Title: COMPOSITIONS AND METHODS FOR MODULATING CANCER CELL METABOLISM

Abstract: Compositions typically including a modulator of cancer cell metabolism and a cancer cell- or a glucose-depleted and/or lactate-rich disease environment targeting moiety, wherein the targeting moiety is associated with, linked, conjugated, or otherwise attached directly or indirectly to the modulator, or to a nanoparticle or other delivery vehicle thereof, and methods of use for treating cancer are provided. Pharmaceutical compositions including the modulator and a pharmaceutically acceptable carrier are also provided. The pharmaceutical compositions can be administered to a subject in need thereof in an effective amount to reduce one or symptoms of the cancer. Such pharmaceutical compositions can be administered prior to or in conjunction with an immune therapy such as adoptive T cell therapy. In an embodiment, PLGA-PEG nanoparticles, which are targeted to the mitochondria by a lipophilic triphenylphosphonium (TPP) cation and which contain a modulator of cancer cell metabolism, are used.
COMPOSITIONS AND METHODS FOR MODULATING CANCER CELL METABOLISM

CROSS-REFERENCE TO RELATED APPLICATIONS
This application claims benefit of U.S. Provisional Application No. 62/262,203, filed December 2, 2015, the contents of which are incorporated by reference herein in their entirety.

FIELD OF THE INVENTION
The field of the invention generally relates to compositions and methods for modulating cancer cell metabolism to increase cancer cell death, reduce cancer cell proliferation, or increase cancer cell sensitivity to conventional cancer therapies.

BACKGROUND OF THE INVENTION
Metabolic aberrations in the form of altered flux through key metabolic pathways are primary hallmarks of many malignant tumors. Among the many adjustments of the metabolic pathways that are found in tumor cells, a key role is played by an enhanced aerobic glycolysis followed by lactic fermentation, which is also known as the Warburg effect (Warburg, O. Science, 123:309 (1956)). Normal cells generally transform glucose into carbon dioxide and water under aerobic conditions, by means of oxidative phosphorylation (OXPHOS). On the contrary, invasive cancer cells mostly produce lactate, even in the presence of sufficient levels of oxygen, even though this glycolytic pathway turns out to be less efficient than OXPHOS in producing ATP units. This apparently counterproductive behavior of cancer cells actually constitutes a survival advantage in rapidly proliferating cells, since it make them insensitive to transient or permanent hypoxic conditions, it contributes to the production of nucleosides and amino acids, and constitutes a very rapid way to produce energy due to the enhanced glucose uptake occurring in cancer tissues. Furthermore, lactate is not just a waste product of this process; on the contrary, it promotes tumor invasion by favoring cell migration, angiogenesis, immune escape and radioresistance (Draoui, N, et al., Dis. Model. Mech., 4:727 (2011)). For example, rather than using lactate as a nutrient, cancer cells generally export lactate, leading
to acidification of the tumor environment and a local inflammatory response that drives tumorigenesis (Doherty and Cleveland, J Clin Invest 2013 Sep 3; 123(9): 3685-3692). Lactate in the tumor cell microenvironment also appears to impair the adaptive immune response, disabling immune surveillance, in part by inhibiting immune cell metabolism.

Although there is a great deal of investigation into these mechanisms of tumor survival, to date no one has used the pathway as a means to selectively kill or inhibit the growth and proliferation of tumor cells.

There remains a need for therapeutic strategies for overcoming the metabolic advantages that promote tumor cell survival.

It is an object of the invention to provide compositions and methods for altering or reducing metabolism in cancer cells, and preferably directly, or indirectly via immune cells, leading to cancer cell death.

It is another object of the invention to reduce by-products and other affects associated with aberrant metabolism in cancer cells in an effective amount to reduce suppression of, or activate an immune response against the cancer cells.

It is a further object of the invention to provide compositions and methods that enhance and/or prolong the activation of T cells (i.e., increase antigen-specific proliferation of T cells, enhance cytokine production by T cells, stimulate differentiation, stimulate effector functions of T cells, and/or promote T cell survival), overcome T cell exhaustion and/or anergy, and combinations thereof.

SUMMARY OF THE INVENTION

Compositions including a modulator of cancer cell metabolism and preferably a cancer cell- or a glucose-depleted and/or lactate-rich disease environment-targeting moiety, wherein the targeting moiety is associated with, linked, conjugated, or otherwise attached directly or indirectly to the modulator, or to a nanoparticle or other delivery vehicle thereof, and methods of use for treating cancer are provided. Preferably, the compositions target cancer cells selectively over immune cells, particularly Tumor Infiltrating Lymphocytes (TIL). In some embodiments, the modulator reduces cancer cell glycolysis. The modulator can be, for
example, a glucose transporter (GLUTs) inhibitor, a hexokinase inhibitor, a phosphofructokinase inhibitor, aglyceraldehyde-3-phosphate dehydrogenase (GAPDH) inhibitor, a phosphoglycerate mutase (PGM) inhibitor, an enolase (ENO) inhibitor, a pyruvate kinase (PK) activator, a lactate dehydrogenase inhibitor, a glutaminase (GLS) inhibitor, a pyruvate dehydrogenase (PDH) activator, a pyruvate dehydrogenase kinase inhibitor or a glucose-phosphate dehydrogenase (G6PD) inhibitor. In some embodiments, the modulator reduces the tricarboxylic acid (TCA) cycle in cancer cells. The modulator can also inhibit a monocarboxylate transporter (MCTs) in cancer cells. The modulator can be, for example, a small molecule or a functional nucleic acid. Formulations may contain two or more inhibitors, selected to maximize tumor death or inhibition of proliferation.

Preferably the modulator, for example via the targeting moiety, is preferentially delivered cancer cells. Preferably the modulator does not target or otherwise more than minimally modulate the metabolism of non-cancer cells, particular immune cells, or does so at a reduced level compared to cancer (e.g., tumor) cells. In this way, by-products and other effects associated with aberrant metabolism in cancer cells are reduced, preferably leading directly or indirectly to cancer cell death. In some embodiments, the modulator is targeted to the mitochondria. In some embodiments, the modulator reduces cancer cell migration, angiogenesis, immune escape and radioresistance. In preferred embodiments, the modulator induces a change in the cancer cell itself or its microenvironment that reduces suppression or induces activation of an immune response against the cancer cell. The immune response can be mediated by, for example, tumor resident or infiltrating immune cells such as Tumor Infiltrating Lymphocytes (TIL). Pharmaceutical compositions including the modulator and a pharmaceutically acceptable carrier are also provided. The pharmaceutical compositions can be administered to a subject in need thereof in an effective amount to reduce one or symptoms of the cancer, for example by increasing an immune response against the cancer cells. Such pharmaceutical compositions can be administered prior to or in conjunction with an immune therapy such as adoptive T cell therapy.
BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a flow diagram showing the glycolytic pathway and its metabolic interconnection with the pentose phosphate pathway. The solid arrows indicate glycolytic reactions, whereas the dashed arrows show the pentose phosphate pathway. The green arrows indicate further metabolism of pyruvate downstream of glycolysis. Pentavalent arsenic compound (H₃AsO₄) abolishes ATP generation by causing arsenolysis in the glyceraldehyde-3-phosphate dehydrogenase reaction. HK, hexokinase; PGI, phosphoglucone isomerase; PFK, phosphofructokinase; TPI, triosephosphate isomerase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; PK, pyruvate kinase; PDH: pyruvate dehydrogenase; LDH: lactate dehydrogenase (Pellicano H, et al, Oncogene, 25:4633-4646 (2006)).

Figures 2A and 2B are illustrations of a tumor microenvironment with metabolic inhibitor targeted to the tumor microenvironment (e.g., cancer cells, a glucose-depleted and/or lactate-rich disease environment, etc.) (2A); or untargeted or otherwise non-specific (2B). Metabolic inhibitors affecting the tumor will adapt the micro-environment and make it more friendly to immune cells both in the tumor and those coming from the outside it. Metabolic inhibitor affecting outside the tumor could inhibit the immune cells and cause the inverse effect, lowering response.

Figures 3A-3C are dot plots showing the percentages of CD4, CD8, and Treg cell populations in tumor of mice. Percentages of CD4 (3A), CD8 (3B), and Treg (3B) populations were calculated relative to the live cell gate in individual samples from Group 3 ("Kula-18", closed circle), Group 6 (vehicle, closed square), and Group 7 ("Free-D", closed triangle). Lines represent mean of each group.

