component. Thus, the device optionally includes a recruitment molecule such as a cytokine. In those situations, polymers were designed to first release a cytokine to recruit and house host dendritic cells (DCs), and subsequently present cancer antigens and danger signals to activate the resident DCs and dramatically enhance their homing to lymph nodes. Specific and protective anti-tumor immunity was generated with these materials. For example, a 90% survival rate was achieved in animals that otherwise die from cancer within 25 days. These materials are useful in cancer and other vaccines to program and control the trafficking of a variety of cell types in the body.

[0170] A polymer system was designed to not only serve as a drug delivery device, but also as a physical, antigen-presenting structure to which the DCs are recruited, and where DCs reside while they are activated using a material (poly[lactide-co-glycolide]) (PLG) and bioactive molecules (e.g., GM-CSF and CpG-ODN). These bioactive molecules have excellent safety profiles. The material system serves as an effective cancer vaccine, eliminating the time, expense and regulatory burden inherent to existing cell therapies and reducing or eliminating the need for multiple, systemic injections and high total drug loading. The devices described herein utilize infection-mimicking materials to program DCs in situ.


[0172] A macroporous poly-lactide-co-glycolide (PLG) matrix presents GM-CSF, danger signals, and cancer antigens in a defined spatiotemporal manner in vivo, and serve as a residence for recruited DCs as they are programmed. GM-CSF is encapsulated into PLG scaffolds using a high pressure C02 foaming process, as described in US 2013-0202707. The GM-CSF release profile from the matrix allows diffusion of the factor through the surrounding tissue to effectively recruit resident DCs.

[0173] In situ dendritic cell targeting systems are utilized to therapeutically manipulate the immune system with TLR agonists. As described in detail in US 2013-0202707 (incorporated herein by reference), macroporous polymeric scaffolds are designed that
deliver three different classes of TLR agonists in vivo: CpG-ODN, monophosphoryl lipid A (MPLA), and polyinosin:polyicidylic acid (P(I:C)) in combination with GM-CSF, Flt3L, or CCL20 to augment DC recruitment and activation. Various subsets of DCs are recruited and utilized for in situ vaccination. The ability of these systems to effect immune protection and tumor regression required CD8(+) DCs and correlates strongly with plasmacytoid DCs(pDCs) and IL-12 production, regardless of the TLR agonist type or dose.

Inflammatory Mediators

Dendritic Cell (DC) proliferation, migration and maturation are sensitive to inflammatory mediators, and granulocyte macrophage colony stimulating factor (GM-CSF) has been identified as a potent stimulator of immune responses, specifically against cancer antigens. GM-CSF also has the ability to recruit and program these antigen-presenting immune cells. Additionally, Cytosine-guanosine (CpG) oligonucleotide (CpG-ODN) sequences found in bacterial DNA are potent immunomodulators that stimulate DC activation, leading to specific T-cell responses. Creating an infection mimicking microenvironment by the presentation of exogenous GM-CSF and CpG-ODN provides an avenue to precisely control the number and timing of DC migration and modulate antigen specific immune responses.

The vertebrate immune system employs various mechanisms for pathogen recognition, making it adept at generating antigen-specific responses and clearing infection. Immunity is controlled by antigen presenting cells (APCs), especially dendritic cells (DCs), which capture antigens and are activated by stimuli, unique ‘danger signals’ of the invading pathogen, such as CpG dinucleotide sequences in bacterial DNA (Banchereau J, and Steinman RM. Nature. 392, 245-252. (1998); Klinman DM. Nat. Rev. Immunol. 4, 249-58 (2004); each incorporated herein by reference).

However, cancerous cells, derived from self-tissues, are void of the danger signals required to signal DC maturation and instead promote an immunosuppressive microenvironment that allows cells to escape immunity. Key elements of infection are inflammatory cytokines and danger signals. A polymeric material system is ideal to present these factors in the required spatiotemporal manner to provide an infection-mimicking microenvironment in situ that useful as a vaccine. These infection mimics provide the continuous programming of host DCs, providing for efficient DC activation and dispersement in situ. These infection-mimicking devices are used for numerous vaccine applications, including melanoma cancer vaccines.
In many infections, inflammatory cytokines and danger signals stimulate specific DC responses that mediate immune recognition and pathogen clearance. For example, upon bacterial invasion and release of toxins, skin cells such as fibroblasts, keratinocytes and melanocytes are damaged, resulting in the release of inflammatory cytokines, such as GM-CSF (Hamilton J. Trends in Immunol. 23, 403-408. (2002); Hamilton J., and Anderson G. Growth Factors. 22(4), 225-231. (2004); each herein incorporated by reference), that act to recruit Langerhans DC (skin) and DC precursors (monocytes; blood) (Hamilton J. Trends in Immunol. 23, 403-408. (2002); Hamilton J., and Anderson G. Growth Factors. 22(4), 225-231. (2004); Bowne W.B., et al. Cytokines Cell Mol Ther. 5(4), 217-25. (1999); Dranoff, G. Nat. Rev. Cancer 4, 11-22 (2004); each herein incorporated by reference). As DCs arrive to the site of infection, they begin to differentiate and increase in phagocytic ability in response to the inflammation (Mellman I., and Steinman R.M. Cell. 106, 255-258. (2001), incorporated herein by reference). DCs that ingest bacteria or their products begin to process antigens, and DC maturation proceeds via endosomal TLR9 signaling stimulated by CpG dinucleotide sequences in bacterial DNA (Krieg A. M., Hartmann G., and Weiner G. J. CpG DNA: A potent signal for growth, activation, and maturation of human dendritic cells. Proc Natl Acad Sci USA. 16, 9305-93 10 (1999), incorporated herein by reference). Mature DCs then home to the lymph nodes where they prime antigen specific T-cell responses that clear infection.

CpG-ODNs are potent "danger signals" that upregulate DC expression of CCR7, CD80/86 costimulatory molecules, and MHC-antigen complexes. Importantly, TLR9 signaling induces DCs into promoting Thl-like, cytotoxic T cell responses by cytokine production (e.g., type I IFN) and cross-presentation of antigen onto MHCI molecules. The presentation of these signals concurrently with tumor antigens provides the danger signal needed to promote immune responses that effectively fight cancerous cells.

Different classes of CPG-ODNs promote different immune responses depending on the ODN’s specific structure and sequence. The ODN utilized in the present invention, CpG-ODN 1826, has been successfully tested in various mouse vaccination models, including melanoma. CpG-ODN 1826 has shown a beneficial effect alone or when used as adjuvant for peptide vaccines and whole cell vaccines. Moreover, ODN 1826 has been shown to directly promote DC maturation and cytokine production. This particular CpG ODN sequence also indirectly activates Thl cells and NK cells and, thus, enhances adaptive cellular immune responses.
Vector systems that promote CpG internalization into DCs to enhance delivery and its localization to TLR9 have been developed. The amine-rich polycation, polyethylenimine (PEI) has been extensively used to condense plasmid DNA, via association with DNA phosphate groups, resulting in small, positively charge condensates facilitating cell membrane association and DNA uptake into cells (Godbey W.T., Wu K.K., and Mikos, A.G. J. of Biomed Mater Res, 1999, 45, 268-275; Godbey W.T., Wu K.K., and Mikos, A.G. Proc Natl Acad Sci U S A. 96(9),5177-81. (1999); each herein incorporated by reference).

Consequently, PEI has been utilized as a non-viral vector to enhance gene transfection and to fabricate PEI-DNA loaded PLG matrices that promoted long-term gene expression in host cells in situ (Huang YC, Riddle F, Rice KG, and Mooney DJ. Hum Gene Ther. 5, 609-17. (2005), incorporated herein by reference). Therefore, CpG-ODNs were condensed with PEI molecules in the present invention. The PEI condensation enhances DC internalization of CpG-ODN, and the subsequent decondensation of PEI-CpG-ODN within DCs promotes DC activation (US 2013-0202707, incorporated herein by reference, e.g., at page 86, lines 1-7; and Figure 3).

To appropriately mimic infection and program cells in situ, the PLG system of the invention was designed to not only serve as a drug delivery device that releases inflammatory cytokines (e.g., GM-CSF), but also as a physical structure to which the DCs are recruited and reside while they are activated by danger signals (e.g., CpG-ODNs). The ability to control DC recruitment to and DC residence within porous PLG matrices is achieved using temporal control over the delivery of GM-CSF in situ, which results in batches of programmed DCs being dispersed only when GM-CSF levels were designed to subside in situ. For example, this system disperses at least 5 % (e.g., about 6%) of programmed DCs to the lymph nodes and induces protective anti-tumor immunity in at least 20% (e.g., about 23%) of mice when applied as a cancer vaccine. The cell programming and dispersement efficiency is improved using an overriding secondary signal (CpG-ODN) that continuously releases DCs from GM-CSF inhibition and promotes DC maturation and dispersement in the presence of high GM-CSF levels in situ. For example, PLG matrices were fabricated to locally present synthetic CpG-ODN with exogenous GM-CSF allowing for DCs recruited by GM-CSF to be stimulated by CpG-ODN in situ.

Dendritic Cells
Dendritic cells (DCs) are immune cells within the mammalian immune system and are derived from hematopoietic bone marrow progenitor cells. More specifically, dendritic cells can be categorized into lymphoid (or plasmacytoid) dendritic cell (pDC) and myeloid
dendritic cell (mDC) subdivisions having arisen from a lymphoid (or plasmacytoid) or myeloid precursor cell, respectively. From the progenitor cell, regardless of the progenitor cell type, an immature dendritic cell is born. Immature dendritic cells are characterized by high endocytic activity and low T-cell activation potential. Thus, immature dendritic cells constitutively sample their immediate surrounding environment for pathogens. Exemplary pathogens include, but are not limited to, a virus or a bacteria. Sampling is accomplished by pattern recognition receptors (PRRs) such as the toll-like receptors (TLRs). Dendritic cells activate and mature once a pathogen is recognized by a pattern recognition receptor, such as a toll-like receptor.

[0183] Mature dendritic cells not only phagocytose pathogens and break them down, but also, degrade their proteins, and present pieces of these proteins, also referred to as antigens, on their cell surfaces using MHC (Major Histocompatibility Complex) molecules (Classes I, II, and III). Mature dendritic cells also upregulate cell-surface receptors that serve as co-receptors for T-cell activation. Exemplary co-receptors include, but are not limited to, CD80, CD86, and CD40. Simultaneously, mature dendritic cells upregulate chemotactic receptors, such as CCR7, that allows the cell to migrate through the blood stream or the lymphatic system to the spleen or lymph node, respectively.

[0184] Dendritic cells are present in external tissues that are in contact with the external environment such as the skin (dendritic cells residing in skin are also referred to as Langerhans cells). Alternatively, dendritic cells are present in internal tissues that are in contact with the external environment such as linings of the nose, lungs, stomach, and intestines. Finally, immature dendritic cells reside in the blood stream. Once activated, dendritic cells from all off these tissues migrate to lymphoid tissues where they present antigens and interact with T cells and B cells to initiate an immune response. One signaling system of particular importance for the present invention involves the chemokine receptor CCR7 expressed on the surface of dendritic cells and the chemokine receptor ligand CCL19 secreted by lymph node structures to attract migrating mature dendritic cells toward high concentrations of immune cells. Exemplary immune cells activated by contact with mature dendritic cells include, but are not limited to, helper T cells, killer T cells, and B cells. Although multiple cell types within the immune system present antigens, including macrophages and B lymphocytes, dendritic cells are the most potent activators of all antigen-presenting cells.

[0185] Dendritic cells earned their name from the characteristic cell shape comprising multiple dendrites extending from the cell body. The functional benefit of this cell shape is a
significantly increased cell surface and contact area to the surroundings compared to the cell volume. Immature dendritic cells sometimes lack the characteristic dendrite formations and are referred to as veiled cells. Veiled cells possess large cytoplasmic veils rather than dendrites.

[0186] Plasmacytoid dendritic cells (pDCs) are innate immune cells that circulate in the blood and are found in peripheral lymphoid organs. They constitute < 0.4% of peripheral blood mononuclear cells (PBMC). In humans, these cells express the surface markers CD123, BDCA-2(CD303) and BDCA-4(CD304), but do not express high levels of CD11c or CD14, which distinguishes them from conventional dendritic cells or monocytes, respectively. Mouse pDC express CD11c, B220, BST-2 (mPDCA) and Siglec-H and are negative for CD1 lb. As components of the innate immune system, these cells express intracellular Toll-like receptors 7 and 9 which detect ssRNA and CpG DNA motifs, respectively. Upon stimulation and subsequent activation, these cells produce large amounts of type I interferon (mainly IFN-a (alpha) and IFN-β (beta)), which are critical pleiotropic anti-viral compounds mediating a wide range of effects. The CD8- subset presents antigen using the class II pathway to CD4+ helper T cells. The CD8+ subset presents antigens using the class I pathway. The peptide/MHC class I molecules are presented to CD8+ T cells which go on to become cytotoxic T lymphocytes (CTL). The CD8 cell surface protein in the mouse corresponds to the CD141 cell surface protein in the human. CD8/CD141-positive cells express TLR3 and are preferentially activated by TLR3 agonists.

Toll-like Receptors (TLRs)

[0187] TLRs are a class of single transmembrane domain, non-catalytic, receptors that recognize structurally conserved molecules referred to as pathogen-associated molecular patterns (PAMPs). PAMPs are present on microbes and are distinguishable from host molecules. TLRs are present in all vertebrates. Thirteen TLRs (referred to as TLRs1-13, consecutively) have been identified in humans and mice. Humans comprise TLRs 1-10.

[0188] TLRs and interleukin-1 (IL-1) receptors comprise a receptor superfamily the members of which all share a TIR domain (Toll-IL-1 receptor). TIR domains exist in three varieties with three distinct functions. TIR domains of subgroup 1 are present in receptors for interleukins produced by macrophages, monocytes, and dendritic cells. TIR domains of subgroup 2 are present in classical TLRs which bind directly or indirectly to molecules of microbial origin. TIR domains of subgroup 3 are present in cytosolic adaptor proteins that mediate signaling between proteins comprising TIR domains of subgroups 1 and 2.
[0199] TLR ligands comprise molecules that are constantly associated with and highly specific for a threat to the host’s survival such as a pathogen or cellular stress. TLR ligands are highly specific for pathogens and not the host. Exemplary pathogenic molecules include, but are not limited to, lipopolysaccharides (LPS), lipoproteins, lipoarabinomannan, flagellin, double-stranded RNA, and unmethylated CpG islands of DNA.

[0190] In one preferred embodiment of the present invention, the Toll-Like receptor 9 (TLR9) is activated by specific unmethylated CpG-containing sequences in bacterial DNA or synthetic oligonucleotides (ODNs) found in the endosomal compartment of dendritic cells. Methylation status of the CpG site is a crucial distinction between bacterial and mammalian DNA, as well as between normal and cancerous tissue. Unmethylated ODNs including one or more CpG motifs mimic the effects of bacterial DNA. Alternatively, or in addition, unmethylated ODNs including one or more CpG motifs occur within oncopgenes present within malignant tumor cells.

[0191] One or more sequences of the TLR-9 receptor recognizes one or more CpG-ODN sequences of the present invention. TLR-9 receptors encompassed by the present invention are described by the following sequences.

[0192] Human TLR-9, isoform A, is encoded by the following mRNA sequence (NCBI Accession No. NM_017442 and SEQ ID NO: 19; the start codon for all mRNA sequences presented herein is bolded and capitalized):

```
1  ggaggtctttg  ttccccqaag  atgttgcaag  gtctgtgtaa  aggcagagtc  agcctagcct
61  cctgtctcaa  gctaccagct  gccctccacg  catagggccc  tgcaagatcc  tggagatgtg
121  gctcaaaagg  gcagaaagg  acgaatgcgc  agccgctgtc  ctgagggcac  caagtgtgtg
181  gcagagccca  agacctggag  gtgaagaagt  cctcttagaag  tggaggagcc  ccaagacagt
241  gtaccgcgct  cttggtctat  ccaagaaaac  cattcctccc  tgtctctcgc  tggactgctcg
301  gcctctagct  cctcctcggg  cttgtctagag  cacagtgtgtg  aggcctcccct  gggatgtagc
361  cttgctcgtgaa  gggtagtgga  aaagagaggg  gttgaaaggg  ctgctcgccaa  tttgactatg
421  caaatgqgct  tgtacctcatg  gcagcctgtc  cttctctactg  ggggcaaggtg  ggaagggagg
481  gggagagact  aggtgctgat  aaaaatactta  cttctctctat  tctcctagccc  gctgtgcccc
541  cttggtgggag  gcagctcgag  tggtagaatc  cctctctcctg  aggtgctgcc  cagctgcccc
601  gcacagacctt  cttgagaagcc  cctggcccccc  cagctggtgtg  ttcctgcccca  ggcctgcccc
661  cccgctgtcct  cttcctggtgct  gcgctacatc  gttgcttcctg  acctctggcc  tggctccct
721  cctgctgcctc  ttaccctgtgc  agtcggccgc  ccacgccttg  tggtaactcga  actgigtgtt
781  cttgaccagtc  gtgcctcaacct  tttcatgggc  agcagccctgc  ggcgaatgtca  ccaagcccttgc
841  cttgtctctcc  aacgcatgatc  accaactctca  tggcatgctgtg  tttggcctcc  tggccagcct
901  gcgcagactcct  aacattcagtt  gcaagctgcc  ggcttgattg  ctcagccctca  gcaactctccc
961  cttgacccctg  accatcgagc  ccaagccattt  ttcgctgctg  cccacctgtg  aagagcttaaaa
1021  cttgcgactac  aacaacatca  tgactgtgcc  tggctgctccc  aatactctcca  tatccctgcgg
1081  cttcaagccat  acccaacatcc  tgaatgctagta  cttcgccagc  cttgcagggc  tcagtcctccc
1141  gctccctctata  cttctgtggc  caactggtta  ttacaaagttc  ccctcagccg  aggaactggag
1201  gttgcccgcgg  gttgctccccc  ttggcttggg  caaactccac  ccacgtgctac  tcaagatcaca
1261  caaacctctgct  gttgctgcccc  ccaacgtcgc  tttccagctgc  gagtaactcctg  tttgctccat
1321  caaacctcaact  cttgcctacgga  ccctctggact  ctgctccctg  tggctagtctg
1381  ctabgctgggg  gguatctgcc  gcgcctgctg  ccacgtctcct  aaccctccagttc  tggagctgccc
1441  tccgcctactc  cccagcaacgt  atctccgatac  tttggcctccc  cttgctcctg  tggagatgcc
1501  gttgtctgaag  cccagctactc  cttctctggtct  gcagtcggtgt  ggtgtgagaa
1561  cttccagagtg  cttggacctgt  gtgaagactt  cctctacaaa  tgcattacta  aaaccaaggcg
1621  cttccagaggc  cttaacacagc  agcctagcct  ttcaactgcc  tcaacttacct  aaaaaggggt
```
Human TLR-9, isoform A, is encoded by the following amino acid sequence (NCBI Accession No. NP_059138 and SEQ ID NO: 20):

MGFCRSLAHPLSLVQAIIAMLTRANLTLAPFLPCELQPHGLVLNCNWLFLKSYSVFHMAAPRN
GVTSLSNRSRJNHLDHDSFAHPLSRHLNLKWNPPLVGLSPMHPCHMTIPSTFLAVPTLE
ELNLSSYNNITVAPALKLSLSSLHTNLMLDSASLALHRLFMGDNCYYKNPCRQALE
APGALLGLNLTHSLKNMLTVVPRLNPLSSYLLSISYNTRLAPLEDNLTRLVLDYG
NCRRCDHAPNPCMECPRHFQPQLHQIDTFSHLRSLEGVLKDSSLWSWASFRGLGLNLRLVDLS
ENFLYCKITKTAKFQTLRLKNLSFNYKRKSIVAFHSLAPSFGSLVAKLEDMDHIGFRSLD
ETTLRPLRNLMLQTLRLQMNFINAQGLIGIFRAPFGLYRVDLSNDRISGASELTATMGEAEDGG
EKVWLQPGDAPVPYDPSSEDFRPNCTLNFLLDMSNLVTVQPEMFAQLHSLQCLRSHN
CISQAVNQSVQFLPTLGQLVSDLHNKLDLYHESFTPLERLAPLNYLDSNPQFPGMGVHNFNS
VFAHRVLRLHLSSLHNNHSQVVQOLCSTSLRALDFSGNALGHMWAEGDLYHLIFQGQLGSLI
WLDLSNLQRLTHTLPQTLNLRQPLVRLRNLYAFFKWSLHFLPKEVLDDLGNQLKALT
NGSLPAGTRLLRLDVCNSISFVAPGFFSAKELRELNLXANLKTVDHSWGPGLASALQILDV
SANPLHACGCAFMDLFLEVQAAYPLPSRVCkgSPQQLQGLSIFnQDLRLCDEALSQDCF
ALSLLAVGLGVMDHLCGWDLWYCFHLCLAWLPWRGRQSMEDALPYDAFVFDK7