Figures 4A-4F are dot plots showing percentages of PD-1 and PD-CTL A-4 on CD4, CD8, and Treg cell populations in tumor of animals from mice. Percentages of PD-1 (4A-4C) and CTLA-4 (4D-4DE) were calculated relative to the CD4 (4A, 4D), CD8 (4B, 4E), and Treg (4C, 4F) populations in individual samples from Group 3 ("Kula-18", closed circle), Group 6
(vehicle, closed square), and Group 7 ("Free-D", closed triangle). Line represent mean of each group.

Figures 5A-5F are dot plots showing the percentages of LAGS and Tim3 on CD4, CD8, and Treg cell populations in tumor of mice.

Percentages of LAG3 (5A-5C) and Tim3 (5D-5F) were calculated relative to the CD4 (5A, 5D), CD8 (5B, 5E), and Treg (5C, 5E) populations in individual samples from Group 3 ("Kula-18", closed circle), Group 6 (vehicle, closed square), and Group 7 ("Free-D", closed triangle). Line represent mean of each group.

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

As used herein, the term "carrier" or "excipient" refers to an organic or inorganic ingredient, natural or synthetic inactive ingredient in a formulation, with which one or more active ingredients are combined.

As used herein, the term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients.

As used herein, the terms "effective amount" or "therapeutically effective amount" means a dosage sufficient to alleviate one or more symptoms of a disorder, disease, or condition being treated, or to otherwise provide a desired pharmacologic and/or physiologic effect. The precise dosage will vary according to a variety of factors such as subject-dependent variables (e.g., age, immune system health, etc.), the disease or disorder being treated, as well as the route of administration and the pharmacokinetics of the agent being administered.

As used herein, the term "prevention" or "preventing" means to administer a composition to a subject or a system at risk for or having a predisposition for one or more symptom caused by a disease or disorder to cause cessation of a particular symptom of the disease or disorder, a reduction or prevention of one or more symptoms of the disease or disorder, a reduction in the severity of the disease or disorder, the complete ablation of the disease or disorder, stabilization or delay of the development or progression of the disease or disorder.
As used herein, "inhibit" or other forms of the word such as "inhibiting" or "inhibition" means to hinder or restrain a particular characteristic. It is understood that this is typically in relation to some standard or expected value, in other words it is relative, but that it is not always necessary for the standard or relative value to be referred to. For example, "inhibits" an enzyme means hindering or restraining the activity of the enzyme relative to a standard or a control. "Inhibits" can also mean to hinder or restrain the synthesis or expression of the enzyme relative to a standard or control.

As used herein, "treatment" or "treating" means to administer a composition to a subject or a system with an undesired condition (e.g., cancer or oilier proliferative disorder). The condition can include a disease.

"Parenteral administration", as used herein, means administration by any method other than through the digestive tract or non-invasive topical or regional routes. For example, parenteral administration may include administration to a patient intravenously, intradermally, intraperitoneally, intrapleurally, intratracheally, intramuscularly, subcutaneously, subjunctivally, by injection, and by infusion.

"Topical administration", as used herein, means the non-invasive administration to the skin, orifices, or mucosa. Topical administrations can be administered locally, i.e. they are capable of providing a local effect in the region of application without systemic exposure. Topical formulations can provide systemic effect via adsorption into the blood stream of the individual. Topical administration can include, but is not limited to, cutaneous and transdermal administration, buccal administration, intranasal administration, intravaginal administration, intravesical administration, ophthalmic administration, and rectal administration.

"Enteral administration", as used herein, means administration via absorption through the gastrointestinal tract. Enteral administration can include oral and sublingual administration, gastric administration, or rectal administration.

"Pulmonary administration", as used herein, means administration into the lungs by inhalation or endotracheal administration. As used herein,
the term "inhalation" refers to intake of air to the alveoli. The intake of air can occur through the mouth or nose.

The terms "bioactive agent" and "active agent", as used interchangeably herein, include, without limitation, physiologically or pharmacologically active substances that act locally or systemically in the body. A bioactive agent is a substance used for the treatment (e.g., therapeutic agent), prevention (e.g., prophylactic agent), diagnosis (e.g., diagnostic agent), cure or mitigation of disease or illness, a substance which affects the structure or function of the body, or pro-drugs, which become biologically active or more active after they have been placed in a predetermined physiological environment.

The terms "sufficient" and "effective", as used interchangeably herein, refer to an amount (e.g. mass, volume, dosage, concentration, and/or time period) needed to achieve one or more desired result(s).

The term "biocompatible", as used herein, refers to a material that along with any metabolites or degradation products thereof are generally non-toxic to the recipient and do not cause any significant adverse effects to the recipient. Generally speaking, biocompatible materials are materials which do not elicit a significant inflammatory or immune response when administered to a patient.

The term "biodegradable" as used herein, generally refers to a material that will degrade or erode under physiologic conditions to smaller units or chemical species that are capable of being metabolized, eliminated, or excreted by the subject. The degradation time is a function of composition and morphology. Degradation times can be from hours to weeks.

The term "pharmaceutically acceptable", as used herein, refers to compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio, in accordance with the guidelines of agencies such as the Food and Drug Administration. A "pharmaceutically acceptable
carrier”, as used herein, refers to all components of a pharmaceutical formulation which facilitate the delivery of the composition \textit{in vivo}.

\textbf{Pharmaceutically} acceptable carriers include, but are not limited to, diluents, preservatives, binders, lubricants, disintegrators, swelling agents, fillers, stabilizers, and combinations thereof.

The term "molecular weight", as used herein, generally refers to the mass or average mass of a material. If a polymer or oligomer, the \textbf{molecular} weight can refer to the relative average chain length or relative chain mass of the bulk polymer. In practice, the molecular weight of polymers and oligomers can be estimated or characterized in various ways including gel permeation chromatography (GPC) or capillary \textbf{viscometry}. GPC molecular weights are reported as the weight-average molecular weight \((M_w)\) as opposed to the number-average molecular weight \((M_n)\). Capillary \textbf{viscometry} provides estimates of molecular weight as the inherent viscosity determined from a dilute polymer solution using a particular set of concentration, temperature, and solvent conditions.

The term "small molecule"; as used herein, generally refers to an organic molecule that is less than about 2.000 g/mol in molecular weight, less than about 1500 g/mol, less than about 1000 g/mol, less than about 800 g/mol, or less than about 500 g/mol. Small molecules are non-polymeric and/or non-oligomeric.

The term "copolymer" as used herein, generally refers to a single polymeric material that is comprised of two or more different monomers. The copolymer can be of any form, such as random, block, graft, etc. The copolymers can have any end-group, including capped or acid end groups.

The term "hydrophilic", as used herein, refers to substances that have strongly polar groups that readily interact with water.

The term "hydrophobic", as used herein, refers to substances that lack an affinity for water; tending to repel and not absorb water as well as not dissolve in or mix with water.

The term "lipophilic", as used herein, refers to compounds having an affinity for lipids.
The term "amphiphilic", as used herein, refers to a molecule combining hydrophilic and lipophilic (hydrophobic) properties.

The term "mean particle size", as used herein, generally refers to the statistical mean particle size (diameter) of the particles in the composition.

The diameter of an essentially spherical particle may be referred to as the physical or hydrodynamic diameter. The diameter of a non-spherical particle may refer preferentially to the hydrodynamic diameter. As used herein, the diameter of a non-spherical particle may refer to the largest linear distance between two points on the surface of the particle. Mean particle size can be measured using methods known in the art, such as dynamic light scattering.

Two populations can be said to have a "substantially equivalent mean particle size" when the statistical mean particle size of the first population of nanoparticles is within 20% of the statistical mean particle size of the second population of nanoparticles; more preferably within 15%, most preferably within 10%.

The terms "monodisperse" and "homogeneous size distribution", as used interchangeably herein, describe a population of particles, microparticles, or nanoparticles all having the same or nearly the same size. As used herein, a monodisperse distribution refers to particle distributions in which 90% of the distribution lies within 5% of the mean particle size.

The term "targeting moiety", as used herein, refers to a moiety that binds to or localizes to a specific locale. The moiety may be, for example, a protein, nucleic acid, nucleic acid analog, carbohydrate, or small molecule. The locale may be a tissue, a particular cell type, or a subcellular compartment. The targeting moiety or a sufficient plurality of targeting moieties may be used to direct the localization of a particle or an active entity. The active entity may be useful for therapeutic, prophylactic, or diagnostic purposes.

The term "reactive coupling group", as used herein, refers to any chemical functional group capable of reacting with a second functional group to form a covalent bond. The selection of reactive coupling groups is within the ability of the skilled artisan. Examples of reactive coupling groups can include primary amines (~NH₂) and amine-reactive linking groups such as...
isothiocyanates, isocyanates, acyl azides, NHS esters, sulfonyl chlorides, aldehydes, glyoxals, epoxides, oxiranes, carbonates, aryl halides, iinidoesters, carbodiimides, anhydrides, and fluorophenyl esters. Most of these conjugate to amines by either acylation or alkylation. Examples of reactive coupling groups can include aldehydes (-COH) and aldehyde reactive linking groups such as hydrazides, alkoxyamines, and primary amines. Examples of reactive coupling groups can include thiol groups (-SH) and sulphydryl reactive groups such as maleimides, haloacetyl, and pyridyl disulfides. Examples of reactive coupling groups can include photoreactive coupling groups such as aryl azides or diazirines. The coupling reaction may include the use of a catalyst, heat, pH buffers, SIGHT, or a combination thereof.

The term "protective group", as used herein, refers to a functional group that can be added to and/or substituted for another desired functional group to protect the desired functional group from certain reaction conditions and selectively removed and/or replaced to deprotect or expose the desired functional group. Protective groups are known to the skilled artisan. Suitable protective groups may include those described in Greene, T.W. and Wuts, P.G.M., Protective Groups in Organic Synthesis, (1991). Acid sensitive protective groups include dimethoxytrityl (DMT), tert-butylicarbamate (tBoc) and trifluoroacetyl (tFA). Base sensitive protective groups include 9-fluorenylmethoxycarbonyl (Fmoc), isobutyryl (iBu), benzoyl (Bz) and phenoxyacetyl (pac). Other protective groups include acetamidomethyl, acetyl, tert-amyloxycarbonyl, benzyl, benzoyloxycarbonyl, 2-(4-biphenylyl)-2-propyloxycarbonyl, 2- bromobenzyloxycarbonyl, left-buty? tert-butylxycarbonyl, 1-carbobenzoxamido-2,2,2-trifluoroethyl, 2,6- dichlorobenzyl, 2-(3,5-dimethoxyphenyl)-2-propyloxycarbonyl, 2,4- dinitrophenyl, dithiasuccinyl, formyl, 4-methoxybenzenesulfonyl, 4- methoxybenzyl, 4-methylbenzyl, o-nitrophenylsulfenyl, 2-phenyl-2 propyloxycarbonyl, a-2,4,5- tetramethylbenzyloxycarbonyl, p- toluenesulfonyl, xanthien, benzyl ester, N-hydroxysuccinimide ester, p-nitrobenzyl ester, p-nitrophenyl ester, phenyl ester, p-nitrocarbonate, p-nitrobenzylcarboxylic, trimethylsilyl and pentachlorophenyl ester.
II. Compositions

Direct or indirect inhibition of glucose uptake and/or lactate production by cancer cells can influence the tumor microenvironment and thereby activate exhausted or dormant immune cells and/or help to influence the number of immune cells in the microenvironment. Therefore, energetics can be used to influence the microenvironment of cancers with immunological activity or inactivity.

Compositions and methods of use thereof for preferentially modulating metabolism in cancer cells are provided. The compositions generally include a ceil metabolism modulator optionally in, on, or otherwise associated with a delivery vehicle such a nanoparticle, microsphere, dendrimer, antibody, or conjugate. In some embodiments, the compositions are targeted to a glucose-depleted and/or lactate-rich disease environment.

Typically the composition includes a moiety or other modification that increases delivery of the modulator to the cancer cells relative to non-cancer cells, and particularly immune cells. In some embodiments, the composition include a moiety (e.g., a mitochondrial localization signal) or other characteristic (e.g., EPR, zeta potential of the delivery vehicle, etc.), or combination thereof that enhances delivery to the mitochondria. In the most preferred embodiments, the composition is designed for delivery to (a) the tumor microenvironment generally or preferentially; and/or (b) preferentially or generally to the cancer cells in the microenvironment.

In the most preferred embodiments, the compositions and methods of use thereof lead to (a) direct or indirect inhibition of glycolysis or oxidative phosphorylation or any other form of metabolism that utilizes glucose or equivalents from the disease area's microenvironment or direct or indirect inhibition of the uptake of glucose or equivalents from the disease area microenvironment; and/or (b) direct or indirect inhibition of the production of lactate or equivalents, or the direct or indirect inhibition of the release of lactate or equivalents into the disease microenvironment.

Modulating cancer metabolism can modify the tumor microenvironment to make it more amenable for an immune response against the cancer cells. In some embodiments, the compositions and methods
increase the number or activity of immune cells, for example tumor associated immune cells, relative to the number or activity of the immune cells prior to administration of the modulator, or compared to administration of the modulator absent a targeting moiety, a delivery vehicle, or a combination thereof. The immune cells can include, but are not limited to, CD4 cells and CDS cells. In some embodiments, the compositions and methods increase the number of CD4 or CDS cells in the tumor. In some embodiments, the increase in immune cells is an increase in the total number of immune cells. In some embodiments, the increase in immune cells is an increase in the ratio of immune cells to tumor cells. Accordingly, in some embodiments, the increase in immune cells is actually the results of a reduction in tumor cells. In some embodiments, the compositions and methods lead to a decrease in expression of a regulator of immune suppression (or suppressor of immune activation) such as PD-1, CTLA4, or a combination thereof.

**Therapeutic** targets that effect metabolism include, but are not limited to, glucose transporters (GLUT family), lactate transporters (MCT family), proteins involved in glycolysis such as hexokinase (HK family), lactate dehydrogenase (LDH family), pyruvate dehydrogenase (PDH), pyruvate dehydrogenase kinase (PDK family), members of the citric acid cycle such as succinate dehydrogenase (SDH family), isocitrate dehydrogenase (IDH family) and members involved in oxidative phosphorylation such as Complex I proteins; and (b) can be delivered to the tumor microenvironment via methods that include but are not limited to small molecules, conjugates, antibodies, nanoparticles, liposomes, microspheres, patches, gels, injections and others, whether alone or in combinations thereof, including or excluding use in combination with linkers or other targeting mechanisms that can include targeting surface proteins of cells, differences in physiology or morphology. Each of these aspects is discussed in more detail below.

Exemplary preferred embodiments include, but are not limited to, mitochondrial-targeting nanoparticle containing dichloroacetate, mitochondrial-targeting nanoparticle containing lonidamine, mitochondrial targeting nanoparticle containing 3-bromopyruvate, mitochondrial-targeting
nanoparticle containing galloflavin or oxalate. Exemplary preferred delivery vehicles are discussed in U.S. Published Application No. 2014/0303081, which is specifically incorporated by reference herein in its entirety. In some embodiments, the delivery vehicle is a mitochondrial-targeted PLGA-PEG-TPP nanoparticle containing a metabolic modulating compound such as those disclosed herein.

A. Modulators of Cancer Cell Metabolism

The increased dependence of cancer cells on the glycolytic pathway for ATP generation provides a biochemical basis for the design of therapeutic strategies to preferentially kill cancer cells.

Compositions and methods for direct or indirect pharmacological inhibition of cancer cell metabolism, including glycolysis in cancer cells, are provided. Targets of inhibitions include: (1) glycolytic enzymes directly-involved in glycolysis, and glucose transporters (GLUTs), which control the availability of glucose for tumor growth; (2) enzymes involved in interconnected pathways such as pentose phosphate pathway and TCA cycle: (3) monocarboxyiate transporters, which transport lactate in or from tumor cells (e.g., PPP pathway, glutamine pathway, etc.).

In the most preferred embodiments, the modulator is a small molecule, such as those discussed in more detail below. The term "small molecule" refers to small organic compounds having a molecular weight of more than 100 and less than about 2,500 daitons, preferably between 100 and 2000, more preferably between about 100 and 1250, more preferably between about 100 and 750, more preferably between about 200 and about 500 daitons. The small molecules often include cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more functional groups.