(SEQ ID NO: 19)
QSAVADWVYNELRGQLEERCRGWLCEERDLWARELPGKTFLPWLSVYGSRKTLFVLAHT
DRVSSLRLRSLAQQRILLERDKVDVVLISLPDGRSRSYVRLRQLRCSVLLLLWHQPSGQR
SFWAQLGMLALTDDNHHFYNRFNCQGPTAE (SEQ ID NO: 20)

[0194] Human TLR3 is encoded by the following mRNA sequence (GenBank Accession No. NM_003265.2 (01:19718735), incorporated herein by reference; SEQ ID NO: 21):

```
1 cacttttcgag agttgctgctt atttgccaca cactttccctg atgaaatgtc tggattttgga
61 ctaaagaaaa aaggaagagc tagcagctcat ccaacagaaact cATGacacag actttgtgccct
121 gtatctactt ttgggggggc ctttttgccct ttgggtattgt gttgctcacc tccacccacca
181 agtctcctgt tagcagctcat ttggctgtact gcagccacact gaaggtctact caggttacccgt
241 atgatctcacc ccaaaaacat caagttgtga accttacccca taataacact caagaagattc
301 cagccgcaca cttccacaagg tattagcgcg ctaactgctgt cattagcgga tttacccaca
361 ttcacaacact ggagccgaag atttgtcagag aacctcccatg attaacaagtt ttgacccct
421 agcacagctga gttacttcca atttgcattg cagaaaaagtc aacttcccatg cagaaaaattt
gtctcctatat ttgtctctaaag ggctgactagg ggccacccct tacaaatcgaagagtggctt
581 cagtgccgcat gtttcagcgc atttgggtctc taacctgata cagccagcctg cggcagtc
641 aactttgtgg actaaattgg ccaatccata cttacgtcct ctcttcttac aagacaagagttt
gtttcagctac atgaaatgtc cagctccattg cccttctcttg acatctttcg cagactttcc
701 atataaatt acacgttctt cttctccac tttccacact ccaacagaaact cACGtggatcc
761 tgaatattgaa atttgggtccg ccataacagttt cttcagccct ccaacagaaact cACGtggatcc
821 atgatctcacc ccaaaaacat caagttgtga accttacccca taataacact caagaagattc
881 cagccgcaca cttccacaagg tattagcgcg ctaactgctgt cattagcgga tttacccaca
941 ttcacaacact ggagccgaag atttgtcagag aacctcccatg attaacaagtt ttgacccct
1001 agtctcctgt tagcagctcat ttggctgtact gcagccacact gaaggtctact caggttacccgt
1061 gatgctgcctttcctctctctc gccctcgatattcttctttcctggt gttgcttcagtttctctttt
1121 ttcacaacact ggagccgaag atttgtcagag aacctcccatg attaacaagtt ttgacccct
1181 atgatctcacc ccaaaaacat caagttgtga accttacccca taataacact caagaagattc
1241 cagccgcaca cttccacaagg tattagcgcg ctaactgctgt cattagcgga tttacccaca
1301 ttcacaacact ggagccgaag atttgtcagag aacctcccatg attaacaagtt ttgacccct
1361 aactttgtgg actaaattgg ccaatccata cttacgtcct ctcttcttac aagacaagagttt
gtttcagctac atgaaatgtc cagctccattg cccttctcttg acatctttcg cagactttcc
1421 ttatagctttttctctgtctttagttgatggcattgcattgctattgcattgcattgctattgcattg
1481 cagtgccgcat gtttcagcgc atttgggtctc taacctgata cagccagcctg cggcagtc
1541 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
1601 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
1661 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
1721 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
1781 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
1841 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
1901 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
1961 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2021 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2081 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2141 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2201 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2261 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2321 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2381 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2441 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2501 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2561 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2621 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2681 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2741 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2801 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2861 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
2921 cactttgtcg agtcttgcctgcatta acatcaat ctttttctctcttctctctcttctctctttctct
...```
Human TLR3 is encoded by the following amino acid sequence (GenBank Accession No. ABC86910.1 (GI:86161330), incorporated herein by reference; SEID No: 22):

1 mrtqtlpcyf wggllpfglm casatstktcv shevadcshl kltqvpddlp tnttvnlnth
61 nqlrlrlpaan ftrotsqtsl dvqfntskl epelcqlkpm lvkvnqlghne lsqladktfa
121 fctntelhl msnsigikln npfvkqknli tldisnhngls stklqgtqvl enlqelllan
181 nkigalksee ldifanslkh kleiasqnik efspgcffai grlfgflinn vqglpsitek
241 lclelantsi rlrlsnsqsl ststntflgl kwnlntmlsl lsmynlnvvg ndsfawlpql
301 eyffleynni qhlfshahlg ifnvrlnlk rfttkqsisl asplkiddfs fqwklczehl
361 nmmednpqi ksmftglin kylsasnf tsarltnet vslsahspil iniitknkis
421 kiesdafswl ghlevldlgi neigqeltgq ewrglenife iylysnyklyq ltrnsfalvlp
481 slqrmlrrv alknvdsapsp pfqpilrlnti ldlanvnian indmdlqgole kleidigqhn
541 nlariwkhan pgppiyflkg lshhihnlne snfdeipve vflkflfelki idlglnnint
601 lpasyvnlngv slklknlgkhn lntsvekkvf gpafrntel dmrntqcdct cesiafsvn
661 inethtnipe lasylcntnp phygqfpvrl ftsttcckdaa peflfmmint sillififiv
721 lllhfegwri sfywnsvshr vlgkfeirdq tegfeyayai ihaykddkwv wehrsmsmeke
781 dqgilkfclee rdfeaqvfl elainvksiks rkiifvithh lkkdplckfr kvhnavvqai
841 eqnldsiivl fleeipdykl nhalolrgm fkskhcinwp vqkergafr hklqvalgsk
901 nsvh (SEQ ID NO: 22)

The nucleic acid sequence of human TLR1 is provided in GenBank Accession No. NM_003263.3 (GI:41350336), incorporated herein by reference. The amino acid sequence of human TLR1 is provided in GenBank Accession No. NP_003254.2 (GI:41350337), incorporated herein by reference.

The nucleic acid sequence of human TLR2 is provided in GenBank Accession No. NM_003264.3 (GI:68160956), incorporated herein by reference. The amino acid sequence of human TLR2 is provided in GenBank Accession No. NP_003255.2 (GI:19718734), incorporated herein by reference.

The nucleic acid sequence of human TLR4 is provided in GenBank Accession No. NM_138554.4 (01:37342600), incorporated herein by reference. The amino acid sequence of human TLR4 is provided in GenBank Accession No. NP_612564.1 (01:19924149), incorporated herein by reference.

The nucleic acid sequence of human TLR5 is provided in GenBank Accession No. NM_003268.5 (01:281427130), incorporated herein by reference. The amino acid sequence of human TLR5 is provided in GenBank Accession No. NP_003259.2 (01:16751843), incorporated herein by reference.

The nucleic acid sequence of human TLR6 is provided in GenBank Accession No. NM_006068.4 (01:318067953), incorporated herein by reference. The amino acid sequence of human TLR6 is provided in GenBank Accession No. NP_006059.2 (01:20143971), incorporated herein by reference.
[0201] The nucleic acid sequence of human TLR7 is provided in GenBank Accession No. NM_016562.3 (GI:67944638), incorporated herein by reference. The amino acid sequence of human TLR7 is provided in GenBank Accession No. NP_057646.1 (GI:7706093), incorporated herein by reference.

[0202] The nucleic acid sequence of human TLR8 is provided in GenBank Accession No. NM_138636.4 (GI:257196253), incorporated herein by reference. The amino acid sequence of human TLR8 is provided in GenBank Accession No. NP_619542.1 (GI:20302168), incorporated herein by reference.

[0203] The nucleic acid sequence of human TLR10 is provided in GenBank Accession No. NM_030956.3 (GI:306140488), incorporated herein by reference. The amino acid sequence of human TLR10 is provided in GenBank Accession No. NP_112218.2 (GI:62865618), incorporated herein by reference.

[0204] The nucleic acid sequence of mouse TLR11 is provided in GenBank Accession No. NM_205819.3 (01:408684412), incorporated herein by reference. The amino acid sequence of mouse TLR11 is provided in GenBank Accession No. NP_991388.2 (01:408684413), incorporated herein by reference.

[0205] The nucleic acid sequence of mouse TLR12 is provided in GenBank Accession No. NM_205823.2 (GI:148539900), incorporated herein by reference. The amino acid sequence of mouse TLR12 is provided in GenBank Accession No. NP_991392.1 (01:45430001), incorporated herein by reference.

[0206] The nucleic acid sequence of mouse TLR13 is provided in GenBank Accession No. NM_205820.1 (01:45429998), incorporated herein by reference. The amino acid sequence of mouse TLR13 is provided in GenBank Accession No. NP_991389.1 (01:45429999), incorporated herein by reference.

[0207] A representative list of TLR agonists (both synthetic and natural ligands), along with their corresponding receptor is provided in the table below.
**Granulocyte Macrophage Colony Stimulating Factor (GM-CSF)**

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a protein secreted by macrophages, T cells, mast cells, endothelial cells and fibroblasts. Specifically, GM-CSF...
is a cytokine that functions as a white blood cell growth factor. GM-CSF stimulates stem cells to produce granulocytes and monocytes. Monocytes exit the blood stream, migrate into tissue, and subsequently mature into macrophages.

[0209] Scaffold devices described herein comprise and release GM-CSF polypeptides to attract host DCs to the device. Contemplated GM-CSF polypeptides are isolated from endogenous sources or synthesized in vivo or in vitro. Endogenous GM-CSF polypeptides are isolated from healthy human tissue. Synthetic GM-CSF polypeptides are synthesized in vivo following transfection or transformation of template DNA into a host organism or cell, e.g. a mammal or cultured human cell line. Alternatively, synthetic GM-CSF polypeptides are synthesized in vitro by polymerase chain reaction (PCR) or other art-recognized methods Sambrook, J., Fritsch, E.F., and Maniatis, T., Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, NY, Vol. 1, 2, 3 (1989), herein incorporated by reference.

[0210] GM-CSF polypeptides are modified to increase protein stability in vivo. Alternatively, GM-CSF polypeptides are engineered to be more or less immunogenic. Endogenous mature human GM-CSF polypeptides are glycosylated, reportedly, at amino acid residues 23 (leucine), 27 (asparagine), and 39 (glutamic acid) (see US Patent No. 5,073,627). GM-CSF polypeptides of the present invention are modified at one or more of these amino acid residues with respect to glycosylation state.

[0211] GM-CSF polypeptides are recombinant. Alternatively GM-CSF polypeptides are humanized derivatives of mammalian GM-CSF polypeptides. Exemplary mammalian species from which GM-CSF polypeptides are derived include, but are not limited to, mouse, rat, hamster, guinea pig, ferret, cat, dog, monkey, or primate. In a preferred embodiment, GM-CSF is a recombinant human protein (PeproTech, Catalog # 300-03). Alternatively, GM-CSF is a recombinant murine (mouse) protein (PeproTech, Catalog #315-03). Finally, GM-CSF is a humanized derivative of a recombinant mouse protein.

[0212] Human Recombinant GM-CSF (PeproTech, Catalog # 300-03) is encoded by the following polypeptide sequence (SEQ ID NO: 24):

MAPARSPSPS TQPWEHVNAI QEARRLLNLS RDTAAEMNET VEVISEMFDL QEPTCLQTRL ELYKQGLRGS LTKLKGPLTMASHYKQHCP PTPETSCATQ HTFESFKEN LKDFLLVIPF DCWEPVQE (SEQ ID NO: 24)

[0213] Murine Recombinant GM-CSF (PeproTech, Catalog # 315-03) is encoded by the following polypeptide sequence (SEQ ID NO: 25):

MAPTRSPITV TRPWKHEVAI KEALNLLDDM PVTLNREEVE VSNESFSDKL TVQTRKLIF EQGLRGNFTK LKGALNMTAS YYQTYCPTPT ETDCEQVT TT YADFIDSLKT FLTDIPFECK KPQK (SEQ ID NO: 25)
Human Endogenous GM-CSF is encoded by the following mRNA sequence (NCBI Accession No. NM_000758 and SEQ ID NO: 26):

```
1 acacagagag aagagctaaa gttgtcttga ggtatgttgc gcagagctcg ctgctcttgg
121 ggacagtctg ctaggccccct cccgctcgcg ccagccccag acgcagccct
241 cctgcttaca gaccgctctg gaagctgata agcaggcrcg cggagggcag cttacacagc
361 tcaagggcct cttgaccactg atggccagcc actacaagcc gcactgccct ccaaccccgg
481 aaactttcttg tgcacaaccct attatacctt ttgaaagttt ccaaggaagc ctaggagact
601 tcatttggag ggaccaaggg tggggcacac gcctgagctc gtagaagtca gtagaagtca
721 catatttcat atttttcatt ttatatatttt aaaaatatatt tattatatatt tatattaattt
781 a (SEQ ID NO: 26)
```

Human Endogenous GM-CSF is encoded by the following amino acid sequence (NCBI Accession No. NP_000749.2 and SEQ ID NO: 27):

```
MWLQSSLLELTVACSISAPARSPSPSTQPWEHVAIQEARRLLNLSDTAAEMNETVEVISEMF DLQEP
TCLQTRLELYQGQLRSLTKGTGKLMMASHYQKQHCPPTPETSCATQHTFESFKNLKDFLLVPPDCW
EPVQE (SEQ ID NO: 27)
```

GM-CSF signaling is a potent chemotactic factor for conventional DCs and significantly enhanced surface expression of MHC(II) and CD86(+), which are utilized for priming T cell immunity. In contrast, Flt3L vaccines led to greater numbers of plasmacytoid DCs (pDCs), correlating with increased levels of T cell priming cytokines that amplify T cell responses. Thus, as described in US 2013-0202707, incorporated herein by reference, 3D polymer matrices modified to present inflammatory cytokines are utilized to effectively mobilize and activate different DC subsets in vivo for immunotherapy.

An exemplary amino acid sequence of human Flt3 is provided below (GenBank Accession No.: P49771.1 (GI:1706818), incorporated herein by reference; SEQ ID NO: 28):

```
1 mtvlapawsp ttyllllllll ssglsqtqcd sfgqspisss sfavkreisd yllqdpvttv
61 asnlqdeelc gglwlrlvaq rwmerlkvta gskmqgiller vnteihftvk cagpqppsc1
121 rfcqtnrisl lqetseqlva lkpwitrqnf srclslqcpq dstllppws prpleatapt
181 apqppli1ll 1lpvgl11la aawclhwqrt rrrprrpgcq vppvpspql1 llveh (SEQ ID NO: 28)
```

Cytosine-Guanosine (CpG) Oligonucleotide (CpG-ODN) Sequences

CpG sites are regions of deoxyribonucleic acid (DNA) where a cysteine nucleotide occurs next to a guanine nucleotide in the linear sequence of bases along its length (the "p" represents the phosphate linkage between them and distinguishes them from a cytosine-guanine complementary base pairing). CpG sites play a pivotal role in DNA methylation, which is one of several endogenous mechanisms cells use to silence gene expression.
Methylation of CpG sites within promoter elements can lead to gene silencing. In the case of cancer, it is known that tumor suppressor genes are often silenced while oncogenes, or cancer-inducing genes, are expressed. Importantly, CpG sites in the promoter regions of tumor suppressor genes (which prevent cancer formation) have been shown to be methylated while CpG sites in the promoter regions of oncogenes are hypomethylated or unmethylated in certain cancers. The TLR-9 receptor binds unmethylated CpG sites in DNA.

[0219] The present invention comprises CpG dinucleotides and oligonucleotides. Contemplated CpG oligonucleotides are isolated from endogenous sources or synthesized in vivo or in vitro. Exemplary sources of endogenous CpG oligonucleotides include, but are not limited to, microorganisms, bacteria, fungi, protozoa, viruses, molds, or parasites. Alternatively, endogenous CpG oligonucleotides are isolated from mammalian benign or malignant neoplastic tumors. Synthetic CpG oligonucleotides are synthesized in vivo following transfection or transformation of template DNA into a host organism.


[0220] CpG oligonucleotides are presented for cellular uptake by dendritic cells. In one embodiment, naked CpG oligonucleotides are used. The term "naked" is used to describe an isolated endogenous or synthetic polynucleotide (or oligonucleotide) that is free of additional substituents. In another embodiment, CpG oligonucleotides are bound to one or more compounds to increase the efficiency of cellular uptake. Alternatively, or in addition, CpG oligonucleotides are bound to one or more compounds to increase the stability of the oligonucleotide within the scaffold and/or dendritic cell.

[0221] CpG oligonucleotides are condensed prior to cellular uptake. In one preferred embodiment, CpG oligonucleotides are condensed using polyethylimine (PEI), a cationic polymer that increases the efficiency of cellular uptake into dendritic cells.

[0222] CpG oligonucleotides of the present invention can be divided into multiple classes. For example, exemplary CpG-ODNs encompassed by compositions, methods and devices of the present invention are stimulatory, neutral, or suppressive. The term "stimulatory" used herein is meant to describe a class of CpG-ODN sequences that activate TLR9. The term "neutral" used herein is meant to describe a class of CpG-ODN sequences that do not activate TLR9. The term "suppressive" used herein is meant to describe a class of CpG-ODN
sequences that inhibit TLR9. The term "activate TLR9" describes a process by which TLR9 initiates intracellular signaling.

[0223] Simulatoy CpG-ODNs can further be divided into three types A, B and C, which differ in their immune-stimulatory activities. Type A stimulatory CpG ODNs are characterized by a phosphodiester central CpG-containing palindromic motif and a phosphorothioate 3’ poly-G string. Following activation of TLR9, these CpG ODNs induce high IFN-a production from plasmacytoid dendritic cells (pDC). Type A CpG ODNs weakly stimulate TLR9-dependent NF-κB signaling.

[0224] Type B stimulatory CpG ODNs contain a full phosphorothioate backbone with one or more CpG dinucleotides. Following TLR9 activation, these CpG-ODNs strongly activate B cells. In contrast to Type A Cpg-ODNs, Type B CpG-ODNS weakly stimulate IFN-a secretion.

[0225] Type C stimulatory CpG ODNs comprise features of Types A and B. Type C CpG-ODNs contain a complete phosphorothioate backbone and a CpG containing palindromic motif. Similar to Type A CpG ODNs, Type C CpG ODNs induce strong IFN-a production from pDC. Similar to Type B CpG ODNs, Type C CpG ODNs induce strong B cell stimulation.

[0226] Exemplary stimulatory CpG ODNs comprise, but are not limited to, ODN 1585, ODN 1668, ODN 1826, ODN 2006, ODN 2006-G5, ODN 2216, ODN 2336, ODN 2395, ODN M362 (all InvivoGen). The present invention also encompasses any humanized version of the preceding CpG ODNs. In one preferred embodiment, compositions, methods, and devices of the present invention comprise ODN 1826 (the sequence of which from 5’ to 3’ is tccatgacgttcctgacgtt, wherein CpG elements are bolded, SEQ ID NO: 29).

[0227] Neutral, or control, CpG ODNs that do not stimulate TLR9 are encompassed by the present invention. These ODNs comprise the same sequence as their stimulatory counterparts but contain GpC dinucleotides in place of CpG dinucleotides.

[0228] Exemplary neutral, or control, CpG ODNs encompassed by the present invention comprise, but are not limited to, ODN 1585 control, ODN 1668 control, ODN 1826 control, ODN 2006 control, ODN 2216 control, ODN 2336 control, ODN 2395 control, ODN M362 control (all InvivoGen). The present invention also encompasses any humanized version of the preceding CpG ODNs.

[0229] Suppressive CpG ODNs that inhibit TLR9 are encompassed by the present invention. Exemplary potent inhibitory sequences are (TTAGGG), (SEQ ID NO: 30) (oligonucleotide TTAGGG, InvivoGen), found in mammalian telomeres and ODN 2088 (InvivoGen), derived
from a murine stimulatory CpG ODN by replacement of 3 bases. Suppressive ODNs disrupt the colocalization of CpG ODNs with TLR9 in endosomal vesicles without affecting cellular binding and uptake. Suppressive CpG ODNs encompassed by the present invention are used to fine-tune, attenuate, reverse, or oppose the action of a stimulatory CpG-ODN. Alternatively, or in addition, compositions, methods, or devices of the present invention comprising suppressive CpG ODNs are used to treat autoimmune conditions or prevent immune responses following transplant procedures.

Cancer Antigens

[0230] Compositions, methods, and devices of the present invention comprise cancer antigens with means to vaccinate and/or provide protective immunity to a subject to whom such a device was administered. Cancer antigens are used alone or in combination with GM-CSF, CpG-ODN sequences, or immunomodulators. Moreover, cancer antigens are used simultaneously or sequentially with GM-CSF, CpG-ODN sequences, or immunomodulators.

[0231] Exemplary cancer antigens encompassed by the compositions, methods, and devices of the present invention include, but are not limited to, tumor lysates extracted from biopsies (e.g., from melanoma tumor biopsies, or from B16-F10 tumors isolated from mice challenged with B16-F10 melanoma tumor cells), irradiated tumor cells (e.g., irradiated melanoma cells), antigens from lung cancer, antigens from breast cancers (e.g., Her2, e.g., purified Her2 or a fragment thereof), antigens from glioma cancers, prostate (e.g., prostate cancer) antigens (e.g., prostatic acid phosphatase), MAGE series of antigens (MAGE-1 is an example), MART-1/melanA, tyrosinase, ganglioside, gp100, GD-2, O-acetylated GD-3, GM-2, MUC-1, Sos1, Protein kinase C-binding protein, Reverse transcriptase protein, AKAP protein, VRK1, KIAA1735, T7-1, T11-3, T11-9, Homo Sapiens telomerase ferment (hTRT), Cytokeratin-19 (CYFRA21-1), SQUAMOUS CELL CARCINOMA ANTIGEN 1 (SCCA-1), (PROTEIN T4-A), SQUAMOUS CELL CARCINOMA ANTIGEN 2 (SCCA-2), Ovarian carcinoma antigen CA125 (1A1-3B) (KIAA0049), MUCIN 1 (TUMOR-ASSOCIATED MUCIN), (CARCINOMA-ASSOCIATED MUCIN), (POLYMORPHIC EPITHELIAL MUCIN),(PEM),(PEMT),(EPISIALIN), (TUMOR-ASSOCIATED EPITHELIAL MEMBRANE ANTIGEN), (EMA), (H23AG), (PEANUT-REACTIVE URINARY MUCIN), (PUM), (BREAST CARCINOMA-ASSOCIATED ANTIGEN DF3), CTCL tumor antigen sel-1, CTCL tumor antigen sel4-3, CTCL tumor antigen se20-4, CTCL tumor antigen se20-9, CTCL tumor antigen se33-1, CTCL tumor antigen se37-2, CTCL tumor antigen se57-1, CTCL tumor antigen se89-1, Prostate-specific membrane antigen, 5T4 oncofetal trophoblast glycoprotein, Orf73 Kaposi’s sarcoma-associated herpesvirus, MAGE-C1 (cancer/testis
antigen CT7), MAGE-B1 ANTIGEN (MAGE-XP ANTIGEN) (DAM10), MAGE-B2 ANTIGEN (DAM6), MAGE-2 ANTIGEN, MAGE-4a antigen, MAGE-4b antigen, Colon cancer antigen NY-CO-45, Lung cancer antigen NY-LU-12 variant A, Cancer associated surface antigen, Adenocarcinoma antigen ART1, Paraneoplastic associated brain-testis-cancer antigen (onconeuronal antigen MA2; paraneoplastic neuronal antigen), Neuro-oncological ventral antigen 2 (NOVA2), Hepatocellular carcinoma antigen gene 520, TUMOR-ASSOCIATED ANTIGEN CO-029, Tumor-associated antigen MAGE-X2, Synovial sarcoma, X breakpoint 2, Squamous cell carcinoma antigen recognized by T cell, Serologically defined colon cancer antigen 1, Serologically defined breast cancer antigen NY-BR-15, Serologically defined breast cancer antigen NY-BR-16, Chromogranin A; parathyroid secretory protein 1, DUPAN-2, CA 19-9, CA 72-4, CA 195, Carcinoembryonic antigen (CEA).

\[0232\] The amino acid sequence of human prostatic acid phosphatase is provided by Genbank Accession No. AAA60022.1, and is shown below (SEQ ID NO: 31), with the signal peptide shown in underlined font and the mature peptide shown in italicized font.