In other embodiments, the modulator can be a functional nucleic acid such as those discussed in more detail below. Suitable targets, and inhibitors and activators thereof, are discussed in Pelicano, et al, Oncogene (2006) 25, 4633-4646 and in more detail below.
1. Modulators of Glycolysis

Glycolysis is a series of metabolic processes by which one molecule of glucose is catabolized to two molecules of pyruvate with a net gain of two ATP. The glycolytic pathway is also known as the Embden-Meyerhof pathway, which has two phases, the priming phase and the energy-yielding phase. As illustrated in Figure 1, the priming phase uses two molecules of ATP to convert glucose to fructose-1,6-bisphosphate through sequential reactions catalyzed by hexokinase, phosphoglucone isomerase, and phosphofructokinase. In the second phase, fructose-1,6-bisphosphate is further converted stepwise into pyruvate with the production of four molecules of ATP and two molecules of NADH. During this process, two ADP and two NAD+ are consumed. In the absence of oxygen, NAD+ is regenerated from NADH by reduction of pyruvate to lactic acid catalyzed by lactate dehydrogenase (LDH). Under aerobic conditions, pyruvate can be further oxidized to CO₂ and H₂O in the mitochondria through the tricarboxylic acid (TCA) cycle and the respiratory chain, yielding large amount of ATP. As illustrated in Figure 1, each reaction in the glycolytic pathway is catalyzed by a specific enzyme or enzyme complex.

a. Glucose transporter (GLUTs) Inhibitors

Glucose transporters (GLUTs) constitute a family of proteins that regulate the transport of glucose across the hydrophobic cell membranes. Fourteen isoforms of the GLUT genes have been identified, which show similar structural architecture but different cellular and sub-cellular localization, kinetic properties and affinity for glucose and other hexoses. Different GLUTs have been found to be overexpressed in a wide variety of cancer types, and their level of expression often correlates with the metastatic potential and worse prognosis of the tumor (Adekola, K et al., Curr. Opin. Oncol., 24:650 (2012)). In particular, GLUT1 is regarded as a potential target in oncology drug discovery (Granchi, et al., Bioorganic and Medicinal Chemistry Letters, 24(21):4915-25 (2014)).

In some embodiments, the modulator directly or indirectly inhibits a glucose transporter. For example, the inhibitor can be WZB17 (compound 1). A series of polyphenolic esters were found to inhibit glucose transport...
through the cell membrane, and to exert a certain antiproliferative activity in
the H1299 lung cancer cell line. The initial development of this class led to
WZB17, which showed 93% inhibition in a standard glucose uptake assay
and 41% inhibition of cancer cell growth rate (IC50 = 10 µM in cell viability
assays) in lung cancer cells, with a more pronounced antiproliferative effect
under hypoxic conditions.

The inhibitor can be the natural compound (+)-cryptocaryone
(compound 2). (+)-cryptocaryone was identified among the components of
an extract isolated from the leaves and twigs of Cryptocarya rubra, a tropical
plant belonging to the Lauraceae family. This extract was found to be
cytotoxic on HT-29 human colon cancer cells with an IC50 value of
0.32 µM, and was found to cause a significant reduction of the uptake of
glucose, implying that its anti-proliferative activity could be ascribed, at least
in part, to GLUT inhibition.

The inhibitor can be an oxime derivative such as an aldoxime (e.g.,
compounds 3 and 4, Figure 2) or ketoxime (e.g., compounds 5 and 6). Some
members of a class of oxime derivatives, which were previously designed as
estrogen receptor (ER) ligands revealed to be active as GLUTI-inhibitors.
These compounds show some common pharmacophoric similarities with
WZB117-like inhibitors, mainly consisting in the presence of similarly-
spaced peripheral 'phenol-type' OH groups. Aldoximes which differ only for
a fluorine atom in meta position of the distal phenyl ring, displayed IC50
values of 8.5 µM, and 23.4 µM, in the glucose uptake assay, whereas
ketoximes, which, similarly to the other pair of compounds, differ only for
the substitution on the distal phenyl, showed IC50 values of 15.5 and
10.6 µM, respectively, with inhibition potencies generally comparable to that
of WZB117 (IC50 = 10.9 µM).

The inhibitor can be a thiazolidinedione (e.g., compounds 7 and 8). Thiazolidinediones were developed following the observation that a
peroxisome proliferator-activated receptor γ (PPARγ) agonist exerted part of
its action through inhibition of glucose transport. Compound 7 exhibited an
excellent suppression of glucose uptake (IC50 = 2.5 µM) in LNCaP prostate
cancer cells. This activity led to an efficient antiproliferative activity on the
same cell line, with no evident toxicity on healthy prostate and mammary epithelial cells. GLUT1 isoform has been identified as the preferential target of compound 7. Thiazolidimedione (compound 8) displayed a slightly lower activity (IC50 = 6 µM in glucose uptake inhibition, TC50 = 5 µM in GLUT1 inhibition) than that of compound 7.

The inhibitor can be a methylxanthine. Methyloxanthines are a family of natural compounds with a vast variety of physiological effects. In particular caffeine (1,3,7-trimethylxanthine - compound 9), theophylline (1,3-dimethylxanthine - compound 10) and the synthetic methylxanthine pentoxifylline [1-(5-oxoexil)-3,7-dimethylxanthine, pentoxifylline - compound 11] are also known to be inhibitors of glucose transport. Compounds 9, 10 and 11 were able to displace the GLUT1 inhibitor cytochalasm B from the protein, thus confirming their direct interaction with GLUT1.

The inhibitor can be a natural phytalexin such as resveratrol (compound 12). A study of resveratrol showed that it was able to block glucose uptake and hinder glucose accumulation in HL-60 and U-937 leukemia cells, two cell lines that both express mainly the GLUT1 isoform (Salas M., et al., Am. J. Physiol. Cell Physiol, 305: C90 (2013)).

The inhibitor can be STF-31 (compound 13). STF-31, a GLUT inhibitor discovered by Giaccia and co-workers, has been elaborated to obtain affinity chromatography reagents for target identification. A long poly-ether amino-alkyl chain was introduced in the para position of the phenyl sulfonamide terminal moiety to obtain STF-31. The selective retention of GLUT1 protein by the 13-conjugated resin reagent confirmed that GLUT1 is the target of these 3-pyridylphenylsulfonyl benzamide derivatives (Bonnet, M et al, Bioorg. Med. Chem., 22:711(2014)).
b. **Hexokinase (HK) Inhibitors**

The first step in glycolysis is catalyzed by hexokinase (HK) and consists of the transfer of one phosphate group from ATP to glucose, yielding glucose-6-phosphate. Isoform 2 of hexokinase (HK2) is considered to play two crucial roles in the reprogrammed glycolytic metabolism of tumor cells. First, HK2 up-regulation results in increased glycolysis rates and, second, association of HK2 in a complex with a voltage dependent anion channel (VDAC) on the external mitochondrial membrane contributes to inhibition of apoptosis through block of cytochrome c release from mitochondria. Moreover, HK2-bound to mitochondria is insensitive to product inhibition and gains preferential access to newly synthesized ATP for phosphorylating glucose (Mathupala, SP et al., *Senna. Cancer Biol*, 19:17 (2009)).

In some embodiments, the modulator directly or indirectly inhibits a hexokinase. There are a number of chemical inhibitors designed to reduce glycolytic flux governing the rate-limiting step of hexokinase (HK) activity. One such therapeutic is a glucose analog 2-deoxy-d-glucose (2-DG), which is a competitive inhibitor for normal glucose metabolism. 3-bromopyruvate is also an inhibitor of hexokinase and has been shown to abolish ATP production and cause severe depletion of cellular ATP). Like 2-DG, 3-BrPA also exhibits potent cytotoxic activity against cancer cells with mitochondrial respiratory defects and cells in hypoxic environment (Xu RH et al, *Cancer Res* 65:613-621 (2005)). 3-bromopyruvate is currently under clinical trial.

The inhibitor can be Lonidamine. Lonidamine (also known as 1H -070) is a derivative of indazole-3-carboxylic acid, and has been known for a long time to inhibit aerobic glycolysis in cancer cells (Floridi et al., *Cancer Res.*, 41(11 Pt l):4661-6 (1981)). The proven ability of lonidamine to inhibit energy metabolism in cancer cells and to enhance the activity of other anticancer agents has led to extensive clinical trial in many cancer models.

The inhibitor can be a glucosamine derivative or a spirooxindole derivative. Several glucosamine derivatives including compound 14, exhibit pIC50 values higher than 6.5 for HK2 inhibition. A series of spirooxindole derivatives, such as compound 15, displaying micromolar potencies in HK2-
inhibition assays, were identified by scientists of the Okinawa Institute of Science And Technology.

In addition, HK2 inhibitory activity was demonstrated for anticancer agents with complex mechanism of action, such as: (1) alkylating agent and glycolysis inhibitor 3-bromo-pyruvate (compound 16), which has been recently granted FDA orphan drug designation for liver cancer; (2) copper-phenanthroline complex Casiopeina Ugly (compound 17); (3) antidiabetic drug metformin (compound 18), whose anti-tumoral properties are under intensive investigation. Thus, HK2 inhibition likely contributes to the multiple mechanisms underlying the anticancer action of these agents.

c. **Phosphofructokinase (PFK) Inhibitors**

In some embodiments, the modulator directly or indirectly inhibits phosphofructokinase. Isoform 1 of phosphofructokinase (PFK1) catalyzes one of the most critical steps of glycolysis, the conversion of fructose-6-phosphate and ATP to fructose-1,6-bisphosphate and ADP. PFK1 activity is enhanced by the allosteric activator fructose-2,6-bisphosphate, whose production is regulated by phosphofructokinase 2, also named fructose-2,6-bisphatase (PFK2/FBPase), due to its dual function as a kinase and as a
phosphatase. In fact PFK2/FBPase controls both the production of fructose-
2,6-bisphosphatase from fructose-6-phosphate and its reverse hydrolytic
reaction. Among the four isoenzymes belonging to the PFK2/FBPase family,
PFKFB3 is overexpressed in hypoxic tumors under HIF-1α regulation and,
thanks to its increased kinase activity, PFKFB3 is the enzyme that most
likely contributes to the high glycolytic activity in these tumors.

The inhibitor can be a PFKFB3 inhibitor. The initial reference
compound for PFKFB3-inhibition, 1,3-diarylpropenone 3PO, was discovered
in the group of Dr. Chesney through computational modeling and virtual
screening of chemical databases (Clem B et al, Mol. Cancer Ther., 7:110
(2008)). The optimization of this class led firstly to PFK15 (compound 19),
which showed increased potency for the inhibition of the recombinant human
enzyme (IC50 = 207 nM vs 22.9 μM of 3PO), and high selectivity for
PFKFB3 when tested in a wide panel of different kinases (Clem B et al.,
Mol. Cancer Ther., 12:11461 (2013)). Further optimization led to PFK158,
which was reported to be efficacious (approximately 80% growth inhibition)
in several mouse models on human-derived tumors, and well tolerated in rats
and dogs. PFK158 in combination with vemurafenib significantly increased
the apoptotic death of several melanoma cell lines, in vitro. In March 2014, a
phase 1 trial with PFK158 was initiated in patients with advanced solid
malignancies.

A series of ammofurazan-triazoles was discovered by virtual
screening of commercially available libraries of compounds, and, to the best
of our knowledge, they are the first class of structurally related compounds
comprising both activators and inhibitors of PFKFB3 (compound 20).
Compound 20 is the most potent PFKFB3 inhibitor of this series, by
reducing the PFKFB activity in enzymatic assays and by decreasing the
glycolytic flux in rat muscle cell lysates (Pyrkov TV et al, ChemMedChem,
8:1322 (2013)).

A recent high-throughput screen produced 5-triazolo-2-
aiylpyridazinone (compound 21) as a fairly potent PFKFB3 inhibitor (IC50 =
7.4 μM), but unable to inhibit glycolysis in vitro at non-toxic concentrations
In some embodiments the modulator is one that directly or indirectly inhibits glyceraldehyde-3-phosphate dehydrogenase (GAPDH). GAPDH is a key enzyme in the glycolytic pathway. However, GAPDH is implicated in diverse functions independent of its role in energy metabolism; the expression status of GAPDH is also deregulated in various cancer cells. One of the most common effects of GAPDH is its inconsistent role in the determination of cancer cell fate (Zhang JY et al., Cancer Biol Med. 12(1): 10-22 (2015)). Studies have described GAPDH as a regulator of cell death; other studies have suggested that GAPDH participates in tumor progression and serves as a new therapeutic target. However, related regulatory mechanisms of its numerous cellular functions and deregulated expression levels remain unclear. One study showed GAPDH inhibition caused by antisense oligonucleotides in human cervical carcinoma affects cell proliferation and induces apoptosis (Kim JW et al., Antisense Nucleic Acid Drug Dev 1999;9:507-513). However, the mechanism and precise role of GAPDH in tumor cells remains unclear.

In addition to inhibiting HK as discussed above, 3-bromopyruvate is also a potent inhibitor of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by reacting with the -SH nucleophile sites of these enzymes through the rapid displacement of its bromo-leaving group.
e. **Phosphoglycerate mutase (PGM) Inhibitors**

The modulator can be one that directly or indirectly inhibits phosphoglycerate mutase (PGM). PGM catalyzes the reversible conversion of 3-phosphoglycerate to 2-phosphoglycerate. In mammals, PGM exists as two homodimers consisting of two muscle-type monomers (PGM-MM), two brain-type monomers (PGM-BB), or as a heterodimer (PGM-MB). One of the homodimers, PGM-BB is also named PGM1 in humans and is ubiquitously expressed. It has been reported that PGM1 is usually up-regulated in human cancer tissues and plays an important, albeit not yet fully investigated role in cancer cell metabolism.

The inhibitor can be a dihydroxyanthraquinone derivative such as PGMI-004A. Dihydroxyanthraquinone derivatives were developed starting from dye Alizarin, and led to the identification of PGM1-inhibitor PGMI-004A (compound 22) (Hitosugi T et al, *Cancer Cell*, 22: 585(2012)). In enzymatic assays compound 22 displayed a PGM1-inhibition activity in the low micromolar range (IC50 = 13.1 µM, Ki = 3.91 µM), and turned out to be more cytotoxic than parent compound Alizarin in cancer cells, possibly due to a higher cell membrane permeability conferred by the hydrophobic 4-(trifluoromethyl)phenylaminoisulfonyl substituent.

\[
\text{PGMI-004A}
\]

22
f,  Ensase (ENO) Inhibitors

The modulator can be one that directly or indirectly inhibits enolase (ENO). Enolase is the glycolytic enzyme responsible for the conversion of 2-phosphoglycerate to 2-phosphoenolpyruvate. Increased expression of the alpha-enolase isoform (ENO1) has been detected in several tumors and recently Muiler et al. have validated enolase as an anti-cancer target (Muiler FL, et al. Nature, 488:337 (2012)). ENO1 gene is deleted in glioblastoma and this lack is counterbalanced by the expression of the isoform ENO2, however if ENO2 was selectively blocked by shRNA the result was inhibition of growth and survival of the ENO1-deleted tumor cells. Similarly, ENO1-deleted cells proved to be more sensitive to the cytotoxic action of phosphonoaceto-hydroxarnate, a substrate analogue enolase inhibitor (Muiler FL, et al. Nature, 488:337 (2012)).

The inhibitor can be, for example, a substrate analogue or a non-substrate analogue such as ENOblock. Until recently, ENO inhibitors had only been found among substrate analogues. Jung et al. reported tri-substituted tazine ENOblock (compound 23, Figure 6) as the first non-substrate analogue ENO1 inhibitor that directly binds the enzyme (IC50 = 0.576 uM), as confirmed by affinity chromatography experiments (Jung DW et al. ACS Chem. Biol, 8: 1271 (2013)).

PEP is formed from the decarboxylation of oxaloacetate and hydrolysis of a guanosine triphosphate molecule in a reaction catalyzed by the enzyme phosphoenolpyruvate carboxykinase (PEPCK, PEPCK2; PEPCK-M). This reaction is a rate-limiting step in gluconeogenesis. In some embodiments, the modulator directly or indirectly inhibits phosphoenolpyruvate carboxykinase.
g. Pyruvate Kinase (PK) Activators

The modulator can be one that directly or indirectly activates pyruvate kinase (PK). Pyruvate kinase catalyzes the final rate-limiting step of glycolysis, which consists in the transfer of a phosphate group from phosphoenolpyruvate (PEP) to ADP, to give pyruvate and ATP. Isoform PKM2 may exist in both a high-activity tetrameric and a low-activity dimeric form. Moreover, glycolytic intermediate fructose-1,6-biphosphate and natural aminoacid serine are allosteric activators of PKM2. Increased levels of the less active dimeric PKM2 lead to a decreased rate of glycolysis, and an up-regulation of PKM2 has been observed in many tumor cells. This fact may appear as paradoxical given the high rate of lactate production in these cells. However, dimeric PKM2 may allow all glycolytic intermediates above the PKM2 reaction to accumulate, thereby providing a high level of metabolic precursors available for the synthetic anabolic processes, in addition to energy production. These findings explain why both inhibition and activation of PKM2 have so far been considered as valid anticancer approaches (Anastasiou D et al. Nat: Chem. Biol., 8: 839 (2012)).