```
1 mraaplllar asialalascf ccfcwldrsv lakelkftvl vfrhgdrspl dtftpdpike
61 sswwqgffgql tlqgmeqhy lgeyikrky kfldnsyke qvyirstdvd rtlmsrntnl
121 aallppepsv iwnnpllwgp ipvhtvpisae dlqlylpfrn cprfgelese tikseefqkr
181 lhykdfiat lgklsglhhg dlfgiwsyv dplysesvhn flipswated tmtklreise
241 lsllslyih kqkeksrlqg gvlvneilnh mkratqipsy kklimysahd ttvtgqlmal
301 dvyngllppy aschitletyf egkvefymeny ynetqhepy plmlpgcps cplerfaelv
361 gpvipqdwdst evmtnshqg testd (SEQ ID NO: 31)
```

The mRNA sequence encoding human prostatic acid phosphatase is provided by Genbank Accession No. M24902.1, and is shown below (SEQ ID NO: 32), with the start and stop codons in bold.

```
1 gcggccagaaac agctctcttc aacatgagag cttgacccct cctctctggcc agggcagcaa
61 gccttgccttg ggttcttttt ttggttgtgt atctcagcag attggctgcag ctttgcaaaac
121 aggaagtgga aattggtgca cttgccctcgag gcatgctctg gccaatcagca ggtctgatag
181 ttcactctga cccccataag gatactctat gggccacaag attggctgcag ctttgcaaaac
241 ttggtctggga gacagctatt gactctggag gagttataa gcggccagaa ctttgcaaaac
301 sgatgagctc ctataaacat gaagctgttt cctggagctgtt cctgcagcag ctttgcaaaac
361 tgaatgtagc tctgagcttt gatgagcttt gaagctgttt cctgcagcag ctttgcaaaac
421 atcttctcttc acctcrtgca cccctcccgcg tggccacagt tctcctcttc cttggctttta
481 tgcctatcct gcctcctcag aacgtcgctct gtttccaaga actggatag tagctgttag
541 gattcgagaa gttccgaaga aggctgcccc tttataagga ttctataaggg accttgggaa
601 aatctcctcag aatctcctcag gctgtgtctc aggttaagctt taatagactt aatctcctcag
661 tatattctag gaagttgatat cttgaccaag ggtctgatag ggtctgatag ggtctgatag
721 ctagagtatg gatttgtcga gattgtcgtc cttgtcgtcag gtttctgttgc cttgtcgtcag
781 agagaagaaat ctagctcagag cgggtgtgcc ggtgctgctg actctcctcag ggtctgatag
841 gagctaacctca gttatccagcc tccaaagaaa tatttcgtcttg tttggtgcct ggtctgatag
901 tgggtctggcc cagaggtgct ggttctgttgc cttggtctcttg ctttgcctct ctttgcctct
961 gcggccagaa cggagttgctc ctttgagagg ggggtgtctc tgggtctgatag tgggtctgatag
1021 atgagacgca gcggccagaa cttgctcagag ggtctgatag ggtctgatag ggtctgatag
```

-59-
The amino acid sequence of human Her2 is provided by Genbank Accession No.

P04626.1, and is shown below (SEQ ID NO: 33).

```
[0233]  1 melaalcrwg lallalppga astqvtcgtd mkdlrlpaspe thdlmlrhly qgcqgqvgqgnl  
  61 eltylptnas lsflqdiqev qgyvliahnq vrqvpqlqrl irvgtqfled nyalavlxdng  
 121 dplnnttpwt gaspggqreil qslrtesilq ggvlqirnpq lcyqtdilwq difhknqla  
 181 lltldtnrnr aqpcapcmk gsrcgweese dqcalrttrvc aggacckgqp lptdccheqg  
 241 aacgqckqks dzlcshfn aqigcelpa ltylntdflte qmpmqnpyt fgascvtacp  
 301 ynlstdvgws clvpvgqemt eavtqedtq clckskpcar vcyglmgqnl revravtsa  
 361 igefagckki gflhflplues fgdpasanta plqegpqqev eletiegtly yisawpdslp  
 421 disqvnqivk irgrihngs yslrlqmqtt gqlgqhqlqgq sqhglhglq nfhcfevhv  
 481 pwqdlrfrph qallhtanrp edecvqegia qhclqarchq wgpqptqcvn caqfrqgeq  
 541 veevrllqlq lpvynarhgc lpcpchpqegq qngvctfqug lpdqgcvlpqa qmrilketel  
 601 psypvikdlay mpikwpdfepe gacgcppcinh thscvdlddk gqcaqrpasap ltsiisavvg  
 661 illyvlgvgv fiqlrrlqirk ykytmrlqll qetlevpfl tspagnqna qmrilketel  
 721 rkvkgisiqga dfgqyqgivi qdpgenkipv alkvrents qkaneilide ayvmqgvp  
 781 yvrslligcic tsvsqtvqtrq pmpclfslldq renrrgqsq dlnwcmqlia kmayledv  
 841 lhvrilardn vlkvqsklnh mtidflqarlq dideteyhdg ggkvkpkwma lessrffr  
 901 hqsdwsvygv ftwvtmgfa kpydipare ipdileker ipcppidct vymirvkwra  
 961 idsecrpfr eulsefrma rdqrfqvqvlq nedigpqsl dstfylsirldldmgdldva  
1021 eeyeivqqqf cfcpdpagqag gmhvhrhrass stsgsrqldgq iglepsesea praplapase  
1081 agsdfvdgqdl gmgakqlqgs lpthsdpgqpl psedgypvap ltcagpgeyv
1141 ngpdvrrpqqq spregilpaa rpqastlerp ktlspgknnv kvdusqfga vneylpqtpq  
1201 ggagagqggttgctgtacgt gttggctcct tgactccctca agactggctc acggaggtta
```

ID NO: 33)
The mRNA sequence encoding human Her2 is provided by Genbank Accession No. NM_004448.3, and is shown below (SEQ ID: 34), with the start and stop codons in bold.

```
gcttgctcccc aatcacagga aaggagagag tgtgagggac aggctgctct g6.ggaagitatta
1
aagagtgaag tgtgtagauct gaggattcccc tcctattggga cggagggaaac caggaggaccc
61
ccccgggacag cccgggccc cttccacctac ggcccttctcc tgccctgccg gcccgggccc
121
cacccctggc acgcaccccg gcgccccgcct cttccagcgc ggtcctcaggg gacccatggg
181
cggcgacgcag cagtgacatc gctgaggagct gcggcagaggat gccgctctcc gcctgcctccct
241
gcccctgctt ccagctcggc gacaggccag caagttgcag caggagaggt gaccaagttctct
301
gggtctccttg ccagttcggc gaaggcctgcc gactgcttga gcaaccctg gcccctctctt cagagggctgc
361
tcatgcctgtc agggtagctg cagcagggtt aacataacat ccaccctgcag agggccctcc
421
cagcggcttgc aggaaactct gaaacctctg tctggctuct ccagtgcctcc ccaagctgcct cctgcctccct
481
tatcagacag cggagccgct gctgacctct ctgctggtga tgtgcggaca gttggagggcc
541
tcgcacgggtg aagatggtcag gcgctgctgt ggtcctcctg gccctctctc cagctgctcct
601
tcgccctgag cagacccgag caccctcgct gcctgcctcc ggtgggggacc gcctgctgccgc
661
```

The mRNA sequence encoding human Her2 is provided by Genbank Accession No. NM_004448.3, and is shown below (SEQ ID: 34), with the start and stop codons in bold.
In some embodiments, tumor antigens are classified into 4 major groups based on their expression profile. See van der Bruggen P, Stroobant V, Vigneron N, Van den Eynde B. Peptide database: T cell-defined tumor antigens. Cancer Immun 2013. URL: www.cancerimmunity.org/peptide/, incorporated herein by reference. Exemplary tumor antigens and their classification are summarized below as follows:

1) Unique Antigens: Unique to tumor cells (Table 1). These antigens arise from mutations in the gene that encodes the protein antigen. Commonly, the mutation(s) affects the coding sequence of the gene. In some examples, the mutation(s) are unique to the tumor of an individual subject or a small number of subjects. These unique antigens are generally not shared by tumors from different subjects.

2) Shared Antigens: Tumor specific antigens (Table 2). Shared antigens, unlike unique antigens, are expressed in multiple independent tumors. Tumor specific antigens are expressed in multiple tumors but not in normal cells. For example, these antigens are encoded by "cancer-germline" genes.

3) Shared Antigens: Differentiation antigens (Table 3). Differentiation antigens are expressed in the tumor as well as in the normal tissue from which the tumor originated. For example, these antigens are expressed in a particular lineage of cells during a developmental stage. Since these antigens are not tumor-specific, targeting these antigens for cancer immunotherapy may cause autoimmunity toward the corresponding normal tissue, depending on whether the normal tissue is dispensable and whether the tissue expressing the antigen is surgically removed during the course of cancer treatment.
4) Shared Antigens: Overexpressed antigens (Table 4). These antigens are expressed in
a variety of normal tissues and are overexpressed in tumor cells.

For example, tables of tumor peptides, e.g., considered to be tumor antigens based on their
recognition by T lymphocytes that also recognize tumor cells expressing the parent proteins.
Each table below includes the protein or gene name, GeneCard information about the
protein/gene, a peptide sequence from the protein (e.g., a minimum sequence for antigen
specificity, e.g., recognized by T cells), and the position of the peptide in the full length
protein sequence. In some examples, the peptide shown in the tables below is a human
leukocyte antigen (HLA) presenting molecule, e.g., the peptide is presented onto a major
histocompatibility complex (MHC) molecule. In Table 1, the underlined amino acid(s) are
those that are different from the sequence of the version of the protein found in non-tumor
cells, i.e., tumor cells contain a mutated form of the protein(s) where the mutation(s) are
underlined in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Gene/protein</th>
<th>GeneCard information, incorporated herein by reference</th>
<th>Peptide</th>
<th>SEQ ID NO:</th>
<th>Position</th>
</tr>
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<tbody>
<tr>
<td>alpha-actinin-4</td>
<td><a href="http://www.genecards.org/cgi-bin/carddisp.pl?gene=ACTN4">http://www.genecards.org/cgi-bin/carddisp.pl?gene=ACTN4</a></td>
<td>FIASNGVKLV</td>
<td>35</td>
<td>118-127</td>
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<td>ARTC1</td>
<td><a href="http://www.genecards.org/cgi-bin/carddisp.pl?gene=BBX">http://www.genecards.org/cgi-bin/carddisp.pl?gene=BBX</a></td>
<td>YSVYFNLPADTIYT</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>BCR-ABL fusion protein (b3a2)</td>
<td><a href="http://www.genecards.org/cgi-bin/carddisp.pl?gene=ABL1">http://www.genecards.org/cgi-bin/carddisp.pl?gene=ABL1</a></td>
<td>SSKALQRPV, GFKQSSKAL</td>
<td>37, 38</td>
<td>926-934, 922-930</td>
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<td></td>
<td>ATGFKQSSKALQRPVAS</td>
<td>39</td>
<td>920-936</td>
<td></td>
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<tr>
<td></td>
<td>ATGFKQSSKALQRPVAS</td>
<td>40</td>
<td>920-936</td>
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<td>EDLTVKIGDFGLAT, EKSRWSGSHQFEQLS</td>
<td>41</td>
<td>586-614</td>
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<td>FLIWQNTM, FLIWQNTMc</td>
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<td>Description</td>
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<tr>
<td>CASP-8</td>
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<td>FPSDSWCYF</td>
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<tr>
<td>beta-catenin</td>
<td>g/cgi-bin/carddisp.pl?gene=CTNNB1</td>
<td>SYLDSGIHE</td>
<td>44</td>
<td>29-37</td>
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<tr>
<td>Cdc27</td>
<td>g/cgi-bin/carddisp.pl?gene=CD C27</td>
<td>FSWAMDLPKGAb</td>
<td>45</td>
<td>760-771</td>
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<tr>
<td>CDK4</td>
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<td>ACDPHSGHFV</td>
<td>46</td>
<td>23-32</td>
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<td>CDKN2A</td>
<td>g/cgi-bin/carddisp.pl?gene=CDKN2A</td>
<td>AVCPWTWLRc</td>
<td>47</td>
<td>125-133 (pl4ARF-0RF3)</td>
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<td>CDKN2A</td>
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<td>AVCPWTWLRc</td>
<td>47</td>
<td>111-119 (pl6INK4a-0RF3)</td>
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<td>CLPP</td>
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<td>ILDKVLVHLc</td>
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<td>240-248</td>
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<tr>
<td>COA-1</td>
<td>g/cgi-bin/carddisp.pl?gene=UBXN11</td>
<td>TLYQDDTLTLQAA Gb</td>
<td>49</td>
<td>447-460</td>
</tr>
<tr>
<td>COA-1</td>
<td>g/cgi-bin/carddisp.pl?gene=UBXN11</td>
<td>TLYQDDTLTLQAA Gb</td>
<td>49</td>
<td>447-460</td>
</tr>
<tr>
<td>dek-can fusion protein</td>
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<td>TMKOICKKEIRRLHQY</td>
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<td>342-357</td>
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<td>KILDAVVAQK</td>
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<td>668-677</td>
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<td>Elongation factor 2</td>
<td>g/cgi-bin/carddisp.pl?gene=ETV6-AML1 fusion</td>
<td>ETVSEQSNV</td>
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<td>581-589</td>
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<td>IGRIAECILGMNPSR</td>
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<td>332-346</td>
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<td>FLT3-ITD</td>
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<td>YVDFREYEYY</td>
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<td>591-600</td>
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<td>FN1</td>
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<td>MIFEKHGFRRTPPP</td>
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<td>2050-2063</td>
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<td>GPNMB</td>
<td><a href="http://www.genecards.org/cgi-bin/carddisp.pl?gene=GP">http://www.genecards.org/cgi-bin/carddisp.pl?gene=GP</a> NMB TDWLLQTPK</td>
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<td>179-188</td>
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<tr>
<td>LDLR-fucosyltransferaseASfusion protein</td>
<td><a href="http://www.genecards.org/cgi-bin/carddisp.pl?gene=LD">http://www.genecards.org/cgi-bin/carddisp.pl?gene=LD</a> LR WRRAPAPGA</td>
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<td>60</td>
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<td><a href="http://www.genecards.org/cgi-bin/carddisp.pl?gene=FU">http://www.genecards.org/cgi-bin/carddisp.pl?gene=FU</a> T1 PVTWRAPAPA</td>
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<td>HLA-A2a</td>
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<td>AEPINIQTW</td>
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<td>MART2</td>
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<td>126-134</td>
</tr>
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<td>EAFIQPITR</td>
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<sup>a</sup>The mutation affects the HLA gene itself.
<sup>b</sup>The mutation is not located in the region encoding the peptide.
<sup>c</sup>Frameshift product.
<sup>d</sup>The mutation creates a start codon (ATG) that opens an alternative open reading frame (ORF) encoding the antigenic peptide, which is recognized by regulatory T cells (Tregs).
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Same peptide as MAGE-A3/A2 (aa 271-279).

Aberrant transcript of N-acetyl glucosaminyl transferase V (GnTV) that is found only in melanomas.

Incompletely spliced transcript found only in melanomas.

The processing of this peptide requires the immunoproteasome.

This peptide is encoded by allele MAGE-4a, which is expressed in one third of MAGE-4 positive tumor samples. The other allele, namely MAGE-4b, encodes peptide EVDPTSNTY.

Mucin underglycosylation also occurs in breast duct epithelial cells during lactation, but only at the extracellular apical surface, which is not accessible to T cells.

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**mammaglobin-A**

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- **http://www.geneCards.org/cgi-bin/carddisp.pl?gene=SCGB2A2**

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- **ILTVILGVL**
- **285**
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- **EAAGIGILTV**
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- **AEEAAGIGIL(T)**
- **287**
- **24-33(34)**
- **RNYRALMDKS**
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- **YTTAEEAAGIGILTVILGVLLLIGCWYCRR**
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<sup>d</sup> Different alleles encoding tyrosinase have been described. In 50% of Caucasians, the serine residue of nonapeptide SErWRDIDF is replaced by a tyrosine.

<sup>c</sup> The peptide is composed of two non-contiguous fragments that are spliced by the proteasome.

<sup>f</sup> Phosphopeptide.

<sup>g</sup> Seems to be poorly processed by tumor cells (Fauquembergue et al. J. Immunother. 33.4(2010):402-13).

Table 4

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<sup>c</sup>The antigen is recognized by CTLs bearing an NK inhibitory receptor that prevents lysis of
cells expressing certain HLA-C molecules.

\[ ^a \text{Poorly or not processed (Parkhurst, 2004; Ayyoub, 2001).} \]
\[ ^b \text{The peptide is composed of two non-contiguous fragments that are spliced.} \]
\[ ^c \text{Alternative transcript.} \]
\[ ^d \text{MMP-2 is expressed ubiquitously but melanoma cells cross-present, in an avP3-dependent manner, an antigen derived from secreted MMP-2.} \]
\[ ^e \text{The epitope is located in the untranslated region.} \]

**Immunomodulators**

[0235] Compositions, methods, and devices of the present invention comprise immunomodulators including, but not limited to, TLR ligands, growth factors, and products of dying cells, e.g. heat shock proteins, with means to stimulate dendritic cell activation. Immunomodulators are used alone or in combination with GM-CSF, CpG-ODN sequences, or cancer antigens. Immunomodulators are used simultaneously or sequentially with GM-CSF, CpG-ODN sequences, or cancer antigens.

[0236] All known TLR ligands found either on a cell surface or an internal cellular compartment are encompassed by the compositions, methods, and devices of the present invention. Exemplary TLR ligands include, but are not limited to, triacyl lipoproteins (TLR1); lipoproteins, gram positive peptidoglycan, lipteichoic acids, fungi, and viral glycoproteins (TLR2); double-stranded RNA, poly I:C (TLR 3); lipopolysaccaride, viral glycoproteins (TLR 4); flagellin (TLR5); diacyl lipoproteins (TLR6); small synthetic compounds, single-stranded RNA (TLR7 and TLR 8); unmethylated CpG DNA (TLR9); Profilin (TLR11). Also included as TRL ligands are host molecules like fibronectin and heat shock proteins (HSPs). Host TLR ligands are also encompassed by the present invention. The role of TLRs in innate immunity and the signaling molecules used to activate and inhibit them are known in the art (for a review, see Holger K. Frank B., Hessel E., and Coffman RL. Therapeutic targeting of innate immunity with Toll-like receptor agonists and antagonists. Nature Medicine 13, 552-559 (2007), incorporated herein by reference).

[0237] All known growth factors are encompassed by the compositions, methods, and devices of the present invention. Exemplary growth factors include, but are not limited to, transforming growth factor beta (TGF-β), granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), nerve growth factor (NGF), neurotrophins, Platelet-derived growth factor (PDGF), erythropoietin (EPO), thrombopoietin (TPO), myostatin (GDF-8), growth differentiation factor-9 (GDF9), acidic fibroblast growth factor (aFGF or FGF-1), basic fibroblast growth factor (bFGF or FGF-2), epidermal growth factor (EGF), hepatocyte growth factor (HGF). The present invention encompasses cytokines as well as growth factors for stimulating dendritic cell activation. Exemplary cytokines
include, but are not limited to, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, TNF-a, IFN-\(\gamma\), and IFN-a.

[0238] Indications of cell death and products of dying cells stimulate dendritic cell activation. As such, all products of dying cells are encompassed by the compositions, methods, and devices of the present invention. Exemplary cell death products include, but are not limited to, any intracellular feature of a cell such as organelles, vesicles, cytoskeletal elements, proteins, DNA, and RNA. Of particular interest are heat shock proteins expressed when a cell is under stress and which are released upon cell death. Exemplary heat shock proteins include, but are not limited to, Hsp10, Hsp20, Hsp27, Hsp33, Hsp40, Hsp60, Hsp70, Hsp71, Hsp72, Grp78, Hsp70, Hsp84, Hsp90, Grp94, Hsp100, Hsp104, Hsp110.

Microenvironments and Vaccine Efficiency

[0239] The devices/scaffold described herein represent an infection-mimicking microenvironment. Each device constitutes a factory that attracts/accepts, educates/stimulates and sends forth to surrounding bodily tissues activated dendritic cells that are capable of stimulating/enhancing an immune response to a particular antigen. Specifically, the scaffold devices are implanted or coated with pathogenic molecules to mimic and infectious microenvironment to further activate the dendritic cell response.

[0240] Appropriately mimicking aspects of infection with material systems dramatically impacts tumor progression when applied as cancer vaccines by continuously recruiting, activating and homing DCs to LNs. The first PLG vaccine, using GM-CSF alone, led to a batch process where host DCs were recruited by GM-CSF to reside at a site of tumor antigen presentation, and were trapped until GM-CSF levels fell and the cells could become activated and disperse (see US 2008/0044900 A1, incorporated herein by reference). Temporal variation of the local GM-CSF concentration allowed control over the number of recruited DCs, and the timing of their activation and dispersement. Although the best GM-CSF-based vaccine was able to confer protective immunity in nearly a quarter of the animals tested, approximately 26% of the recruited DCs were activated (-240,000 DCs) and approximately 6% of DCs dispersed to the LNs. High levels of GM-CSF recruited large numbers of DC, but also limited DC activation, leaving potentially therapeutic DCs entrapped within scaffolds. These results motivated the development of an improved system that mimicked bacterial infection by locally presenting CpG-ODNs as an overriding 'danger signal', that opposed GM-CSF inhibition of DC activation and dispersement. These devices described herein represent significant advances by mediating increased and continuous egress of DCs.
CpG-ODN molecules were condensed with PEI to not only promote ODN uptake into DCs and localization to its TLR-9 receptor, but also to electrostatically immobilize it in PLG matrices to be presented simultaneously with tumor antigens. In vitro results indicated that PEI-CpG-ODN condensates can decondense within DCs and stimulate TLR signaling that promoted DC activation and dispersement toward the lymph node derived chemokine, CCL19, in the presence of inhibitory levels of GM-CSF (500ng/ml) (US 2013-0202707, incorporated herein by reference).

As described in detail in US 2013-0202707, the vaccine devices of the invention advantageously allow for fine control of cell behavior and programming in situ.

Scaffold Compositions and Architecture

Components of the scaffolds are organized in a variety of geometric shapes (e.g., discs, beads, pellets), niches, planar layers (e.g., thin sheets). For example, discs of about 0.1-200 millimeters in diameter, e.g., 5, 10, 20, 40, 50 millimeters are implanted subcutaneously. The disc may have a thickness of 0.1 to 10 millimeters, e.g., 1, 2, 5 millimeters. The discs are readily compressed or lyophilized for administration to a patient. An exemplary disc for subcutaneous administration has the following dimensions: 8 millimeters in diameter and 1 millimeter in thickness. Multicomponent scaffolds are optionally constructed in concentric layers each of which is characterized by different physical qualities (% polymer, % crosslinking of polymer, chemical composition of scaffold, pore size, porosity, and pore architecture, stiffness, toughness, ductility, viscoelasticity, and or composition of bioactive substances such as growth factors, homing/migration factors, differentiation factors. Each niche has a specific effect on a cell population, e.g., promoting or inhibiting a specific cellular function, proliferation, differentiation, elaboration of secreted factors or enzymes, or migration. Cells incubated in the scaffold are educated and induced to migrate out of the scaffold to directly affect a target tissue, e.g., and injured tissue site. For example, stromal vascular cells and smooth muscle cells are useful in sheetlike structures are used for repair of vessel-like structures such as blood vessels or layers of the body cavity. For example, such structures are used to repair abdominal wall injuries or defects such as gastroscisis. Similarly, sheetlike scaffolds seeded with dermal stem cells and/or keratinocytes are used in bandages or wound dressings for regeneration of dermal tissue. The device is placed or transplanted on or next to a target tissue, in a protected location in the body, next to blood vessels, or outside the body as in the case of an external wound dressing. Devices are introduced into or onto a bodily tissue using a variety of known methods and tools, e.g., spoon, tweezers or graspers, hypodermic needle, endoscopic manipulator, endo-
trans-vascular catheter, stereotaxic needle, snake device, organ-surface-crawling robot
(United States Patent Application 20050154376; Ota et al., 2006, Innovations 1:227-231),
minimally invasive surgical devices, surgical implantation tools, and transdermal patches.
Devices can also be assembled in place, for example by sequentially injecting or inserting
matrix materials. Scaffold devices are optionally recharged with cells or with bioactive
compounds, e.g., by sequential injection or spraying of substances such as growth factors or
differentiation factors.

[0244] A scaffold or scaffold device is the physical structure upon which or into which cells
associate or attach, and a scaffold composition is the material from which the structure is
made. For example, scaffold compositions include biodegradable or permanent materials
such as those listed below. The mechanical characteristics of the scaffold vary according to
the application or tissue type for which regeneration is sought. It is biodegradable (e.g.,
collagen, alginates, polysaccharides, polyethylene glycol (PEG), poly(glycolide) (PGA),
poly(L-lactide) (PLA), or poly(lactide-co-glycolide) (PLGA), poly lactic-coglycolic acid, or
permanent (e.g., silk). In the case of biodegradable structures, the composition is degraded
by physical or chemical action, e.g., level of hydration, heat or ion exchange or by cellular
action, e.g., elaboration of enzyme, peptides, or other compounds by nearby or resident cells.
The consistency varies from a soft/pliable (e.g., a gel) to glassy, rubbery, brittle, tough,
elastic, stiff. The structures contain pores, which are nanoporous, microporous, or
macroporous, and the pattern of the pores is optionally homogeneous, heterogenous, aligned,
repeating, or random.

[0245] Alginates are versatile polysaccharide based polymers that may be formulated for
specific applications by controlling the molecular weight, rate of degradation and method of
scaffold formation. Coupling reactions can be used to covalently attach bioactive epitopes,
such as the cell adhesion sequence RGD to the polymer backbone. Alginate polymers are
formed into a variety of scaffold types. Injectable hydrogels can be formed from low MW
alginate solutions upon addition of a cross-linking agents, such as calcium ions, while
macroporous scaffolds are formed by lyophilization of high MW alginate discs. Differences
in scaffold formulation control the kinetics of scaffold degradation. Release rates of
morphogens or other bioactive substances from alginate scaffolds is controlled by scaffold
formulation to present morphogens in a spatially and temporally controlled manner. This
controlled release not only eliminates systemic side effects and the need for multiple
injections, but can be used to create a microenvironment that activates host cells at the
implant site and transplanted cells seeded onto a scaffold.
The scaffold comprises a biocompatible polymer matrix that is optionally biodegradable in whole or in part. A hydrogel is one example of a suitable polymer matrix material. Examples of materials which can form hydrogels include polylactic acid, polyglycolic acid, PLGA polymers, alginates and alginate derivatives, gelatin, collagen, agarose, natural and synthetic polysaccharides, polyamino acids such as polypeptides particularly poly(lysine), polyesters such as polyhydroxybutyrate and poly-epsilon-caprolactone, polyanhydrides; polyphosphazines, poly(vinyl alcohols), poly(alkylene oxides) particularly poly(ethylene oxides), poly(allylamines)(PAM), poly(acrylates), modified styrene polymers such as poly(4-aminomethylstyrene), pluronic polyols, polyoxamers, poly(uron acids), poly(vinylpyrrolidone) and copolymers of the above, including graft copolymers.
[0247] The scaffolds are fabricated from a variety of synthetic polymers and naturally-occurring polymers such as, but not limited to, collagen, fibrin, hyaluronic acid, agarose, and laminin-rich gels. One preferred material for the hydrogel is alginate or modified alginate material. Alginate molecules are comprised of (1-4)-linked \( \beta \)-D-mannuronic acid (M units) and a L-guluronic acid (G units) monomers, which can vary in proportion and sequential distribution along the polymer chain. Alginate polysaccharides are polyelectrolyte systems which have a strong affinity for divalent cations (e.g., \( Ca^{2+} \), \( Mg^{2+} \), \( Ba^{2+} \)) and form stable hydrogels when exposed to these molecules. See Martinsen A., et al., Biotech. & Bioeng., 33 (1989) 79-89.) For example, calcium cross-linked alginate hydrogels are useful for dental applications, wound dressings chondrocyte transplantation and as a matrix for other cell types.