In some embodiments, the activator is an allosteric sulfonamide quinolone. A series of allosteric sulfonamido quinoline-based PKM2 activators has been explored by Agios Pharmaceuticals (WG20 14074848). The class representative (compound 24), which shows an AC50 of 0.017 µM, was able to activate the enzyme in cancer cells, with AC50 of 45 nM in A549 lung adenocarcinoma cells.

Pyrazoie-carboxamide SGI-10067 (compound 25) from Astex Pharmaceuticals shows excellent activation potency both on the isolated enzyme (AC50 = 11 nM) and in cell-based PKM2 activity assays (AC50 = 0.22-0.26 µM) (Xu Y et al. Bioorg. Med. Chem. Lett., 24: 515(2014)).

Benzimidazole-pyrimidone (compound 26), discovered by Pfizer scientists, showed good activation potency of PKM2 (AC50 = 0.159 µM) and favorable ADME and pharmaceutical properties (Guo C et al, Bioorg. Med. Chem. Lett, 23: 3358 (2013)). Furthermore, compound 26 was found to be highly selective for PKM2 when tested on a wide panel of kinases.
Dynamix Pharmaceuticals developed a class of arylsulfonyl indoline PKM2 activators, whose representative (compound 27, Figure 7) efficiently activates PKM2 (AC50 = 45 nM) with high selectivity for isoform M2 versus the other PK isofonnrs (Yacovan A et al, Bioorg. Med. Chem. Lett., 22: 6460 (2012)).

h. Lactate dehydrogenase (LDH)

The modulator can be one that directly or indirectly inhibits the enzyme lactate dehydrogenase (LDH). LDH catalyzes the reversible conversion of pyruvate to lactate with the simultaneous oxidation of the cofactor NADH to NAD+. The human isoform LDH-A or LDH5 is composed of four A subunits (LDH-A4) and is mainly expressed in liver and muscle. Several evidences indicate that LDH-A, which is up-regulated in invasive glycolytic cancers, plays an important role in cell proliferation, allowing the survival of tumors even in conditions of low oxygen concentration. Hence the inhibition of this enzyme is of great interest for cancer treatment.

A fragment-based approach accompanied by a virtual screen of commercially available databases was used by ARJAD Pharmaceuticals to identify bifunctional LDH-A inhibitors, which are able to bind both in the substrate and in the cofactor binding sites of the enzyme. Among them, compound 28 (Figure 8) showed an IC50 of 0.12 μM against LDH-A in enzymatic assays (Kohlmann A et al, J. Med. Chem., 56: 1023 (2013)).
Ward et al. at AstraZeneca UK discovered one of the most potent bifunctional LDH-A inhibitors by a fragment-based approach, the diacid malonate-based compound 29. This inhibitor showed an IC50 value of 0.27 µM and a Kd value of 0.008 µM in the BIAcore binding affinity assay but lacked of any cellular activity, probably because of the diacid functionality that hinders membrane permeability (Ward RA et al., J Med. Chem., 55:3285 (2012)).

Screening of the corporate compound collection and lead optimization efforts produced two potent LDH-A inhibitors: compound 30 possessing a 2-thio-6-oxo-1,6-dihy(kopyrimidine structure and the 2-amino-5-aryl-pyrazine (compound 31). Both of them showed nearly identical IC50 values in the low micromolar range on LDH-A (IC50 =0.48 and 0.50 µM, for compound 30 and 31, respectively), with good selectivity over the heart LDH isoform (LDHl or LDH-B) and the structurally similar enzyme malate dehydrogenases 1 and 2 (MDH1 and MDH2) (Dragovich PS et al., Bioorg. Med. Chem. Lett, 23:3186 (2013); Fauber BP et al., Med. Chem. Lett, 23:5533 (2013)).

GNE-140 (compound 32), a piperidindione derivative inhibitor of LDH-A developed by Genentech, was identified by the optimization of a HTS hit. Compound 32 is a nanomolar inhibitor of LDH-A (EC50 = 5 iiM), which proved to be effective also in inhibiting MiaPaCa-2 pancreatic cell proliferation with an EC50 of 0.25 µM. A similar class of LDH-A inhibitors characterized by a 3-hydroxy-2-mercaptocyclohexenone scaffold, includes the representative compound 33, which showed an IC50 of 0.87 µM on LDH-A, with an 8-fold selectivity for this isoform over LDH-B (1C50 = 6.9 µM) (Dragovich PS et al., Bioorg. Med. Chem. Lett., 24:3764 (2014)).
Quinoline 3-sulfonamide (compound 34) developed by GlaxoSmithKline is one of the most potent NADH-competitive LDH-A-inhibitor discovered so far, with an IC50 of 2.6 nM (K_i = 4.8 nM), a sixteen-fold selectivity over LDH-B (IC50 = 43 nM). Compound 34 inhibited lactate production in several cancer cell lines (Xie H et al., Cell Metab., 19:795 (2014)).

Recent studies of the chemical class of N-hydroxyindole-based LDH-A-inhibitors produced several compounds that inhibit LDH-A in the low micromolar range. A recent development of this chemical class led to the discovery of glucose-conjugated methyl ester (compound 35, Figure 9), which inhibits LDH-A with a Ki value of 37.8 μM and is therefore a weaker
inhibitor than the N-OH analogue (compound 36, $K_i = 5.1 \mu M$) on the isolated enzyme (Calvaresi EC et al., ChemBioChem, 14:2263 (2013)). Nevertheless, compound 35 proved to be able to cross the cell membrane very efficiently by means of GLUT transporters, which actively promoted its uptake in cancer cells.

In the past few years, there has been a growing interest in the discovery of natural products showing inhibitory properties against LDH-A. In some cases only commonly used plant and herbal extracts were identified by HTS procedure to be active on LDH-A (Deiab S et al., Eur. J. Med. Plants, 3:603(2014)). In other cases, a known $\alpha,\beta$-unsaturated aldehyde, such as 4-hydroxy-2-nonenal, which usually forms in meat products by lipid peroxidation, was found to be able to bind to histidine and cysteine residues of LDH-B (Ramanathan R et al., J. Agnc. Food Chem., 62:212 (2014)).

Manganese(II) complex 37 containing a di(pyndylmethyl)amine and a pyrrol-ketone moiety was developed as a dual drug. It is a mimic of catalase, the enzyme catalyzing the decomposition of hydrogen peroxide to water and oxygen, thus being responsible for protecting cells from oxidative damage by reactive oxygen species; additionally, this complex is also an inhibitor of LDH-A. Kinetic studies were performed to evaluate the LDH-A-inhibition potency of 37 (caution note: a rabbit LDH isoform was used in this assay), revealing $K_i$ values of 41.7 and 21.4 $\mu M$ versus cofactor and substrate, respectively. Complex 37 displayed a good antiproliferative activity against HepG-2 cells, and an additional inhibitory effect on hypoxia inducible factor 1a (HIF-1a) expression (Xue JJ, et al., Eur. J. Med. Chem., 80:1(2014)).
Galloflavin (CAS 568-80-9), an inhibitor targets both human LDH isoforms (LDH-A and LDH-B) by preferentially binding the free enzyme, without competing with the substrate or cofactor. The calculated Ki values for pyruvate were 5.46 µM (LDH-A) and 15.06 µM (LDH-B). In cultured tumor cells, galloflavin blocked aerobic glycolysis at micromolar concentrations, did not interfere with cell respiration, and induced cell death by triggering apoptosis (Manerba M et al., ChemMedChem. 7(2):3 11-7 (2012)).

Oxamaie, a classical inhibitor of LDH-A, has been shown to induce G2/M cell cycle arrest via downregulation of the CDK1/cyclin B1 pathway and promote apoptosis through enhancement of mitochondrial ROS generation in various tumor models (Zhai X et al, Oncol Rep. 30(6):2983-91 (2013); Yang Y etai, Oncotarget. 5(23): 11886-11896 (2014)).