[0248] An exemplary device utilizes an alginate or other polysaccharide of a relatively low molecular weight, preferably of size which, after dissolution, is at the renal threshold for clearance by humans, e.g., the alginate or polysaccharide is reduced to a molecular weight of 1000 to 80,000 daltons. Preferably, the molecular mass is 1000 to 60,000 daltons, particularly preferably 1000 to 50,000 daltons. It is also useful to use an alginate material of high guluronate content since the guluronate units, as opposed to the mannurionate units, provide sites for ionic crosslinking through divalent cations to gel the polymer. U.S. Patent Number 6,642,363, incorporated herein by reference, discloses methods for making and using polymers containing polysaccharides such as alginites or modified alginates that are particularly useful for cell transplantation and tissue engineering applications.

[0249] Useful polysaccharides other than alginates include agarose and microbial polysaccharides such as those listed in the table below.

<table>
<thead>
<tr>
<th>Polysaccharide Scaffold Compositions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymers(^{3})</td>
</tr>
<tr>
<td>Fungal</td>
</tr>
<tr>
<td>Pullulan (N)</td>
</tr>
<tr>
<td>Scleroglucan (N)</td>
</tr>
<tr>
<td>Chitin (N)</td>
</tr>
<tr>
<td>Chitosan (C)</td>
</tr>
<tr>
<td>Elsinan (N)</td>
</tr>
<tr>
<td>Bacterial</td>
</tr>
<tr>
<td>Xanthan gum (A)</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Acid as side groups

Curdlan (N) 1,3-β-D-Glucan (with branching)

Dextran (N) 1,6-α-D-Glucan with some 1,2; 1,3-; 1,4- linkages

Gellan (A) 1,4-β-D-Glucan with rhamose, D-glucuronic acid

Levan (N) 2,6-β-D-Fructan with some β-2,1-branching

Emulsan (A) Lipoheteropoly saccharide

Cellulose (N) 1,4-β-D-Glucan

aN-neutral, A = anionic and C=cationic.

[0250] The scaffolds of the invention are porous or non-porous. For example, the scaffolds are nanoporous having a diameter of less than about 10 nm; microporous wherein the diameter of the pores are preferably in the range of about 100 nm-20 μm; or macroporous wherein the diameter of the pores are greater than about 20 μm, more preferably greater than about 100 μm and even more preferably greater than about 400 μm. In one example, the scaffold is macroporous with aligned pores of about 400-500 μm in diameter. The preparation of polymer matrices having the desired pore sizes and pore alignments are described in the Examples. Other methods of preparing porous hydrogel products are known in the art. (U.S. Pat. No. 6,511,650, incorporated herein by reference).

Bioactive compositions

[0251] The device includes one or more bioactive compositions. Bioactive compositions are purified naturally-occurring, synthetically produced, or recombinant compounds, e.g., polypeptides, nucleic acids, small molecules, or other agents. For example, the compositions include GM-CSF, CpG-ODN, and tumor antigens or other antigens. For example, the compositions described herein include an inhibitor of an immune inhibitory protein (e.g., an inhibitor of CTLA4, PD1, PDL1, B7-H3, B7-H4, LAG3, 2B4, BTLA, TIM3, A2aR, or a killer inhibitory receptor). For example, the composition includes an antibody or fragment thereof or a protein that binds to an immune inhibitory protein (e.g., CTLA4, PD1, PDL1, B7-H3, B7-H4, LAG3, 2B4, BTLA, TIM3, A2aR, or a killer inhibitory receptor). In preferred embodiments, the composition includes an antibody of fragment thereof that binds to CTLA4, PD1, or PDL1.
The compositions described herein are purified. Purified compounds are at least 60% by weight (dry weight) the compound of interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. Purity is measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

Coupling of the polypeptides, antibodies, or fragments thereof to the polymer matrix is accomplished using synthetic methods known to one of ordinary skill in the art. Approaches to coupling of peptides to polymers are discussed in Hirano and Mooney, Advanced Materials, p.17-25 (2004). Other useful bonding chemistries include those discussed in Hermanson, Bioconjugate Techniques, p. 152-185 (1996), particularly by use of carbodiimide couplers, DCC and DIC (Woodward's Reagent K). Polypeptides contain a terminal amine group for such carbodiimide bonding. The amide bond formation is preferably catalyzed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), which is a water soluble enzyme commonly used in peptide synthesis.

Control of release kinetics of bioactive compositions.

The release profile of bioactive compositions such as GM-CSF is controlled using a number of different techniques, e.g., encapsulation, nature of attachment/association with the scaffold, porosity of the scaffold, and particle size of the bioactive compositions.

For example, GM-CSF is encapsulated as one means by which to incorporate GM-CSF into the scaffolds. GM-CSF was first encapsulated into PLG microspheres, and then these GM-CSF loaded microspheres were then in a gas foaming process to develop macroporous PLG scaffolds. The incorporation of GM-CSF into the microspheres causes the GM-CSF to be more deeply embedded into the polymer, which causes the device to sustain the initial pulse of GM-CSF delivery over days 1-5. Other incorporation methods are optionally used to alter or fine tune the duration of the GM-CSF pulse as desired, which would in turn change the kinetics of DC recruitment. For example, foaming PLG particles mixed with lyophilized GM-CSF results in GM-CSF that is associated more with the surface of the polymer scaffold, and the protein diffuses more quickly.

Alternative methods for scaffold fabrication that modify release kinetics include modifying the physical structure of the scaffolds pores, thereby leading to different degradation times and release kinetics (change pore size or total porosity as a percentage of volume), e.g., as described in Riddle et al., Role of poly(lactide-co-glycolide) particle size on gas-foamed scaffolds. J Biomater Sci Polym Ed. 2004;15(12):1561-70. Another way to alter release kinetics is to modify the composition, i.e., the raw materials from which the scaffold
is made, thereby altering the release properties. For example, different polymers, e.g., alginate, PLA, PGA, or using PLGA are used. Also, use of the polymers with different ratios of glycolic and lactic acid) leads to different release profiles. For example, a variety of PLGs, differing in composition (lactide to glycolide ratio) and molecular weight are used to prepare microspheres (5-50 μm) using known double emulsion (water/oil/water) process, followed by preparation of scaffolds using particulate PLG and PLG microspheres using gas foaming/particulate leaching techniques (Ennett et al., Temporally regulated delivery of VEGF in vitro and in vivo. J Biomed Mater Res A. 2006 Oct;79(l)). Another technique involves incorporating the protein into different compartments (e.g., encapsulating proteins PLG microspheres or simple mixing and lyophilizing with the polymer before foaming). Methods of making a scaffold described herein include using gas foaming, e.g., as described in detail in Harris et al. J. Biomed. Materials Res. Part A. 42.3(1998)396-402 and Sheridan et al. J. Control. Rel. 64(2000)91-102, both incorporated herein by reference. In other embodiments, wires (e.g., a template containing multiple wires) are used as porogens, i.e., to create pores in the scaffold, e.g., to create aligned pores.

Charging and/or recharging the device

[0257] A bioactive composition such as GM-CSF is incorporated within different layers/compartment of the device, thereby allowing multiple pulses of GM-CSF to be delivered. Each pulse charges (or recharges) the device with an influx of DCs. Scaffolds are fabricated using a variety of methods to create multiple pulses of GM-CSF (or other bioactive agents). For example, such devices are made by incorporating the protein into different compartments (e.g., encapsulating proteins PLG microspheres or simple mixing and lyophilizing with the polymer before foaming) thereby creating 2 or more distinct release profiles (i.e., pulses) of the protein (e.g., as described in Richardson et al., Polymeric system for dual growth factor delivery. Nat Biotechnol. 2001 Nov;19(II)).

[0258] Alternatively, the protein is encapsulated in fast degrading PLG microspheres (e.g. low MW, 50:50 ratio) and slow degrading PLG microspheres (high MW, 85:15 ratio). Then these microspheres are mixed together to be used later to fabricate the scaffolds. Therefore, the protein is encapsulated in both fast a degrading polymer and a slow degrading polymer, thereby resulting in at least 2 distinct releases kinetics and pulses of delivery. This method is utilized to create 3, 4, 5, or more different kinds of microspheres, the ratiometric characteristics of which differ, thereby leading to 3, 4, 5 or more pulses of release of the bioactive composition such as GM-CSF.
Another approach to making a device that delivers more than one pulse is to fabricate a layered scaffold. Layered scaffolds are made by compression molding on different scaffold formulations with another. For example, the raw materials (sucrose + PLG1 + Protein) is compressed in a mold and a slightly varied formulation (sucrose + PLG2 + Protein) is also compressed in a mold. Then these two layers are compressed together and then foamed, resulting in a bilayered scaffold with distinct spatial control of the concentration of the protein, e.g., as described in Chen et al., Pharm Res. Spatio-temporal VEGF and PDGF delivery patterns blood vessel formation and maturation. 2007 Feb;24(2):258-64.

Device construction

The scaffold structure is constructed out of a number of different rigid, semi-rigid, flexible, gel, self-assembling, liquid crystalline, or fluid compositions such as peptide polymers, polysaccharides, synthetic polymers, hydrogel materials, ceramics (e.g., calcium phosphate or hydroxyapatite), proteins, glycoproteins, proteoglycans, metals and metal alloys. The compositions are assembled into cell scaffold structures using methods known in the art, e.g., injection molding, lyophilization of preformed structures, printing, self-assembly, phase inversion, solvent casting, melt processing, gas foaming, fiber forming/processing, particulate leaching or a combination thereof. The assembled devices are then implanted or administered to the body of an individual to be treated.

The device is assembled in vivo in several ways. The scaffold is made from a gelling material, which is introduced into the body in its ungelled form where it gels in situ. Exemplary methods of delivering device components to a site at which assembly occurs include injection through a needle or other extrusion tool, spraying, painting, or methods of deposit at a tissue site, e.g., delivery using an application device inserted through a cannula. In one example, the ungelled or unformed scaffold material is mixed with bioactive substances and cells prior to introduction into the body or while it is introduced. The resultant in vivo/in situ assembled scaffold contains a mixture of these substances and cells.

In situ assembly of the scaffold occurs as a result of spontaneous association of polymers or from synergistically or chemically catalyzed polymerization. Synergistic or chemical catalysis is initiated by a number of endogenous factors or conditions at or near the assembly site, e.g., body temperature, ions or pH in the body, or by exogenous factors or conditions supplied by the operator to the assembly site, e.g., photons, heat, electrical, sound, or other radiation directed at the ungelled material after it has been introduced. The energy is directed at the scaffold material by a radiation beam or through a heat or light conductor, such as a wire or fiber optic cable or an ultrasonic transducer. Alternatively, a shear-thinning
material, such as an amphiphile, is used which re-cross links after the shear force exerted upon it, for example by its passage through a needle, has been relieved.


[0264] A multiple compartment device is assembled in vivo by applying sequential layers of similarly or differentially doped gel or other scaffold material to the target site. For example, the device is formed by sequentially injecting the next, inner layer into the center of the previously injected material using a needle, forming concentric spheroids. Non-concentric compartments are formed by injecting material into different locations in a previously injected layer. A multi-headed injection device extrudes compartments in parallel and simultaneously. The layers are made of similar or different scaffolding compositions differentially doped with bioactive substances and different cell types. Alternatively, compartments self-organize based on their hydrophilic/phobic characteristics or on secondary interactions within each compartment.

**Compartmentalized device**

[0265] In certain situations, a device containing compartments with distinct chemical and/or physical properties is useful. A compartmentalized device is designed and fabricated using different compositions or concentrations of compositions for each compartment.

[0266] Alternatively, the compartments are fabricated individually, and then adhered to each other (e.g., a "sandwich" with an inner compartment surrounded on one or all sides with the second compartment). This latter construction approach is accomplished using the intrinsic adhesiveness of each layer for the other, diffusion and interpenetration of polymer chains in each layer, polymerization or cross-linking of the second layer to the first, use of an adhesive (e.g., fibrin glue), or physical entrapment of one compartment in the other. The compartments self-assemble and interface appropriately, either in vitro or in vivo, depending on the presence of appropriate precursors (e.g., temperature sensitive oligopeptides, ionic strength sensitive oligopeptides, block polymers, cross-linkers and polymer chains (or combinations thereof), and precursors containing cell adhesion molecules that allow cell-controlled assembly).

[0267] Alternatively, the compartmentalized device is formed using a printing technology. Successive layers of a scaffold precursor doped with bioactive substances is placed on a
substrate then cross-linked, for example by self-assembling chemistries. When the cross linking is controlled by chemical-, photo- or heat-catalyzed polymerization, the thickness and pattern of each layer is controlled by a masque, allowing complex three dimensional patterns to be built up when un-cross-linked precursor material is washed away after each catalyzation. (WT Brinkman et al., Photo-cross-linking of type I collagen gels in the presence of smooth muscle cells: mechanical properties, cell viability, and function. Biomacromolecules, 2003 Jul-Aug;4(4): 890-895.; W. Ryu et al., The construction of three-dimensional micro-fluidic scaffolds of biodegradable polymers by solvent vapor based bonding of micro-molded layers. Biomaterials, 2007 Feb;28(6): 1174-1184; Wright, Paul K. (2001). 21st Century manufacturing. New Jersey: Prentice-Hall Inc.) Complex, multi-compartiment layers are also built up using an inkjet device which "paints" different doped-scaffold precursors on different areas of the substrate. Julie Phillippi (Carnegie Mellon University) presentation at the annual meeting of the American Society for Cell Biology on December 10, 2006; Print me a heart and a set of arteries, Aldhouse P., New Scientist 13 April 2006 Issue 2547 p 19.; Replacement organs, hot off the press, C. Choi, New Scientist, 25 Jan 2003, v2379. These layers are built-up into complex, three dimensional compartments. The device is also built using any of the following methods: Jetted Photopolymer, Selective Laser Sintering, Laminated Object Manufacturing, Fused Deposition Modeling, Single Jet Inkjet, Three Dimensional Printing, or Laminated Object Manufacturing.

[0268] The release profiles of bioactive substances from scaffold devices is controlled by both factor diffusion and polymer degradation, the dose of the factor loaded in the system, and the composition of the polymer. Similarly, the range of action (tissue distribution) and duration of action, or spatiotemporal gradients of the released factors are regulated by these variables. The diffusion and degradation of the factors in the tissue of interest is optionally regulated by chemically modifying the factors (e.g., PEGylating growth factors). In both cases, the time frame of release determines the time over which effective cell delivery by the device is desired.

[0269] The bioactive substances are added to the scaffold compositions using known methods including surface absorption, physical immobilization, e.g., using a phase change to entrap the substance in the scaffold material. For example, a growth factor is mixed with the scaffold composition while it is in an aqueous or liquid phase, and after a change in environmental conditions (e.g., pH, temperature, ion concentration), the liquid gels or solidifies thereby entrapping the bioactive substance. Alternatively, covalent coupling, e.g.,
using alkylating or acylating agents, is used to provide a stable, long term presentation of a bioactive substance on the scaffold in a defined conformation. Exemplary reagents for covalent coupling of such substances are provided in the table below.

**Methods to covalently couple peptides/proteins to polymers**

<table>
<thead>
<tr>
<th>Functional Group of Polymer</th>
<th>Coupling reagents and cross-linker</th>
<th>Reacting groups on protein/peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>-OH</td>
<td>Cyanogen bromide (CNBr)</td>
<td>-NH₂</td>
</tr>
<tr>
<td></td>
<td>Cyanuric chloride</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-[4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMT-MM)]</td>
<td></td>
</tr>
<tr>
<td>-NH₂</td>
<td>Dicarbonyl compounds</td>
<td>-NH₂</td>
</tr>
<tr>
<td></td>
<td>Dithiocarbonyl compounds</td>
<td>-OH</td>
</tr>
<tr>
<td></td>
<td>Glutaraldehyde</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Succinic anhydride</td>
<td></td>
</tr>
<tr>
<td>-NH₂</td>
<td>Nitrous Acid</td>
<td>-NH₂</td>
</tr>
<tr>
<td></td>
<td>Hydrazine + nitrous acid</td>
<td>-SH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Ph-OH</td>
</tr>
<tr>
<td>-NH₂</td>
<td>Carboximid compounds (e.g., EDC, DCC)[a]</td>
<td>-COOH</td>
</tr>
<tr>
<td></td>
<td>DMT-MM</td>
<td></td>
</tr>
<tr>
<td>-COOH</td>
<td>Thionyl chloride</td>
<td>-NH₂</td>
</tr>
<tr>
<td></td>
<td>N-hydroxysuccinimide</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N-hydroxysulfo succinimide + EDC</td>
<td></td>
</tr>
<tr>
<td>-SH</td>
<td>Disulfide compound</td>
<td>-SH</td>
</tr>
</tbody>
</table>

[a] EDC: l-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; DCC: dicyclohexylcarbodiimide

**[0270]** Bioactive substances suitable for use in the present invention include, but are not limited to: interferons, interleukins, chemokines, cytokines, colony stimulating factors, chemotactic factors, granulocyte/macrophage colony stimulating factor (GM-CSF). Splice variants of any of the above mentioned proteins, and small molecule agonists or antagonists thereof that may be used advantageously to activate dendritic cells are also contemplated herein.

**[0271]** Exemplary bioactive substances suitable for use in, on, or in combination with the vaccine device of the invention include an inhibitor of an immune-inhibitory protein. Exemplary immune-inhibitory proteins include immune checkpoint proteins (e.g., CTLA4, PD1, PDL1, and PDL2). Other exemplary immune inhibitory proteins include B7-H3, B7-H4, LAG3, 2B4, BTLA, TIM3, A2aR, and/or a killer inhibitory receptor. Exemplary
inhibitors include small molecules, proteins, peptides, antibodies or fragments thereof, and nucleic acids. For example, an inhibitor is a nucleic acid, protein, antibody, or fragment thereof that binds to CTLA4, PD1, PDL1, PDL2, B7-H3, B7-H4, LAG3, 2B4, BTLA, TIM3, A2aR, and/or a killer inhibitory receptor. For example, an inhibitor is a nucleic acid that binds to a mRNA that encodes CTLA4, PD1, PDL1, PDL2, B7-H3, B7-H4, LAG3, 2B4, BTLA, TIM3, A2aR, and/or a killer inhibitory receptor. In some embodiments, the nucleic acid that binds to a mRNA of the inhibitor downregulates inhibitor expression at the mRNA and/or protein level.

[0272] A small molecule is a low molecular weight compound of less than 1000 Daltons, less than 800 Daltons, or less than 500 Daltons. Antibodies and fragments thereof described herein include, but are not limited to, polyclonal, monoclonal, chimeric, dAb (domain antibody), single chain, Fab, Fab’ and F(ab’)2 fragments, Fv, scFvs. A fragment of an antibody possess the immunological activity of its respective antibody. In some embodiments, a fragment of an antibody contains 1500 or less, 1250 of less, 1000 or less, 900 or less, 800 or less, 700 or less, 600 or less, 500 or less, 400 or less, 300 or less, 200 or less amino acids. For example, a protein or peptide inhibitor contains 1500 or less, 1250 of less, 1000 or less, 900 or less, 800 or less, 700 or less, 600 or less, 500 or less, 400 or less, 300 or less, 200 or less, 100 or less, 80 or less, 70 or less, 60 or less, 50 or less, 40 or less, 30 or less, 25 or less, 20 or less, 10 or less amino acids. For example, a nucleic acid inhibitor of the invention contains 400 or less, 300 or less, 200 or less, 150 or less, 100 or less, 90 or less, 80 or less, 70 or less, 60 or less, 50 or less, 40 or less, 35 or less, 30 or less, 28 or less, 26 or less, 24 or less, 22 or less, 20 or less, 18 or less, 16 or less, 14 or less, 12 or less, 10 or less nucleotides.

[0273] In some cases, a compound (e.g., small molecule) or macromolecule (e.g., nucleic acid, polypeptide, or protein) of the invention is purified and/or isolated. As used herein, an "isolated" or "purified" small molecule, nucleic acid molecule, polynucleotide, polypeptide, or protein (e.g., antibody or fragment thereof), is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized. Purified compounds are at least 60% by weight (dry weight) the compound of interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. For example, a purified compound is one that is at least 90%, 91%, 92%, 93%, 94%, 95%, 98%, 99%, or 100% (w/w) of the desired compound by weight. Purity is measured by any appropriate standard method, for example, by column chromatography, thin layer
chromatography, or high-performance liquid chromatography (HPLC) analysis. A purified or isolated polynucleotide (ribonucleic acid (RNA) or deoxyribonucleic acid (DNA)) is free of the genes or sequences that flank it in its naturally occurring state. Purified also defines a degree of sterility that is safe for administration to a human subject, e.g., lacking infectious or toxic agents.

[0274] By "substantially pure" is meant a nucleotide or polypeptide that has been separated from the components that naturally accompany it. Typically, the nucleotides and polypeptides are substantially pure when they are at least 60%, 70%, 80%, 90%, 95%, or even 99%, by weight, free from the proteins and naturally-occurring organic molecules with they are naturally associated.

[0275] For example, a nucleic acid inhibitor is a short interfering RNA, a short hairpin RNA, antisense RNA, aptamers, peptide nucleic acids (PNAs), microRNAs (miRNAs), or locked nucleic acids (LNAs). In some embodiments, the nucleic acid comprises modified oligonucleotides (e.g., 2′-O-methyl RNA).

[0276] Examples of cytokines as mentioned above include, but are not limited to IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), interferon-γ (IFN-γ), IFN-α, tumor necrosis factor (TNF), TGF-β, FLT-3 ligand, and CD40 ligand.

[0277] Scaffolds of the invention optionally comprise at least one non-viral gene therapy vector such that either the transplanted cells or host cells in the vicinity of the implant would take up and express gene that lead to local availability of the desired factor for a desirable time frame. Such non-viral vectors include, but are not limited to, cationic lipids, polymers, targeting proteins, and calcium phosphate.

Scaffold Fabrication.

[0278] A 85:15, 120 kD copolymer of D.L.-lactide and glycolide (PLG) (Alkermes, Cambridge, MA) was utilized in a gas-foaming process to form scaffolds with open, interconnected pores (Cohen S., Yoshioka T., Lucarelli, M., Hwang L.FL, and Langer R. Pharm. Res. 8,713-720 (1991); herein incorporated by reference). PLG microspheres encapsulating GM-CSF were made using standard double emulsion (Hams, L.D., Kim, B.S., and Mooney, D.J. J. Biomed. Mater. Res. 42,396-402 (1998); herein incorporated by reference). 16 mg of PLG microspheres were then mixed with 150 mg of the porogens, NaCl or sucrose (sieved to a particle size between 250 μm and 425 μm), and compression molded. The resulting disc was allowed to equilibrate within a high-pressure CO₂ environment, and a
rapid reduction in pressure causes the polymer particles to expand and fuse into an interconnected structure. The NaCl was leached from the scaffolds by immersion in water yielding scaffolds that were 90% porous. To incorporate tumor lysates into PLG scaffolds, biopsies of B16-F10 tumors, that had grown subcutaneously in the backs of C57BL/6J mice (Jackson Laboratory, Bar Harbor Maine), were digested in collagenase (250 U/ml) (Worthington, Lakewood, NJ) and suspended at a concentration equivalent to 10⁷ cells per ml after filtration through 40 μm cell strainers. The tumor cell suspension was subjected to 4 cycles of rapid freeze in liquid nitrogen and thaw (37°C) and then centrifuged at 400 rpm for 10 min. The supernatant (1ml) containing tumor lysates was collected and lyophilized with the PLG microspheres and the resulting mixture was used to make PLG scaffold-based cancer vaccines. To incorporate CpG-ODNs into PLG scaffolds, PEI-CpG-ODN condensate solutions were vortexed with 60 μl of 50% (wt/vol) sucrose solution, lyophilized and mixed with dry sucrose to a final weight of 150 mg. The sucrose containing PEI-CpG-ODN condensate was then mixed with blank, GM-CSF and/or tumor lysate loaded PLG microspheres to make PLG cancer vaccines.

[0279] Scaffold compositions of the present invention comprise GM-CSF, Flt3L, and/or CCL20, and CpG-ODN sequences. A range of concentrations of each element are contemplated. In a preferred embodiment, the scaffold composition comprises PLG. With respect to GM-CSF, Flt3L, and/or CCL20, per 40 mg polymeric scaffold composition, 0-100 μg of GM-CSF, Flt3L, and/or CCL20 polypeptide is incorporated into or coated onto the scaffold composition. Alternatively, doses comprising 0-50 μg, 0-25 μg, 0-10 μg, 0-5 μg, and 0-3 μg of GM-CSF, Flt3L, and/or CCL20 are incorporated into the scaffold composition. In a preferred embodiment, 0-3 μg of GM-CSF, Flt3L, and/or CCL20 are incorporated into the scaffold composition. With respect to CpG-ODN sequences, or PEI-CpG-ODN condensates, per 40 mg polymeric scaffold composition, 0-1000 μg of PEI-CpG-ODN is incorporated into or coated onto the scaffold composition. Alternatively, doses comprising 0-500 μg, 0-250 μg, 0-100 μg (e.g., 100 μg), 0-50 μg, 0-25 μg, 0-10 μg, and 0-5 μg of PEI-CpG-ODN are incorporated into the scaffold composition. In a preferred embodiment, 0-50 μg of PEI-CpG-ODN are incorporated into the scaffold composition.

Vaccine device

[0280] The biocompatible scaffolds are useful as delivery vehicles for cancer vaccines. The cancer vaccine stimulates an endogenous immune response against cancer cells. Currently produced vaccines predominantly activate the humoral immune system (i.e., the antibody dependent immune response). Other vaccines currently in development are focused on
activating the cell-mediated immune system including cytotoxic T lymphocytes which are capable of killing tumor cells. Cancer vaccines generally enhance the presentation of cancer antigens to both antigen presenting cells (e.g., macrophages and dendritic cells) and/or to other immune cells such as T cells, B cells, and NK cells. Although cancer vaccines may take one of several forms, their purpose is to deliver cancer antigens and/or cancer associated antigens to antigen presenting cells (APC) in order to facilitate the endogenous processing of such antigens by APC and the ultimate presentation of antigen presentation on the cell surface in the context of MHC class I molecules. One form of cancer vaccine is a whole cell vaccine which is a preparation of cancer cells which have been removed from a subject, treated ex vivo and then reintroduced as whole cells in the subject. These treatments optionally involve cytokine exposure to activate the cells, genetic manipulation to overexpress cytokines from the cells, or priming with tumor specific antigens or cocktails of antigens, and expansion in culture. Dendritic cell vaccines activate antigen presenting cells directly, and their proliferation, activation and migration to lymph nodes is regulated by scaffold compositions to enhance their ability to elicit an immune response. Types of cancers to be treated include central nervous system (CNS) cancers, CNS Germ Cell tumor, lung cancer, Leukemia, Multiple Myeloma, Renal Cancer, Malignant Glioma, Medulloblastoma, and Melanoma.

[0281] For the purpose of eliciting an antigen-specific immune response, a scaffold device is implanted into a mammal. The device is tailored to activate immune cells and prime the cells with a specific antigen thereby enhancing immune defenses and destruction of undesired tissues and targeted microorganisms such as bacterial or viral pathogens. The device attracts appropriate immune cells, such as macrophages, T cells, B cells, NK cells, and dendritic cells, by containing and/or releasing signaling substances such as GM-CSF. These signaling substances are incorporated in the scaffold composition in such a way as to control their release spatially and temporally using the same techniques used to integrate other bioactive compounds in the scaffold composition.

[0282] Once the immune cells are inside the device, the device programs the immune cells to attack or cause other aspects of the immune system to attack undesired tissues (e.g., cancer, adipose deposits, or virus-infected or otherwise diseased cells) or microorganisms. Immune cell activation is accomplished by exposing the resident immune cells to preparations of target-specific compositions, e.g., ligands found on the surface of the undesired tissues or organisms, such as cancer cell surface markers, viral proteins, oligonucleotides, peptide
sequences or other specific antigens. For example, useful cancer cell-specific antigens and other tissue or organism-specific proteins are listed in the table below.

[0283] The device optionally contains multiple ligands or antigens in order to create a multivalent vaccine. The compositions are embedded in or coated on the surface of one or more compartments of the scaffold composition such that immune cells migrating through the device are exposed to the compositions in their traverse through the device. Antigens or other immune stimulatory molecules are exposed or become exposed to the cells as the scaffold composition degrades. The device may also contain vaccine adjuvants that program the immune cells to recognize ligands and enhance antigen presentation. Exemplary vaccine adjuvants include chemokines/cytokines, CpG rich oligonucleotides, or antibodies that are exposed concurrently with target cell-specific antigens or ligands.

[0284] The device attracts immune cells to migrate into a scaffold where they are educated in an antigen-specific manner and activated. The programmed immune cells are then induced to egress towards lymph nodes in a number of ways. The recruitment composition and deployment signal/composition, e.g., a lymph node migration inducing substance, is released in one or more bursts, programmed by the method of incorporation and/or release from the scaffold material, or controlled by the sequential degradation of scaffold compartments which contain the attractant. When a burst dissipates, the cells migrate away. Compartments containing repulsive substances are designed to degrade and release the repulsive substance in one or more bursts or steadily over time. Relative concentration of the repulsive substances cause the immune cells to migrate out of the device. Alternatively, cells which have been placed in or have migrated into the device are programmed to release repulsive substances or to change their own behavior. For example, localized gene therapy is carried out by cell exposure to plasmid DNA attached to the scaffold. Useful repulsive substances include chemokines and cytokines. Alternatively, the device may cause immune cells to egress by degrading and releasing them.

[0285] Target disease states, stimulatory molecules and antigens useful in vaccine device construction are listed below.

Bioactive factors to promote immune responses

a. Interleukins: IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18 etc.
b. TNF-a
c. IFN-γ
d. IFN-a
e. GM-CSF
f. G-CSF
g. Ftl-3 ligand
h. MIP-3 β (CCL19)
i. CCL21
j. M-CSF
k. MIF
l. CD40L
m. CD3
n. ICAM
o. Anti-CTLA4 proteins or antibodies or fragments thereof (e.g., ipilimumab or tremelimumab)
p. TGF-β
q. CPG rich DNA or oligonucleotides
r. Sugar moieties associated with Bacteria: Lipopolysacharides (LPS) is an example
s. Fas ligand
t. Trail
u. Lymphotactin
v. Mannan (M-FP)
w. Heat Shock Proteins (apg-2, Hsp70 and Hsp 90 are examples)
x. anti-PD1 proteins or antibodies (e.g., MDX-1 106, MK3475, CT-01 1, or AMP-224)
y. anti-PDL1 or anti-PDL2 proteins or antibodies (e.g., MDX-1 1105)
z. anti-LAG3 proteins or antibodies or fragments thereof
aa. anti-B7-H3 proteins or antibodies or fragments thereof
bb. anti-B7-H4 proteins or antibodies or fragments thereof
cc. anti-TIM3 proteins or antibodies or fragments thereof
dd. anti-BTLA proteins or antibodies or fragments thereof
ee. anti-A2aR proteins or antibodies or fragments thereof
ff. anti-killer inhibitor receptor (KIR) (e.g., killer cell immunoglobulin-like receptor or C-type lectin receptor) proteins or antibodies or fragments thereof
gg. anti-TIM4 proteins or antibodies or fragments thereof
hh. anti-TIM2 proteins or antibodies or fragments thereof
ii. anti-OX40 proteins or antibodies or fragments thereof
jj. anti-4-1BB proteins or antibodies or fragments thereof
anti-phosphatidylserine proteins or antibodies or fragments thereof (e.g., a monoclonal antibody against phosphatidylserine)

**Diseases and antigens - vaccination targets**

a. Cancer: antigens and their sources
   i. Tumor lysates extracted from biopsies (e.g., from melanoma tumor biopsies)
   ii. Irradiated tumor cells (e.g., irradiated melanoma cells)
   iii. Melanoma
      1. MAGE series of antigens (MAGE-1 is an example)
      2. MART-1/melanA
      3. Tyrosinase
      4. ganglioside
      5. gp100
      6. GD-2
      7. O-acetylated GD-3
      8. GM-2
      9. B16-F10 tumor lysate, e.g., from mice challenged with B16-F10 melanoma tumor cells (ATCC, Manassas, NJ)
      10. tyrosinase-related protein (TRP)-2
      11. lung cancer cell lysate or lung cancer cell antigen
      12. glioma cancer cell lysate or glioma cancer cell antigen
      13. prostate cancer cell lysate or prostate cancer cell antigen

iv. Breast cancer
   1. MUC-1
   2. Sos1
   3. Protein kinase C-binding protein
   4. Reverse transcriptase protein
   5. AKAP protein
   6. VRK1
   7. KIAA1735
   8. T7-1, T11-3, T11-9
   9. Her2 (also known as CD340)
v. Other general and specific cancer antigens
1. Homo Sapiens telomerase ferment (hTRT)
2. Cytokeratin-19 (CYFRA2-1-1)
3. SQUAMOUS CELL CARCINOMA ANTIGEN 1 (SCCA-1), (PROTEIN T4-A)
4. SQUAMOUS CELL CARCINOMA ANTIGEN 2 (SCCA-2)
5. Ovarian carcinoma antigen CA125 (1A1-3B) (KIAA0049)
6. MUCIN 1 (TUMOR-ASSOCIATED MUCIN), (CARCINOMA-ASSOCIATED MUCIN), (POLYMORPHIC EPITHELIAL MUCIN), (PEM), (PEMT), (EPISIALIN), (TUMOR-ASSOCIATED EPITHELIAL MEMBRANE ANTIGEN), (EMA), (H23AG), (PEANUT-REACTIVE URINARY MUCIN), (PUM), (BREAST CARCINOMA-ASSOCIATED ANTIGEN DF3)
7. CTCL tumor antigen sel-1
8. CTCL tumor antigen sel4-3
9. CTCL tumor antigen se20-4
10. CTCL tumor antigen se20-9
11. CTCL tumor antigen se33-1
12. CTCL tumor antigen se37-2
13. CTCL tumor antigen se57-1
14. CTCL tumor antigen se89-1
15. Prostate-specific membrane antigen
16. 5T4 oncofetal trophoblast glycoprotein
17. Orf73 Kaposi’s sarcoma-associated herpesvirus
18. MAGE-C1 (cancer/testis antigen CT7)
19. MAGE-B1 ANTIGEN (MAGE-XP ANTIGEN) (DAM10)
20. MAGE-B2 ANTIGEN (DAM6)
21. MAGE-2 ANTIGEN
22. MAGE-4a antigen
23. MAGE-4b antigen
24. Colon cancer antigen NY-CO-45
25. Lung cancer antigen NY-LU-12 variant A
26. Cancer associated surface antigen
27. Adenocarcinoma antigen ART1
28. Paraneoplastic associated brain-testis-cancer antigen (onconeural antigen MA2; paraneoplastic neuronal antigen)
29. Neuro-oncological ventral antigen 2 (NOVA2)
30. Hepatocellular carcinoma antigen gene 520
31. TUMOR-ASSOCIATED ANTIGEN CO-029
32. Tumor-associated antigen MAGE-X2
33. Synovial sarcoma, X breakpoint 2
34. Squamous cell carcinoma antigen recognized by T cell
35. Serologically defined colon cancer antigen 1
36. Serologically defined breast cancer antigen NY-BR-15
37. Serologically defined breast cancer antigen NY-BR-16
38. Chromogranin A; parathyroid secretory protein 1
39. DUPAN-2
40. CA 19-9
41. CA 72-4
42. CA 195
43. Carcinoembryonic antigen (CEA)

b. AIDS (HIV-associated antigens)
   i. Gpl20
   ii. SIV229
   iii. SIVE660
   iv. SHIV89.6P
   v. E92
   vi. HC1
   vii. OKM5
   viii. FVIIIRAg
   ix. HLA-DR (Ia) antigens
   x. OKM1
   xi. LFA-3

c. General infectious diseases and associated antigens
   i. Tuberculosis
      1. Mycobacterium tuberculosis antigen 5
      2. Mycobacterium tuberculosis antigen 85
      3. ESAT-6
4. CFP-10
5. Rv3871
6. GLU-S
ii. Malaria
1. CRA
2. RAP-2
3. MSP-2
4. AMA-1
iii. Possible mutant influenza and meningitis strains
d. Neuro protection - protect against neurological diseases (e.g., Alzheimer's, Parkinson's, Prion disease)
   1. Classes of self CNS antigens
   2. human alpha-synuclein (Parkinson's)
   3. beta amyloid plaques (Alzheimer's)
e. Autoimmune Diseases (multiple sclerosis, Rheumatoid arthritis etc)
   i. Disease linked MHC antigens
   ii. Different classes of Self antigens
   iii. Insulin
   iv. Insulin peptide B9-23
   v. glutamic acid
   vi. decarboxylase 65 (GAD 65)
   vii. HSP 60
Disease linked T-cell receptor (TCR)

[0286] Prior vaccines have been largely ineffective for patients with established cancer, as advanced disease requires potent and sustained activation of CD8+ cytotoxic T lymphocytes (CTLs) to kill tumor cells and clear the disease. Subsets of dendritic cells (DCs) specialize in antigen cross-presentation and in the production of cytokines, which regulate both CTLs and T regulatory (Treg) cells that shut down effector T cell responses. Coordinated regulation of a DC network, and plasmacytoid DCs (pDCs) and CD8+ DCs in particular, enhances host immunity in mice. Functionalized biomaterials incorporating various combinations of an inflammatory cytokine, immune danger signal, and tumor lysates are used in the vaccines described herein to control the activation and localization of host DC populations in situ.
Implantable synthetic polymer matrices (antigen-loaded acellular biomaterial device) that spatially and temporally control the in vivo presentation of cytokines, tumor antigens, and danger signals are utilized. GM-CSF is released from these polylactide-co-glycolide (PLG) [a FDA-approved biomaterial] matrices into the surrounding tissue to recruit DC precursors and DCs. CpG-rich oligonucleotides are immobilized on the matrices as danger signals, and antigen (tumor lysates) is released to matrix-resident DCs to program DC development and maturation. These matrices quantitatively regulate DC activation and trafficking in situ and induce prophylactic immunity against inoculations of murine B16-F10 melanoma cells (P. Schnorrer, G. M. Behrens, N. S. Wilson, J. L. Pooley, C. M. Smith, D. El-Sukkari, G. Davey, F. Kupresanin, M. Li, E. Maraskovsky, G. T. Belz, F. R. Carbone, K. Shortman, W. R. Heath, J. A. Villadangos, The dominant role of CD8+ dendritic cells in cross-presentation is not dictated by antigen capture. Proc. Natl. Acad. Sci. U.S.A. 103, 10729-10734 (2006)). As described herein, this system administered repeatedly over time to controls the recruitment and activation of multiple DC and T cell subsets and is effective as a therapeutic vaccine against established tumors.

**Matrix fabrication**

An exemplary protocol for matrix fabrication is described herein (see, e.g., US 2013-0202707, incorporated herein by reference). An 85:15, 120-kD copolymer of DL-lactide and glycolide (PLG) (Alkermes) was utilized in a gas-foaming process to form porous PLG matrices (L. D. Harris, B. S. Kim, D. J. Mooney, Open pore biodegradable matrices formed with gas foaming. J. Biomed. Mater. Res. 42, 396-402 (1998)). PLG microspheres encapsulating GM-CSF were first made with standard double emulsion (S. Cohen, T. Yoshioka, M. Lucarelli, L. H. Hwang, R. Langer, Controlled delivery systems for proteins based on poly(lactic/glycolic acid) microspheres. Pharm. Res. 8, 713-720 (1991)). PLG micro-spheres were then mixed with 150 mg of the porogen, sucrose (sieved to a particle size between 250 and 425 mm), and compression molded. The resulting disc was allowed to equilibrate within a high-pressure CO2 environment, and a rapid reduction in pressure causes the polymer particles to expand and fuse into an interconnected structure. The sucrose was leached from the scaffolds by immersion in water, yielding scaffolds that were 90% porous. To incorporate tumor lysates into PLG scaffolds, the biopsies of B16-F10 tumors that had grown subcutaneously in the backs of C57BL/6J mice (Jackson Laboratory) were digested in collagenase (250 U/ml) (Worthington) and suspended at a concentration equivalent to 10^7 cells per milliliter after filtration through 40-μm cell strainers. The tumor cell suspension was subjected to four cycles of rapid freeze in liquid nitrogen and thaw (37°C) and then
centrifuged at 400 rpm for 10 min. The supernatant (1 ml) containing tumor lysates was collected, incubated with the PLG microspheres, and lyophilized, and the resulting mixture was utilized in the high-pressure CO₂ process to foam macroporous PLG matrices incorporating tumor lysates.

[0289] To incorporate CpG-ODNs into PLG scaffolds, CpG-ODN 1826, 5’-tctagatctctctagtt-3’ (Invivogen, San Diego, CA; SEQ ID NO: 29) was condensed with poly(ethylenimine) (PEI) (Mₙ-60,000; Sigma Aldrich) molecules by dropping ODN 1826 solutions into PEI solution while vortexing the mixture (L. D. Harris, B. S. Kim, D. J. Mooney, Open pore biodegradable matrices formed with gas foaming. J. Biomed. Mater. Res. 42, 396-402 (1998); S. Cohen, T. Yoshioka, M. Lucarelli, L. H. Hwang, R. Langer, Controlled delivery systems for proteins based on poly(lactic/glycolic acid) microspheres. Pharm. Res. 8, 713-720 (1991); Y. C. Huang, M. Connell, Y. Park, D. J. Mooney, K. G. Rice, Fabrication and in vitro testing of polymeric delivery system for condensed DNA. J. Biomed. Mater. Res. A 67, 1384-1392(2003)). The charge ratio between PEI and CpG-ODN (NH₃⁺:PO₄⁻) was kept constant at 7 during condensation. PEI-CpG-ODN condensate solutions were then vortexed with 60 µl of 50% (w/v) sucrose solution, lyophilized, and mixed with dry sucrose to a final weight of 150 mg. The sucrose containing PEI-CpG-ODN condensate was then mixed with blank, GM-CSF, and/or tumor lysate-loaded PLG microspheres to make PLG cancer vaccines.

[0290] To achieve controlled GM-CSF and TLR agonist presentation, macroporous, poly-lactide-co-glycolide (PLG) matrices quickly release GM-CSF (Ali et al., 2009 Nat Mater, 2: 151-8); e.g., approximately 60% of the protein was released by day 10 (US 2013-0202707, incorporated herein by reference), to induce the recruitment of DCs or their precursors. GM-CSF loaded PLG scaffolds were also modified to present TLR-activating, CpG-ODN, MPLA and P(I:C) molecules, as danger signals. Presentation of the TLR agonists was designed to provide a long-term, local signal to activate DCs. Importantly, the relatively high molecular [0291] weight and composition of the particular PLG chosen to fabricate scaffolds results in slow scaffold degradation, allowing for long-term analysis of the vaccine site and its regulation over DC activation and T cell immunity.

[0292] The vaccine system of the invention is capable of generating prophylactic immunity against poorly immunogenic B16-F10 melanoma (O. A. Ali, N. Huebsch, L. Cao, G. Dranoff, D. J. Mooney, Infection-mimicking materials to program dendritic cells in situ. Nat. Mater. 8, 151-158 (2009) and US 2013-0202707, incorporated herein by reference). As described in US 2013-0202707, incorporated herein by reference, the vaccine system promotes and
extends CTL responses through naïve T cell differentiation induced by pDCs and CD8+ DCs, the corresponding production of type 1 IFNs and IL-12, and inhibition of negative feedback mechanisms.

[0293] As described in US 2013-0202707, incorporated herein by reference, vaccine formulations containing various TLR agonists produce significant and systemic anti-melanoma CTLs in correlation with the activation of specific DC subsets and reduce tumor burden. Inclusion of TLR agonists was activates DCs, in general, increasing their surface expression of MHCII and the costimulatory molecule, CD86, indicating an enhanced capacity to present antigen and activate T cell populations. In particular, appropriate TLR signaling enhanced the generation of CD8(+) and pDC subsets at the vaccine site and stimulated the production of IFNs and the potent T cell growth factor, IL-12.

[0294] In some embodiments, three different types of pathogen associated molecular patterns (PAMPs) are incorporated into or onto structural polymeric devices such as PLG disc structures/scaffolds to act as adjuvants in vaccines (3 types; a short oligonucleotide (CpG-ODN); a synthetic RNA - (Poly(I:C); P(I:C)), a synthetic lipid (monophosphoryl lipid A; MPLA). Such vaccine formulations recruit and activate dendritic cells in situ.

[0295] Vaccine-dependent survival in an aggressive melanoma cancer model correlates strongly with the ability of the vaccine to specifically activate 2 subsets of dendritic cells - CD8(+) DCs and plasmacytoid DCs - regardless of the adjuvant utilized in the vaccine system. This correlation has been confirmed utilizing 4 different vaccine adjuvants in the PLG vaccine. These vaccines induce potent tumor rejection in a therapeutic model of melanoma, by activating specific T cell responses that have been detected at the vaccine site and at tumors. These findings demonstrate the PLG vaccine system’s versatility in incorporating different types of agonists that stimulate different pathways in innate and adaptive immune responses.

The role of dendritic cells in the immune response

[0296] Dendritic cells (DCs) orchestrate immune responses to infection and tumors by priming and propagating specific, cytotoxic T lymphocyte (CTL) responses. Immature DCs residing in peripheral tissue detect foreign substances (i.e., antigens) unique to invading pathogens, and are activated by stimuli, such as pathogen associated molecular patterns (PAMPs) or products of dying cells (i.e., "danger signals"), originating during pathogen induced inflammatory responses. Maturing DCs mature both process and present antigens on major histocompatibility complexes (MHC) receptors, and express the costimulatory molecules CD80 and CD86, both of which are required for effector T-cell stimulation.
Another important result of DC maturation by ‘danger signaling’, is that DCs acquire the ability to home to the lymph nodes to engage and activate naive T-cells, enabling the T cells to recognize the antigens DCs are presenting.

The ability of particular DCs to initiate and control immune responses is a consequence of both their localization within tissues and their specialized capacity for mobilization. DCs originate from pluripotent stem cells in the bone marrow, enter the blood stream and localize into almost all organs. Based on the relative expression of a series of surface markers, different subsets of DCs or DC precursors can be identified in peripheral blood, including plasmacytoid DCs (pDCs) and conventional DCs (cDCs). pDCs are major type I interferon (IFN) producers, and specialize in activating adaptive immune responses to virus challenge via cytokine signaling. CD1c(+) cDCs, such as epidermal DCs, are especially adept at antigen presentation and co-stimulation of T cells.