Gossypol (also known as AT-101), a natural compound present in cottonseeds, displays anti-proliferative and pro-apoptotic effects against various cancer cells. It is a nonselective inhibitor of LDH that blocks the binding of NAD+, with a Kᵯ for LDHA and LDHB of 1.9 and 1.4 µM, respectively. Gossypol also inhibits GAPDH, which is an NAD⁺-dependent enzyme, and thus its antitumor activity may also include the inhibition of GAPDH. In addition, FX-11, a gossypol derivative that shows selectivity for LDHA over LDHB ($Kᵯ$ of 0.05 vs. 20 µM, respectively) has antitumor

Epigallocatechin-3-gallate (EGCG), a polyphenol extracted from green tea, was reported to inhibit tumor invasion and angiogenesis, processes that are essential for tumor growth and metastasis. EGCG impacts on various pathways of the cellular metabolic network and significantly modifies the cancer metabolic phenotype. EGCG exerts these effects, at least in part, via inhibition of LDH-A (Lu QY s. 2015 Feb; 11(1): 71-80.).

i. Pyruvate dehydrogenase Kinase Inhibitors

The modulator can be one that directly or indirectly activates pyruvate dehydrogenase (PDH), or inhibits pyruvate dehydrogenase kinase (PDK). PDH is the first component enzyme of pyruvate dehydrogenase complex. The pyruvate dehydrogenase complex converts cytosolic pyruvate to mitochondrial acetyl-CoA, the substrate for the Krebs' cycle. Pyruvate dehydrogenase kinase (PDK) is a mitochondrial enzyme that is activated in a variety of cancers and results in the selective inhibition of PDH.

inhibition of PDK with either small interfering RNAs or the orphan drug dichloroacetate (DCA) shifts the metabolism of cancer cells from glycolysis to glucose oxidation and reverses the suppression of mitochondria-dependent apoptosis. In addition, the inhibition of PDK increases the production of diffusible Krebs' cycle intermediates and mitochondria-derived reactive oxygen species, activating p53 or inhibiting pro-proliferative and pro-angiogenic transcription factors like nuclear factor of activated T cells and hypoxia-inducible factor la. DCA is a small 150 Da molecule that can penetrate cell membranes and most tissues, including the brain. DCA activates PDH by inhibiting PDK at a concentration of 10-250 uM, in a dose-dependent fashion (Stacpoole, 1989). There are four PDK isoforms that are expressed in most tissues with the most sensitive to DCA being PDK2.

lipophilic triphenylphosphonium (TPP) cation moiety for the targeted delivery and accumulation into the mitochondrial matrix. The study showed that Mito-DCA efficiently reduced glycolytic functions, reduced basal cellular respiration, suppressed the calculated ATP synthesis, and attenuated the spare respiratory capacity in prostate cancer cells (Pathak RK et al., ACS Chem. Biol, 9 (5) 1178-1 187 (2014)). In some embodiments, the composition is a DCA or analog thereof conjugated with the TPP alone or encapsulated in a nanoparticle. In a preferred embodiment, the composition is a TPP-DCA analog (see, e.g., WO2015002996) encapsulated in a PLGA-PEG nanoparticle.

Oxythiamine is a thiamine antagonist and inhibits transketolase and pyruvate dehydrogenase, which require thiamine pyrophosphate (TPP) as a cofactor for their enzyme activity. Early studies suggest that oxythiamine is phosphorylated to yield diphosphate ester which then acts as a strong competitive inhibitor ($K_i=0.07 \mu M$) against the normal cofactor TPP ($K_m=0.11 \mu M$) when highly purified pyruvate dehydrogenase was used (Strumilo SA et al., Biomed Biochim Acta 43: 159-163 (1984)).

Glucose-6-phosphate dehydrogenase (G6PD)

The modulator can be one that directly or indirectly inhibits glucose-6-phosphate dehydrogenase (G6PD). G6PD catalyzes the conversion of G-6-P 6-phosphogluconolactone, the first step of the pentose phosphate pathway. 6-aminonicotinamide (6-AN) has been widely used as a chemical tool in various experimental systems to study the biological consequences of inhibiting pentose phosphate pathway. Because of the essential roles of this pathway in generating reducing power (NADPH) and important metabolic intermediates (pentose-5-phosphate) for synthesis of macromolecules, 6-AN exhibits anticancer activity in vitro, causes oxidative stress, and sensitizes cells to anticancer agents and radiation (Budihardjo II et al, Clin Cancer Res 4:1 17-130 (1998); Varshney R et al, Indian J Exp Biol 41:1384-1 391 (2003)).

Imatinib (Gleevec) is a tyrosine kinase inhibitor designed to specifically target BCR-ABL, which is responsible for the development of
chronic myeloid leukemia (CML). The BCRABL-positive cells express the high-affinity glucose transporter (GLUT-1) and exhibit increased glucose uptake. Imatinib treatment decreased the activity of both hexokinase and glucose-6-phosphate dehydrogenase (G6PD) in leukemia cells, leading to suppression of aerobic glycolysis. (Pelicano H, et al., Oncogene 25:4633-4646 (2006))

2. Tricarboxylic Acid (TCA) Cycle Inhibitors

Beyond glycolysis, targeting the tricarboxylic acid (TCA) cycle is yet another potential direction for cancer therapy. Therefore, in some embodiments, the modulator of cancer cell metabolism is one that directly or indirectly inhibits the TCA cycle, or a component thereof. Examples of suitable targets of inhibition include, but are not limited to, succinate dehydrogenase, isocitrate dehydrogenase, aconitase etc. Arsenic trioxide (ATO), a mitochondrial toxicant, is currently used in the treatment of acute promyelocyte leukemia (APL). ATO has several mechanisms by which APL is targeted. Mutations of isocitrate dehydrogenase (IDH), another component of the TCA cycle, are frequently found in several types of cancer such as glioma and acute myeloid leukemia, inhibitors (e.g. AGI-5198) of IDH mutants by Rohle et al. have been developed and demonstrated anti-cancer activities (Rohle D et al., Science. 340:626-630 (2013)).

3. Electron Transport Chain Inhibitors

In some embodiments, the modulator of cancer cell metabolism is one that directly or indirectly inhibits the electron transport chain, or a component thereof. Suitable modulators include classic Complex I-IV inhibitors and ATP synthase inhibitors. Exemplary inhibitors of the ETC are amytal, rotenone, antimycin A, CO, sodium azide, and cyanides. In a particular example, the modulator is metformin. Metformin is a clinically approved drug by the FDA to treat type II diabetes, targets the mitochondrial complex I and thereby reducing ATP synthesis.
4. Monocarboxylate Transporters (MCTs) Inhibitors

In some embodiments, the modulator of cancer cell metabolism is one that direct or indirectly inhibits a monocarboxylate transporter (MCT). MCTs are responsible for the inwards and outwards cellular transportation of monocarboxylate derivatives, such as lactate, pyruvate, and ketone bodies. Among the 14 known human MCT homologue members, the two isoforms MCT1 and MCT4 are the most frequently overexpressed in many tumors and have been regarded as viable anti-cancer targets. These two isoforms exert complementary roles in tumor cells: MCT1 (high affinity for lactate) enables lactate entry into oxidative/oxygenated cells that use it to produce energy, whereas MCT4 (low affinity for lactate) is mainly designated to export lactate and protons out of glycolytic cells, thus contributing to maintain intracellular pH and avoid cytotoxic accumulation of lactate. The therapeutic strategy of inhibiting MCT4 is aimed to prevent lactate efflux from glycolytic cancer cells, thus leading to intracellular acidification and impairment of cell proliferation. Inhibition of MCT1 primarily targets oxidative/oxygenated cancer cells that use lactate as a source of energy.

The "classic" inhibitors of monocarboxylate transporters have been derivatives of cinnamic acid, first identified by Halestrap and co-workers for their effect on isolated mitochondrial pyruvate transport (Halestrap AP, et al, Biochem J. 1974; 138:313-316.), and by Lehninger and co-workers on intact Ehrich ascites tumor (Spencer TL, et al., Biochem J. 1976; 154:405-414.). The latter study and studies by others (Wahl ML, et al., Mol Cancer Ther. 2002; 1:617-628; Coss RA, et al, Mol Cancer Ther. 2003; 2:383-388.) have indicated the cinnamic acid derivatives to be competitive inhibitors of lactate transport in tumors, with a-cyano-4-hydroxy cinnamic acid (ACCA), a commonly utilized off-the-shelf chemical used as a matrix during mass spectrometry, as one of the more potent inhibitors of lactate transport, with a Ki of 0.5 nM.