Upon microbial invasion and inflammation, DCs rapidly migrate into the draining lymph nodes and primary sites of infection at rates that vastly outnumber other APCs, such as macrophages. The production of most DC subsets, including (pDCs) is controlled in the steady state by the cytokine Fms-related tyrosine kinase 3 ligand ligand (FL). Other cytokines, such as GM-CSF and CCL20, released by damaged or infected cells, actively recruit and localize cDCs to the sites of inflammation. In inflammatory models, both in vivo and in vitro, these inflammatory cytokines have been shown to also enhance DC migration and proliferation and may regulate DC activation state. The quantity of DCs activated during infection or within tumors is correlated with the strength of the subsequent immune response and disease prognosis.

To generate sufficient numbers of dendritic cells (DCs) for immunotherapy, laboratory-based culture of DC precursors with inflammatory cytokines, such as granulocyte macrophage-colony stimulating factor (GM-CSF) and FL (Flt3) has often been used. DCs modified in vitro to present tumor antigens are capable of eliciting antitumor effects in murine models upon transplantation. Initial clinical testing of ex vivo DC-based vaccines has revealed the induction of tumor regression in a subset of cancer patients, but little survival benefit. Protocols involving the ex vivo manipulation of DCs are limited by the quantities and types of DCs that can be produced, poor engraftment efficiency and LN homing, and loss of DC activation upon injection in the in vivo environment.

To address these limitations, infection-mimicking materials of the device present inflammatory cytokines in combination with a danger signal to recruit and activate DCs in vivo. Also, nanoparticles containing cytosine-guanosine (CpG) rich oligonucleotide (CpG-
ODN) sequences were immobilized onto scaffolds, as CpG-ODN are expressed in bacterial DNA, and are potent danger signals that can stimulate activation of matrix resident DCs. CD141+ DCs and plasmacytoid DCs are critical for successful cancer vaccination (prophylactic and therapeutic). Plasmacytoid DCs look like plasma cells, but have certain characteristics similar to myeloid dendritic cells, can produce high amounts of interferon-alpha, and are characterized by TLR7 and TLR9. The TLR agonist, CpG, binds to TLR9. CD8+ DCs in mice are equivalent to CD141+ dendritic cells. CD141+ DCs are found in human lymph nodes, bone marrow, tonsil, and blood. They are characterized by high expression of toll-like receptor 3 (TLR3), production of IL-12p70 and IFN-β, and superior capacity to induce T helper 1 cell responses, when compared with the more commonly studied CD1c+ DC subset.

Polyinosine-polycytidylic acid (poly I:C)-activated CD141+ DCs have a superior capacity to cross-present antigens to CD8+ cytotoxic T lymphocytes than poly PC-activated CD1c+ DCs. Thus, CD141+ DC subset represents an important functionally distinct human DC subtype with characteristics similar to those of the mouse CD8α+ DC subset. CD141+ DCs play a role in the induction of cytotoxic T lymphocyte responses and their activation is important for vaccination against cancers, viruses, and other pathogens.

p(PC) in the vaccine device stimulates CD141+ DCs in humans (CD8+ DCs in mice) and CpG stimulates plasmacytoid DCs. Devices with one or both of these TLR agonists lead to potent DC activation and the generation of significant prophylactic and therapeutic anti-tumor immune responses. A combination of different TLR agonists, e.g., a combination of p(PC) and CpG, in a device leads to a synergistic effect in the activation of a DC immune response against tumors.

PLG vaccines incorporating CpG-ODN and P(I:C) act synergistically to generate significant tumor inhibition, reduced tumor burden, and to generate improved anti-tumor immune responses.

Controlled release of cytokines and in vivo DC recruitment

Macroporous, poly-lactide-co-glycolide (PLG) matrices were designed to provide long-term and sustained release of GM-CSF, FL, and CCL20 and to house DCs for activation. These PLG scaffolds were 80-90% porous with an average pore size between 125-200 um to facilitate dendritic cell infiltration. The in vitro release kinetics for the three cytokines were similar, as the matrices quickly released protein with a burst over the first 5 days followed by sustained release over the next several weeks (US 2013-0202707, incorporated herein by reference).
**In vivo DC Activation**

[0306] PLG scaffolds were modified to present nanoparticles containing TLR-activating, CpG-ODN, as an infection-mimicking danger signal in concert with delivery with inflammatory cytokines. This dramatically enhanced DC activation *in situ* over control conditions lacking cytokine signaling.

[0307] Controlled mobilization and activation of DCs and DC precursors is of particular interest in the development of *ex vivo* DC based vaccines, and more generally the design of material systems that activate the immune system *in vivo*. As described herein, polymers which mimic key aspects of microbial infection effectively recruit DCs for cancer vaccination. PLG scaffolds engineered to release GM-CSF, FL, and CCL20 led to significant numbers of resident DCs, and the co-presentation of danger signals led to DC maturation. Even though all vaccine formulations were capable of inducing tumor protection in a therapeutic model of B16-F10 melanoma, GM-CSF and FL vaccines produced more antigen specific CTLs, higher levels of Th1 priming cytokines, and greater survival rates when compared to CCL20.

[0308] pDCs, and their cDC counterparts are targeted to exploit their specialized abilities to mediate anti-tumor T cell responses. In contrast to nanoparticle targeting systems, the polymer systems described herein not only serve as a antigen delivery devices to recruit and activate DCs, but also serve as a physical structure where DCs temporarily reside while they are activated.

[0309] The systems described herein demonstrated significant anti-tumor activity. In addition to the polymers, *e.g.*, PLG, described herein, matrices are optionally fabricated from other more inflammatory polymers to boost immune responses and DC mobilization. Another important aspect of subsequent T cell priming by these cells is LN homing. The exit or dispersement of DCs after antigen exposure is optimized by incorporating different adjuvants into the material to activate migratory function. Alternatively, other matrix properties, including degradation kinetics and porosity are altered to promote further control over DC trafficking.

[0310] FL, CCL20 and GM-CSF are utilized in biomaterial systems to mimic infection-induced recruitment of DCs *in situ*. As described in US 2013-0202707, *e.g.*, at page 111, line 17-page 113, line 17 (incorporated herein by reference), infection-mimicking porous devices are effective as therapeutic cancer vaccines.

**Antibodies**
[0311] As used herein, the term "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. By "specifically binds" or "immunoreacts with" is meant that the antibody reacts with one or more antigenic determinants of the desired antigen and does not significantly react with other antigens. Antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, dAb (domain antibody), single chain, F_{ab}, F_{ab'} and F_{(ab')2} fragments, scFvs, and F_{ab} expression libraries.

[0312] A single chain Fv ("scFv") polypeptide molecule is a covalently linked V_{H} : V_{L} heterodimer, which can be expressed from a gene fusion including V_{H} and V_{L}-encoding genes linked by a peptide-encoding linker. (See Huston et al. (1988) Proc Nat Acad Sci USA 85(16):5879-5883). A number of methods have been described to discern chemical structures for converting the naturally aggregated, but chemically separated, light and heavy polypeptide chains from an antibody V region into an scFv molecule, which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, *e.g.*, U.S. Patent Nos. 5,091,513; 5,132,405; and 4,946,778.

[0313] The term "antigen-binding site," or "binding portion" refers to the part of the immunoglobulin molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. Three highly divergent stretches within the V regions of the heavy and light chains, referred to as "hypervariable regions," are interposed between more conserved flanking stretches known as "framework regions," or "FRs". Thus, the term "FR" refers to amino acid sequences which are naturally found between, and adjacent to, hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as "complementarity-determining regions," or "CDRs."

[0314] As used herein, the term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin, an scFv, or a T-cell receptor. Epitopic determinants consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three dimensional structural characteristics, as well as specific charge characteristics. For example, antibodies may be raised against N-terminal or C-
terminal peptides of a polypeptide, linear or non-linear peptide sequences of a protein, as well as epitopes that comprise amino acids of a first antigen and those of a second antigen.

[0315] As used herein, the terms "immunological binding," and "immunological binding properties" refer to the non-covalent interactions of the type which occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (Kd) of the interaction, wherein a smaller Kd represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and geometric parameters that equally influence the rate in both directions. Thus, both the "on rate constant" (K_on) and the "off rate constant" (K_off) can be determined by calculation of the concentrations and the actual rates of association and dissociation. (Nature 361:186-87 (1993)). The ratio of K_off/K_on enables the cancellation of all parameters not related to affinity, and is equal to the dissociation constant Kd. Davies et al. (1990) Annual Rev Biochem 59:439-473. An antibody of the present invention is said to specifically bind to an antigen or epitope described herein (e.g., a CTLA, PDL1, or other immune inhibitory protein and/or tumor antigen) when the equilibrium binding constant (Kd) is ≤ 1 \( \mu \text{M} \), preferably ≤ 100 nM, more preferably ≤ 10 nM, more preferably ≤ 1 nM, and most preferably ≤ 100 pM to about 1 pM, as measured by assays such as radioligand binding assays or similar assays known to those skilled in the art.

**Routes of administration**

[0316] A pharmaceutical composition of the invention (e.g., an inhibitor described herein) is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intraperitoneal, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (i.e., topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and
agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form
of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0320] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressurized container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

[0321] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[0322] The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[0323] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as sustained/controlled release formulations, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art.

[0324] For example, the active ingredients can be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxyethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes,
albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions.

Sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) and can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811, incorporated herein by reference.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Dosages

The methods of the invention include administering one or more inhibitors of an immune-inhibitory protein described herein at a dosage of 0.01-10 mg/kg (e.g., 0.1-5 mg/kg) bodyweight. For example, the inhibitor is administered at a dosage of 0.01, 0.02, 0.05, 0.1,
0.3, 0.5, 1, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 mg/kg. In some embodiments, the inhibitor is administered every day, every other day, every 2 days, every 3 days, every 4 days, every 5 days, or every 6 days. In other embodiments, the inhibitor is administered every 1-10 weeks (e.g., every 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks). For example, the inhibitor is administered for a total of 7 days to 3 years (e.g., 7 days, 14 days, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 24 weeks, 36 weeks, 1 year, 1.5 years, 2 years, 2.5 years, or 3 years). For example, the inhibitor is administered indefinitely (e.g., at least 3 years). In some embodiments, the inhibitor is provided in an amount of 0.01-50 mg (e.g., 0.05-30 mg) per dose. For example, the inhibitor is administered in an amount of 0.01, 0.02, 0.05 mg, 0.1 mg, 0.2 mg, 0.4 mg, 0.8 mg, 2 mg, 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 40 mg, or 50 mg) per administration (e.g., per injection). In some cases, the inhibitor is administered biweekly. For example, the inhibitor is administered every other week for a total of 1-20 times (e.g., 1, 2, 4, 6, 8, 10, 15, or 20 times).

[0330] In some examples, the inhibitor (e.g., antibody described herein) is incorporated into or onto the vaccine device. In such cases, 0-100 mg (e.g., 5-100 mg, 10-100 mg, 20-100 mg, 30-100 mg, 40-100 mg, 50-100 mg, 60-100 mg, 70-100 mg, 80-100 mg, 90-100 mg, 1-95 mg, 1-90 mg, 5-95 mg, 5-90 mg, 5-80 mg, 5-70 mg, 5-60 mg, 5-50 mg, 5-40 mg, 5-30 mg, or 5-20 mg) of the inhibitor (e.g., antibody described herein) is present in the device. For example, the inhibitor (e.g., antibody described herein) is present in the device at a weight/weight concentration of at least 5% (e.g., at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or more).

[0331] For example, an inhibitor (e.g., anti-CTLA4 antibody or anti-PD1 antibody) is administered (e.g., systemically) at a dosage of 0.5-5 mg/kg (e.g., 3 mg/kg) body weight, e.g., 43 mg-435 mg per dose for a subject having a body weight of about 87 kg (e.g., 260 mg per dose on average). In some examples, the inhibitor (e.g., anti-CTLA4 antibody or anti-PD1 antibody) is administered (e.g., systemically) for 4 doses, e.g., at 0.5-5 mg/kg per dose (e.g., 3 mg/kg per dose), with a total dose of about 1000 mg after 4 doses. In other examples, the inhibitor (e.g., anti-CTLA4 antibody or anti-PD1 antibody) is administered in more than one dose (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more doses). In other examples, the inhibitor (e.g., anti-CTLA4 antibody or anti-PD1 antibody) is administered in more than one dose (e.g., 1, 2, 3, 4, 5, 6, 7 days or more, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks or more, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or more, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 years or more).
In some embodiments, ipilimumab is administered to a subject in need thereof at a dosage of 0.5-5 mg/kg (e.g., 3 mg/kg) body weight. For example, ipilimumab is administered every day, every other day, every 2 days, every 3 days, every 4 days, every 5 days, or every 6 days. For example, ipilimumab is administered once every 1-6 weeks (e.g., once every 3 weeks). For example, ipilimumab is administered to the subject for a total of 12 weeks or more. Ipilimumab is administered by routes such as injection or infusion. Ipilimumab is administered to a subject in need thereof for a total of 4 doses. For example, ipilimumab is administered at a dosage of 3 mg/kg body weight intravenously over 90 minutes every 3 weeks for a total of 4 doses. In some embodiments, ipilimumab is administered in combination (e.g., simultaneously or sequentially) with a vaccine device described herein.

In some cases, tremelimumab is administered to a subject in need thereof at a dosage of 1-20 mg/kg (e.g., 15 mg/kg) body weight. For example, tremelimumab is administered once every day, every other day, every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, or every week. For example, tremelimumab is administered once every 10-100 days (e.g., 90 days).

In some instances, MDX-1106 is administered to a subject in need thereof at a dosage of 0.01-10 mg/kg (e.g., 0.1-10 mg/kg) body weight (e.g., 0.01-1 mg/kg, 0.5-8 mg/kg, 1-10 mg/kg, or 2-8 mg/kg). For example, MDX-1106 is administered at a dosage of 10 mg/kg. MDX-1106 is administered, e.g., intravenously. In some cases, MDX-1106 is administered once every day, every other day, every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, or every week. In other cases, MDX-1106 is administered every 2 weeks, every 3 weeks, or every 4 weeks. For example, MDX-1106 is administered for a total period of at least 6 months (e.g., 6 months, 1 year, 2 years, 3 years or more).

The invention also contemplates administering MK3475 to a subject in need thereof at a dosage of 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 5 mg/kg, or 10 mg/kg bodyweight. MK3475 is administered once every day, every other day, every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, or every week. In another embodiment, MK3475 is administered every other week, every 2 weeks, or every 3 weeks.

In some cases, CT-011 is administered to a subject in need thereof at a dosage of 0.05-6 mg/kg (e.g., 0.2-6.0 mg/kg) body weight.

In some embodiments, MDX-1105 is administered to a subject in need thereof at a dosage of 0.01-10 mg/kg (e.g., 0.1-10 mg/kg) body weight (e.g., 0.01, 0.05, 0.1, 0.3, 1.3, or 10 mg/kg). For example, MDX-1105 is administered once every day, every other day, every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, or every week. In other cases,
MDX-1105 is administered every other week, every 2 weeks, or every 3 weeks. In a preferred embodiment, MDX-1105 is administered every 14 days for a total of at least 42 days.

[0338] The invention also provides for the administration of IMP321 to a subject in need thereof at a dosage of 0.01-30 mg (e.g., 0.050-30 mg, or 0.01, 0.05, 0.25, 1.25, 6.25, or 30 mg) per administration (e.g., per injection). For example, IMP321 is administered biweekly (e.g., for a total of at least 6 weeks, or at least 12 weeks). In other cases, IMP321 is administered once every day, every other day, every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, or every week. In some cases, IMP321 is administered at a dosage of 5 mg/kg. IMP321 is administered by routes, such as subcutaneous injection.

[0339] In some embodiments, the inhibitor(s) described herein is administered in combination (e.g., simultaneously or sequentially) with a vaccine device described herein. For example, the inhibitor(s) is delivered systemically, while the vaccine is delivered locally. In some embodiments, the inhibitor(s) is included in or on the vaccine device. For example, the inhibitor(s) and the vaccine are delivered locally.

[0340] In other examples, the inhibitor is administered at least 6 hours (e.g., at least 6 hours, at least 12 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 6 months, at least 8 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, at least 6 years, at least 8 years, or more) prior to administration of the vaccine device. In other embodiments, the vaccine device is administered at least 6 hours (e.g., at least 6 hours, at least 12 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 6 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, at least 6 years, at least 8 years, or more) prior to administration of the inhibitor(s).

[0341] For example, an inhibitor (e.g., antibody described herein) is administered systemically prior to administration (e.g., implantation) of the vaccine device. In some cases, the inhibitor (e.g., antibody) causes debulking of a tumor (i.e., regression). For example, the debulking of the tumor occurs prior to, during, and/or after administration of the vaccine device.

[0342] As used herein, the term, "about", is plus or minus 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, or 15%.
The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

**EXAMPLES**

Example 1: Treatment of tumor bearing mice with anti-CTLA4 and anti-PDL antibodies

Mouse models of melanoma tumors were used to determine the effect of blockade antibodies (anti-CTLA4 or anti-PDL antibodies) on tumor growth and survival. To establish melanoma tumors, mice were inoculated with 5x10^5 B16-F10 melanoma cells and allowed to develop for 9 days.

Mice bearing established melanoma tumors were treated with intraperitoneal (i.p.) injections of anti-CTLA4 or anti-PDL antibodies. Antibody treatments were administered every 3 days and initiated on Day 3 of tumor challenge.

Tumor growth and survival of the mice were compared between untreated mice versus antibody-treated mice. Mice that were treated with either anti-CTLA4 antibody or anti-PDL antibody had smaller tumor sizes (Fig. 1A) and longer survival times (Fig. IB) than untreated mice.

The anti-CTLA4 antibody (9D9, catalog # BE0086) and anti-PDL antibody (RMP1-14, catalog # BE0146**) were purchased from Bioxcell.

Example 2: Tumor protection and T cell activity induced by therapeutic PLG vaccination in combination with blockade antibodies

The effect of combining therapeutic PLG vaccination with an anti-PDL or anti-CTLA4 antibody was determined using mouse models of melanoma tumors. To establish melanoma tumors, mice were inoculated with 5x10^5 B16-F10 melanoma cells and allowed to develop for 9 days.

The melanoma tumor bearing mice were either untreated, treated with PLG vaccines alone, or treated with PLG vaccines in combination with anti-PDL or anti-CTLA4 antibodies. The antibody treatments were initiated on Day 3 after tumor challenge (with B16-F10 cells, as described above) and injected i.p. every 3 days for 24 days after tumor challenge. PLG vaccination was performed 9 days after tumor challenge. Tumor size (area in mm^2) and survival were determined for each treatment group. The tumor area is the product of the two longest diameters of the tumor. Tumor diameters were measured using standard methods (e.g., with calipers). Mice treated with vaccine alone survived longer and had tumors with smaller area than untreated mice (Figs. 2A-B). Surprisingly, mice treated with vaccines in
combination with either anti-PD1 or anti-CTLA4 antibodies survived longer and had tumors with smaller area than mice treated with vaccine alone (Figs. 2A-B).

Example 3: Engineered vaccines in combination with blockade antibodies enhances intratumoral effector T cell activity.

[0350] The total number of CD3+CD8+ tumor infiltrating T cells in each treatment group (i.e., untreated, vaccine alone, vaccine + anti-PD1 antibody, or vaccine + anti-CTLA4 antibody) was determined from B16 (a type of melanoma cell) tumors isolated from the mice. CD3, also called the T cell co-receptor, is a marker for T cells, as it is expressed on the surface of all mature T cells and is required for T cell activation. CD8 is a marker for cytotoxic T lymphocytes (CTLs). An increase in the number of CD3+CD8+ T cells that have infiltrated the tumor indicates an increased immune response against the tumor.

[0351] Also, the ratio of CD3+CD8+ T cells to CD3+FoxP3+ T regulatory (Treg) cells isolated from the B16 tumors of the mice was determined in each treatment group. FoxP3 is a marker for Treg cells, which modulate (e.g., suppress) the immune response. Thus, the ratio provides a measure of strength of the CTL versus Treg response. A higher ratio indicates an increased immune response against the tumor.

[0352] Antibodies were administered i.p. every 3 days starting on Day 3 after tumor challenge (with 5x10^5 B16-F10 cells) and vaccination was initiated 9 days after tumor challenge. The B16 tumors were extracted at day 20 to determine the types of T cells that have infiltrated the tumor. The number of tumor infiltrating CD3+CD8+ T cells was significantly higher in the vaccine treated mice than untreated mice (Fig. 3A). Also, the ratio of CD3+CD8+ T cells to CD3+FoxP3+ Treg cells was significantly higher in the vaccine treated mice than untreated mice (Fig. 3B). Surprisingly, the number of tumor infiltrating CD3+CD8+ T cells was significantly higher in the mice treated with a combination of vaccine + antibody compared to those treated with vaccine alone (Fig. 3A). Also, the ratio of CD3+CD8+ T cells to CD3+FoxP3+ Treg cells was significantly higher in the mice treated with a combination of vaccine + antibody compared to those treated with vaccine alone (Fig. 3B).

[0353] Taken together, these results indicate that the combination of PLG vaccines with anti-PD1 or anti-CTLA4 antibodies synergistically decreases tumor size, extends survival time, and enhances the T effector cell activity relative to Treg cell activity in the population of T cells isolated from tumors.

[0354] In addition, scaffold infiltrating leukocytes, specifically, the percentage of CTLs, were
compared by flow cytometry among the treatment groups (i.e., blank matrices, PLG vaccines alone, or vaccines in combination with anti-PDL or anti-CTLA4 antibodies) at 14 days post-implantation in mice. The antibody treatments were administered on days 0, 3, 6, 9 and 12 after vaccination. Single cell suspensions were prepared from scaffolds at Day 14 and stained for activated, CTL markers, CD8 and CD107a. The percentage of the cells in the scaffold that were positive for both markers was greater in the combination therapy than the vaccine alone treated mice (Fig. 5A).

Also, the fold increase (relative to blank controls) of CD8+, scaffold-infiltrating T cells positive for both IFNγ and CD107a was compared in blank matrices, PLG vaccines alone, and vaccines in combination with anti-PDL or anti-CTLA4 antibodies at 14 days post-implantation in mice. The antibody treatments were administered on days 0, 3, 6, 9 and 12 after vaccination. The vaccines were implanted 7 days after tumor challenge. CD107a is a marker for CTLs, and IFNγ is a cytokine involved in the immune response against tumors, and viral and bacterial infections. The fold increase in activated CD8+ T cells positive for IFNγ and CD107a was significantly greater in scaffolds from mice treated with the combination vaccine + antibody than vaccine alone (Fig. 5B).

Thus, the vaccine works synergistically with the blockade antibodies to enhance T effector cell activity locally, i.e., at the site of the implanted vaccine or within a vaccine. The vaccine plus blockade antibody combination also works synergistically to enhance the infiltration of tumors by activated CD8+ T cells (e.g., CTLs) and to enhance the T cell activity at tumor sites.

Example 4: Engineered PLG vaccine in combination with blockade antibodies enhances local T effector cell activity

The effect of the combination of a PLG vaccine with a blockade antibody on local T effector cell activity (i.e., at the site of vaccine scaffold implantation) was determined. Mice were treated with PLG vaccines alone or PLG vaccines in combination with an anti-CTLA4 antibody for 14 days. A subset of mice were treated with antibody and vaccine without tumor challenge to analyze the effects at the vaccine site. Another subset of mice were challenged with 500,000 B16 tumor cells. Vaccines were administered 7 days after tumor challenge. The antibody treatments were administered on days 0, 3, 6, 9 and 12 after vaccination.

Effects at the tumor site were examined, specifically the numbers of cytotoxic T cells, interferon gamma expression, CD107a expression, and Treg cell numbers. Flow cytometry
was used to determine the number of CD3+ T cell infiltrates into the implanted vaccine scaffolds that were isolated from the two treatment groups. Mice treated with the combination (vaccine + anti-CTLA4 antibody) had more T cell infiltrates in the scaffolds than mice treated with vaccine alone (Fig. 4A).

[0359] The total number of CD3+CD8+ T effector cells was also determined in mice implanted with blank matrices without vaccine, PLG vaccines alone, or vaccines in combination with anti-PD1 or anti-CTLA4 antibodies for 14 days. The antibody treatments were administered on days 0, 3, 6, 9 and 12 after vaccination. Surprisingly, the combination treatments led to a significantly higher number of CD3+CD8+ T effector cells infiltrated into the scaffolds than vaccine alone (Fig. 4B).

Example 5: Engineered PLG vaccine in combination with CTLA-4 maintains local T cell activity

[0360] The amount of T cell infiltration into the PLG vaccines implanted in mice for 14 days was determined. Flow cytometry was used to determine the phenotypes (i.e., CD4+CD8+ versus CD4+FoxP3+) of T cell infiltrates isolated from PLG implants in mice treated with PLG vaccines alone (Vax) or in combination with anti-CTLA4 antibody (VAX+CTLA4) or with anti-PD1 antibody (VAX+PD1) (Fig. 6A). The proportion of CD4+ T cells that express CD8 was higher in the VAX+CTLA4 and VAX+PD1 mice than the VAX alone mice. Most of the CD4+ T cells in the VAX+CTLA mice had low expression levels of FoxP3 (Fig. 6A). Also, the ratio of CD3+CD8+ effector T cells to CD4+FoxP3+ T cells was determined for cell isolated from PLG implants in mice treated with PLG vaccines alone (VAX) or in combination with anti-CTLA4 antibody (VAX+CTLA4) or with anti-PD1 antibody (VAX+PD1) for 30 days (Fig. 6B). The ratio of CD3+CD8+ effector T cells to CD4+FoxP3+ T cells in the VAX+CTLA4 mice was significantly higher than VAX mice or VAX+PD1 mice (Fig. 6B). Thus, the combination of the PLG vaccine with an anti-CTLA4 antibody maintains local T cell activity and skews the T cell response toward cytotoxic T cell activity relative to suppressive Treg activity. These activities and responses are maintained for an extended period of time, e.g., for at least 30 days.

Example 6: Effector T cell activity is greater than regulatory T cell activity in vaccine draining lymph nodes

[0361] Mice were treated with vaccine alone or in combination with anti-CTLA4 or anti-PD1 antibodies. The antibody treatments were administered on days 0, 3, 6, 9, and 12 after
vaccination. The vaccine draining lymph nodes were then extracted at day 14 to measure the
degree of T cell infiltration. Flow cytometry was used to quantify the percentage of CD8+ T
cells and FoxP3+ Treg cells in the vaccine draining lymph nodes. The ratio of CD3+CD8+ T
cells to CD3+FoxP3+ Treg cells was also determined.

By flow cytometry, the percentage of CD8+ T effector cells in the lymph nodes of
mice treated with a combination of vaccine + antibody was greater than those from vaccine
alone treated mice (Figs. 7A and 8A). Also, the percentage of FoxP3+ Treg cells in the
lymph nodes of mice treated with the combination vaccine + antibody was lower than that of
the vaccine alone treated mice (Figs. 7B and 8A). The ratio of CD3+CD8+ T cells to
CD3+FoxP3+ Treg cells in the lymph nodes of the combination vaccine + anti-CTLA4
antibody treated mice was significantly higher than that of the vaccine alone or the
combination vaccine + anti-PDL antibody treated mice (Fig. 8B).

Thus, the vaccine works synergistically with the blockade antibodies, in particular, the
anti-CTLA4 antibody, to increase the proportion of T effector cells and decrease the
proportion of Treg cells in the vaccine draining lymph nodes.

Example 7: Combining anti-PDL antibody and anti-CTLA4 antibody with PLG vaccination
enhances T cell activation and tumor inhibition in melanoma models.

Described herein is data related to structural vaccines in combination with checkpoint
antibodies. Combining checkpoint blockade inhibitors, oc-PD-1 and α-CTLA4, with PLG
vaccination had a significant effect on tumor growth in comparison to vaccination with either
antibody alone (Fig. 9A). The antibody treatments were administered i.p. as described for
vaccination experiments until tumor excision at day 35 for T cell infiltration analysis.
Vaccination was initiated 9 days after tumor challenge. At day 35 after tumor challenge,
mice treated with PLG vaccination in combination with either oc-PD-1 or α-CTLA4 antibody
alone had an approximately 2.2 - 2.6 fold inhibition in tumor progression relative to
vaccination alone (Fig. 9A). Combining both antibodies with vaccination resulted in about a
5-fold decrease in B16 tumor growth at day 35 (Fig. 9A). The inhibition of tumor growth
correlated with the magnitude of T cell infiltration into tumors. The combination of all three
treatments (oc-PD-1, α-CTLA4 and PLG vaccination) enhanced the numbers of C8(+) T cells
in tumors, FoxP3(+) Tregs and the CD8 T cell/Treg ratio relative to other treatments (Fig. 9B-
Fig. 9D). These data suggest that the tumor inhibition induced by combining vaccination
with blockade treatments is likely due to enhanced T cell activation and cytotoxicity as
opposed to blocking the immune suppression mediated by Tregs as reported elsewhere.
Example 8: Combining anti-PD1 antibody and anti-CTLA4 antibody with PLG vaccination enhances cytotoxic T cell response

[0365] As described in detail below, combining blockade antibodies with PLG vaccination significantly skewed the tumor infiltrating leukocyte (TIL) response toward active, cytotoxic T cells, relative to suppressive Tregs (Figure 10A-Figure 10E and Figure 11). This is consistent with the finding of tumor regression, as higher CD8/Treg ratios within tumors are indicative of effective vaccination (Curran et al., 2010 PNAS U.S.A., 107, 4275-4280). For Figures 10A-10E, the antibody treatments were administered i.p. as described for vaccination experiments until tumor excision at day 18 for Tcell infiltration analysis. Vaccination was initiated 9 days after tumor challenge. All cellular staining was performed on the total cell suspension extracted from tumors. Similarly, for Figure 11, the antibody treatments were administered i.p. as described for vaccination experiments (every 3 days) until tumor excision at day 30 for Tcell infiltration analysis. Vaccination was initiated 9 days after tumor challenge.

[0366] PLG Vaccination at day 9 after tumor challenge induced significant levels of CD3(+)CD8(+) T cell infiltration into 20-day-old B16 tumors, resulting in approximately 3,500 cytotoxic T cells per mm² of tumor (Figure 10A). The addition of anti-PD-1 treatment to vaccination did not have a significant effect on the total numbers of tumor infiltrating CD3(+)CD8(+) T cells, whereas the addition of anti-CTLA-4 therapy produced cytotoxic T cell levels reaching over 17,000 CD3(+)CD8(+) T cells per mm² of tumor (Figure 11). In contrast, these treatment groups had no effect on the numbers of tumor-resident CD4(+)FoxP3(+) Tregs (Figure 10B). The intratumoral ratio of CD8(+) effectors to Tregs at Day 18 almost doubled with PD-1 antibody administration compared to vaccination alone (Figure 10C). Strikingly, combining anti-CTLA-4 with vaccination resulted in a 9-fold increase in the Teff/Treg ratio compared to vaccination alone at Day 18 (25.3 to 2.8; Figure 10C). The same analysis was conducted at Day 30 after tumor challenge, and only immunizations combined with anti-CTLA-4 were able to generate significant CD8/Treg ratios (approximately 6-fold increase; Figure 11) consistent with the long-term survival data. In addition, supplementing vaccination with PD-1 or CTLA-4 antibody therapy resulted in 3-fold and 8-fold increases in intratumoral, cytotoxic T cell activation, as determined by CD107a and IFN-γ co-expression (Figure 10D and Figure 10E). The addition of checkpoint blockade enhanced not only the density of activated, CD8(+) TILs, but also the percentage of total CD8(+) T cells that were activated (Figure 10D and Figure 10E), indicating that these
treatments promoted T cell cytotoxicity locally, within tumors.

All tumors were pretreated with antibody blockade prior to vaccination because this sequence likely reflects the clinical setting where these antibodies are used to initially treat tumors as they become standards of care. However, if antibody administration is ceased after vaccination, the effects on tumor inhibition are lost (Figure 12), suggesting that blockade treatment significantly augments the subsequent T cell responses induced by vaccination. In Figure 12, four antibody treatments were administered on days 0, 3, 6, and 9. Mice were vaccinated on day 9 after tumor challenge and tumors size measurements were recorded at day 26 after tumor challenge.

OTHER EMBODIMENTS

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 invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All United States patents and published or unpublished United States patent applications cited herein are incorporated by reference. All published foreign patents and patent applications cited herein are hereby incorporated by reference. Genbank and NCBI submissions indicated by accession number cited herein are hereby incorporated by reference. All other published references, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.
CLAIMS

We claim:

1. A device comprising:
   a) an inhibitor of an immune-inhibitory protein;
   b) a scaffold composition;
   c) a cell recruitment composition; and
   d) a bioactive composition, wherein the bioactive composition is incorporated into or coated onto the scaffold composition, and wherein the bioactive composition causes modification of cells in or recruited to the device.

2. The device of claim 1, wherein the immune-inhibitory protein is selected from the group consisting of cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), programmed cell death protein 1 (PD1), programmed cell death protein 1 ligand (PDL1), lymphocyte activation gene 3 (LAG3), B7-H3, B7-H4, and T cell membrane protein 3 (TIM3).

3. The device of claim 2, wherein the immune-inhibitory protein is CTLA4.

4. The device of claim 2, wherein the immune-inhibitory protein is PD1.

5. The device of claim 1, comprising an inhibitor of CTLA4 and an inhibitor of PD1.

6. The device of claim 1, wherein the inhibitor comprises a protein, peptide, or nucleic acid.

7. The device of claim 2, wherein the inhibitor comprises an antibody or fragment thereof.

8. The device of claim 7 wherein the antibody or fragment thereof binds to CTLA4.

9. The device of claim 8, wherein the antibody or fragment thereof is Ipilimumab, Tremelimumab, or a fragment thereof.
10. The device of claim 2, wherein the inhibitor binds to PD1, and wherein the inhibitor is a protein.

11. The device of claim 10, wherein the inhibitor is MDX-1 106, MK3475, CT-011, AMP-224, or a fragment thereof.

12. The device of claim 10, wherein the inhibitor is a PD-L2-immunoglobulin (Ig) fusion protein.

13. The device of claim 2, wherein the inhibitor is a protein, and wherein the inhibitor binds to PDL1.

14. The device of claim 13, wherein the inhibitor is MDX-1 105.

15. The device of claim 2, wherein the inhibitor is a protein, and wherein the inhibitor binds to LAG3.

16. The device of claim 15, wherein the inhibitor is a LAG3-Ig fusion protein.

17. The device of claim 16, wherein the LAG3-Ig fusion protein is IMP321.

18. The device of claim 2, wherein the inhibitor is a protein, and wherein the inhibitor binds to B7-H3.

19. The device of claim 18, wherein the inhibitor is MGA271.

20. The device of claim 1, wherein the cell recruitment composition recruits an immune cell.

21. The device of claim 20, wherein the immune cell comprises an antigen presenting cell.

22. The device of claim 21, wherein the antigen presenting cell comprises a dendritic cell.
23. The device of claim 20, wherein the immune cell comprises a macrophage, a T cell, a B cell, a natural killer (NK) cell, or a dendritic cell.

24. The device of claim 1, wherein the scaffold comprises open, interconnected macropores.

25. The device of claim 1, further comprising a deployment signal capable of inducing or promoting migration of cells.

26. The device of claim 25, wherein the deployment signal comprises a protein, peptide, or nucleic acid.

27. The device of claim 25, wherein the deployment signal comprises
   i) one or more factors that induces migration of cells and has or is capable of forming a gradient;
   ii) a nucleic acid molecule encoding a protein that induces migration of cells out of the device; or
   iii) depletion or diffusion of the cell recruitment composition.

28. The device of claim 1, wherein the cell recruitment composition comprises a cytokine, chemokine, or growth factor.

29. The device of claim 1, wherein the cell recruitment composition comprises GM-CSF, Flt3L, or CCL20.

30. The device of claim 1, wherein the bioactive composition comprises a target antigen composition.

31. The device of claim 1, wherein the cell recruitment composition recruits an immune cell to the device, where the immune cells encounters the target antigen, and where the immune cell resides until a deployment signal induces egress of the immune cell to a lymph node tissue outside of the device.
32. The device of claim 31, wherein the level of immune activation of the immune cell at egress is greater than that prior to entering the device.

33. The device of claim 31, wherein the immune cell is antigen-primed at egress compared to the level of priming prior to entering the device.

34. The device of claim 30, wherein the target antigen composition comprises a cancer antigen or a cancer derived antigen.

35. The device of claim 1, wherein the cancer cell is derived from a melanoma, a central nervous system (CNS) cancer, a CNS germ cell tumor, a lung cancer, leukemia, multiple myeloma, a renal cancer, a malignant glioma, a medulloblastoma, a breast cancer, an ovarian cancer, a prostate cancer, a bladder cancer, a fibrosarcoma, a pancreatic cancer, a gastric cancer, a head and neck cancer, or a colorectal cancer.

36. The device of claim 34, wherein the cancer-derived antigen is selected from the group consisting of MAGE series of antigens, MART-1/melanA, Tyrosinase, ganglioside, gp100, GD-2, O-acetylated GD-3, GM-2, MUC-1, Sosl, Protein kinase C-binding protein, Reverse transcriptase protein, AKAP protein, VRK1, KIAA1735, T7-1, Til-3, Til-9, Homo Sapiens telomerase ferment (hTRT), Cytokeratin-19 (CYFRA21-1), SQUAMOUS CELL CARCINOMA ANTIGEN 1 (SCCA-1), (PROTEIN T4-A), SQUAMOUS CELL CARCINOMA ANTIGEN 2 (SCCA-2), Ovarian carcinoma antigen CA125 (1A1-3B) (KIAA0049), MUCIN 1 (TUMOR-ASSOCIATED MUCIN), (CARCINOMA-ASSOCIATED MUCIN), (POLYMORPHIC EPITHELIAL MUCIN),(PEM),(PEMT),(EPISALIN), (TUMOR-ASSOCIATED EPITHELIAL MEMBRANE ANTIGEN), (EMA),(H23 AG), (PEANUT-REACTIVE URINARY MUCIN), (PUM), (BREAST CARCINOMA-ASSOCIATED ANTIGEN DF3), CTCL tumor antigen sel-1, CTCL tumor antigen sel4-3, CTCL tumor antigen se120-4, CTCL tumor antigen se20-9, CTCL tumor antigen se33-1, CTCL tumor antigen se37-2, CTCL tumor antigen se57-1, CTCL tumor antigen se89-1, Prostate-specific membrane antigen, 5T4 oncofetal trophoblast glycoprotein, Orf73 Kaposi's sarcoma-associated herpesvirus, MAGE-C1 (cancer/testis antigen CT7), MAGE-B1 ANTIGEN (MAGE-XP ANTIGEN) (DAM10), MAGE-B2 ANTIGEN (DAM6), MAGE-2 ANTIGEN, MAGE-4a antigen, MAGE-4b antigen, Colon cancer antigen NY-CO-45, Lung

37. The device of claim 1, wherein the bioactive composition comprises a tumor lysate.

38. The device of claim 1, wherein the bioactive composition comprises irradiated tumor cells.

39. The device of claim 1, wherein the bioactive composition comprises a cancer cell surface antigen.

40. The device of claim 1, wherein the bioactive composition comprises a viral or bacterial antigen.

41. The device of claim 1, wherein the device further comprises an adjuvant.

42. The device of claim 41, wherein the adjuvant comprises a CpG rich oligonucleotide.

43. The device of claim 42, wherein the adjuvant comprises a condensed CpG oligonucleotide.

44. The device of claim 43, wherein the adjuvant comprises a PEI-CpG oligonucleotide.

45. The device of claim 1, wherein the scaffold further comprises an RGD-modified alginate.
46. The device of claim 1, wherein the device further comprises a toll-like receptor (TLR) agonist.

47. The device of claim 46, wherein the TLR agonist preferentially binds to TLR3.

48. The device of claim 46, wherein the TLR agonist comprises a TLR3 agonist.

49. The device of claim 48, wherein the TLR3 agonist comprises polyinosine-polycytidylic acid (poly I:C) or PEI-poly (I:C).

50. The device of claim 1, wherein the scaffold comprises a hydrogel or porous polymer, said scaffold comprising a polymer or co-polymer of polylactic acid, polyglycolic acid, PLGA, alginate, gelatin, collagen, agarose, poly(lysine), polyhydroxybutyrate, poly-epsilon-caprolactone, polyphosphazines, poly(vinyl alcohol), poly(alkylene oxide), poly(ethylene oxide), poly(allylamine), poly(acrylate), poly(4-aminomethylstyrene), pluronic polyol, polyoxamer, poly(uronic acid), poly(anhydride) or poly(vinylpyrrolidone).

51. The device of claim 50, wherein the porous polymer is produced by gas-foaming.

52. The device of claim 1, wherein the device is in the form of a bead, pellet, sheet, or disc.

53. A method of killing a cancer cell in a subject in need thereof comprising administering the device of claim 1.

54. A method of killing a cancer cell in a subject in need thereof comprising administering:
a) an inhibitor of an immune-inhibitory protein; and
b) a device comprising
i) a scaffold composition,
ii) a cell recruitment composition, and
iii) a bioactive composition, wherein the bioactive composition is incorporated into or coated onto the scaffold composition, and wherein the bioactive composition causes modification of
cells in or recruited to the device.

55. The method of claim 54, wherein the scaffold comprises open, interconnected macropores, and wherein migration of the modified cells to another site in the body is promoted by the open, interconnected macropores and by the deployment signal.

56. The method of claim 55, wherein the other site in the body is a nearby or remote tissue target.

57. The method of claim 54, wherein the inhibitor is present in or on the device.

58. The method of claim 57, wherein the inhibitor is coated in or on the scaffold composition.

59. The method of claim 54, wherein the inhibitor is not present in or on the device.

60. The method of claim 59, wherein the inhibitor is not coated in or on the scaffold composition.

61. The method of claim 54, wherein the inhibitor and the device are formulated together.

62. The method of claim 54, wherein the inhibitor and the device are formulated separately.

63. The method of claim 62, wherein the inhibitor and the device are administered to the subject simultaneously.

64. The method of claim 62, wherein the inhibitor and the device are administered to the subject sequentially.

65. The method of claim 53 or 54, wherein the device is implanted subcutaneously into the subject.

66. The method of claim 54, wherein the inhibitor is administered intravenously, intraperitoneally, subcutaneously, orally, intradermally, by inhalation, transmucosally, or rectally.
67. The method of claim 54, wherein the inhibitor is administered by injection, infusion, or inhalation.

68. The method of claim 54, wherein the inhibitor is administered at a dosage of 0.01-10 mg/kg bodyweight.

69. The method of claim 54, wherein the inhibitor is administered in an amount of 0.01-30 mg per dose.

70. The method of claim 53 or 54, wherein the subject comprises a cancer cell, wherein the cancer cell is poorly immunogenic.

71. The method of claim 70, wherein the cancer cell is resistant to cytotoxic T-lymphocyte (CTL)-mediated lysis.

72. The method of claim 70, wherein the cancer cell is resistant to natural killer (NK) cell mediated killing.

73. The method of claim 53 or 54, wherein the subject does not comprise an autoantibody.

74. The method of claim 54, wherein the inhibitor of an immune-inhibitory protein comprises an inhibitor of CTLA4 and an inhibitor of PD1.

75. The method of claim 74, wherein said inhibitor of CTLA4 comprises an anti-CTLA-4 antibody, and wherein said inhibitor of PD1 comprises an anti-PD1 antibody.

76. The method of claim 54, wherein the scaffold comprises a hydrogel or porous polymer, said scaffold comprising a polymer or co-polymer of poly (D,L-lactide-co-glycolide) (PLG).

77. The method of claim 54, wherein cytotoxic T cells are enhanced relative to immunosuppressive Treg cells.
78. The method of claim 54, wherein said inhibitor of an immune-inhibitory protein is administered prior to and subsequent to administration of the device.
FIG. 6A

- VAX
- VAX + CTLA4
- VAX + PD-1
FIG. 6B

CD8(+) CTLs vs Treg Ratio at Vaccine Site

Vax  VAX+CTLA4  Vax+PD1
FIG. 9

A

B

C

D

10/13
FIG. 10

A

CD107a+ Tumor-Infiltrating T cells (cells per mm² tumor)

B

CD107a+ Tumor-Infiltrating Tregs (cells per mm² tumor)

C

CD8+ T cell ratio

D

E

Actuated Tumor-Infiltrating T cells (cells per mm² tumor)
FIG. 11

CD8(+) CTLs vs Treg Ratio at Tumor Site at Day 30

V  V+P  V+C

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