The N-methylbenzyl derivative (compound 38) is a representative compound of a series of 7-substituted carboxycoumarins MCT inhibitors (Draoui N et al, Bioorg. Med. Chem., 21:7107(2013)). Compound 38 inhibits lactate uptake with an IC50 value of 59 nM, measured by detecting
the remaining lactate concentration in human cervix carcinoma SiHa cells (expressing mainly MCT1) after 24 h of treatment.

AZD3965 (compound 39) is a selective MCT1 inhibitor (binding affinity 1.6 nM), which is currently undergoing phase I clinical trial in patients with advanced solid tumors or lymphoma. When tested on a panel of small cell lung cancer cell lines assembled to reflect the mutations in TP53, RB1, and MYC family genes that are common in SCLC, compound 39 induced a wide range of responses, with general greater responses in hypoxic conditions, where in some cases the treatment significantly increased intracellular lactate. COR-L103 (human small cell lung cancer cell line) under hypoxic conditions turned out to be the most sensitive among the cell lines tested. Consistently with the proposed mechanism of action, a higher dose of compound 39 was required to inhibit MCT1 in engineered MCT1-overexpressed cells. In vivo, AZD3965 (100 mg/kg bid × 21dd) induced reduction of tumor growth accompanied by high levels of intratumour lactate concentrations in a COR-L103 xenograft model (Polanski R et al., Clin. Cancer Res., 20:926 (2014)).

In addition to inhibiting hexokinase, lonidamine was also shown to inhibit MCTs, which prevents lactate export from cells and causes intracellular acidification (Ben-Yoseph, O. et al, J. Nejrooncol. 36, 149-157 (1998)). It was also reported that lonidamine could inhibit mitochondrial electron transport chain (Floridi, A. et al., Arch. Biochem. Biophys. 226, 73-83 (1983)). However, the mechanism underlying this inhibition is not clear.

In addition to chemical inhibitors, post-transcriptional gene silencing techniques via siRNA (Fire A, et al., Nature. 1998; 391:806-8 11; Zamore PD, et al., Cell. 2000; 101:25-33.) or miRNA (Lee AH. et ai, Cancer Res. 1998; 58:1901-1908.) could be used as an alternative to specifically target

5. Functional Nucleic Acids

Although in preferred embodiments, the modulator is a small molecule, functional nucleic acids target the proteins, enzymes, and other targets discussed above can be targeted by functional nucleic acids. The functional nucleic acids can be antisense molecules. Antisense molecules are designed to interact with a target nucleic acid molecule through either canonical or non-canonical base pairing. The interaction of the antisense molecule and the target molecule is designed to promote the destruction of the target molecule through, for example, RNase H mediated RNA-DNA hybrid degradation. Alternatively the antisense molecule is designed to interrupt a processing function that normally would take place on the target molecule, such as transcription or replication. Antisense molecules can be designed based on the sequence of the target molecule. There are numerous methods for optimization of antisense efficiency by finding the most accessible regions of the target molecule. It is preferred that antisense molecules bind the target molecule with a dissociation constant (Kd) less than or equal to 10⁶, 10⁻⁸, 10⁻¹⁰, or 10⁻¹².

The functional nucleic acids can be aptamers. Aptamers are molecules that interact with a target molecule, preferably in a specific way. Typically aptamers are small nucleic acids ranging from 15-50 bases in length that fold into defined secondary and tertiary structures, such as stem-loops or G-quartets. Aptamers can bind small molecules, such as ATP and theophiline, as well as large molecules, such as reverse transcriptase and thrombin. Aptamers can bind very tightly with K_d's from the target molecule of less than 10⁻¹² M. It is preferred that the aptamers bind the target molecule with a ½ less than10⁶, 10⁻⁸, 10⁻¹⁰, or 10⁻¹². Aptamers can bind the target molecule with a very high degree of specificity. For example, aptamers have been isolated that have greater than a 10,000 fold difference in binding affinities between the target molecule and another molecule that
differ at only a single position on the molecule. It is preferred that the aptamer have a $K_d$ with the target molecule at least $10^2$, $10^5$, $10^8$, or $10^11$ fold Sower than the $K_d$ with a background binding molecule. It is preferred when doing the comparison for a molecule such as a polypeptide, that the background molecule be a different polypeptide.

The functional nucleic acids can be ribozymes. Ribozymes are nucleic acid molecules that are capable of catalyzing a chemical reaction, either intramolecularly or intermolecularly. It is preferred that the ribozymes catalyze intermolecular reactions. There are a number of different types of ribozymes that catalyze nuclease or nucleic acid polymerase type reactions which are based on ribozymes found in natural systems, such as hammerhead ribozymes. There are also a number of ribozymes that are not found in natural systems, which have been engineered to catalyze specific reactions de novo. Preferred ribozymes cleave RNA or DNA substrates, and more preferably cleave RNA substrates. Ribozymes typically cleave nucleic acid substrates through recognition and binding of the target substrate with subsequent cleavage. This recognition is often based mostly on canonical or non-canonical base pair interactions. This property makes ribozymes particularly good candidates for target specific cleavage of nucleic acids because recognition of the target substrate is based on the target substrates sequence.

The functional nucleic acids can be triplex forming molecules. Triplex forming functional nucleic acid molecules are molecules that can interact with either double-stranded or single-stranded nucleic acid. When triplex molecules interact with a target region, a structure called a triplex is formed in which there are three strands of DNA forming a complex dependent on both Watson-Crick and Hoogsteen base-pairing. Triplex molecules are preferred because they can bind target regions with high affinity and specificity. It is preferred that the triplex forming molecules bind the target molecule with a $K_d$ less than $10^{-6}$, $10^{-8}$, $10^{-10}$, or $10^{-12}$.

The functional nucleic acids can be external guide sequences. External guide sequences (EGSs) are molecules that bind a target nucleic acid molecule forming a complex, which is recognized by RNase P, which
then cleaves the target molecule. EGSs can be designed to specifically target a RNA molecule of choice. RNAse P aids in processing transfer RNA (tRNA) within a cell. Bacterial RNAse P can be recruited to cleave virtually any RNA sequence by using an EGS that causes the target RNA:EGS complex to mimic the natural tRNA substrate. Similarly, eukaryotic EGS/RNAse P-directed cleavage of RNA can be utilized to cleave desired targets within eukarotic cells. Representative examples of how to make and use EGS molecules to facilitate cleavage of a variety of different target molecules are known in the art.


Short Interfering RNA (siRNA) is a double-stranded RNA that can induce sequence-specific post-transcriptional gene silencing, thereby decreasing or even inhibiting gene expression. In one example, a siRNA triggers the specific degradation of homologous RNA molecules, such as mRNAs, within the region of sequence identity between both the siRNA and
the target RNA. For example, WO 02/44321 discloses siRNAs capable of sequence-specific degradation of target mRNAs when base-paired with 3’ overhanging ends, herein incorporated by reference for the method of making these siRNAs.

5 Sequence specific gene silencing can be achieved in mammalian cells using synthetic, short double-stranded RNAs that mimic the siRNAs produced by the enzyme dicer (Elbashir, et al. (2001) Nature, 411:494-498) (Ui-Tei, et al. (2000) FEBS Lett 479:79-82). siRNA can be chemically or in vitro-synthesized or can be the result of short double-stranded hairpin-like RNAs (shRNAs) that are processed into siRNAs inside the cell. Synthetic siRNAs are generally designed using algorithms and a conventional DNA/RNA synthesizer. Suppliers include Ambion (Austin, Texas), ChemGenes (Ashland, Massachusetts), Dharmacon (Lafayette, Colorado), Glen Research (Sterling, Virginia), MWB Biotech (Esbersberg, Germany), Proligo (Boulder, Colorado), and Qiagen (Venlo, The Netherlands). siRNA can also be synthesized in vitro using kits such as Ambion's SILENCER® siRNA Construction Kit.

The production of siRNA from a vector is more commonly done through the transcription of a short hairpin RNA (shRNAs). Kits for the production of vectors comprising shRNA are available, such as, for example, Imgenex's GENESUPPRESSOR™ Construction Kits and Invitrogen's BLOCK-IT™ inducible RNAi plasmid and lentivirus vectors.

In some embodiment, the functional nucleic acid is siRNA, shRNA, miRNA. In some embodiments, the composition includes a vector expressing the functional nucleic acid. Methods of making and using vectors for in vivo expression of functional nucleic acids such as antisense oligonucleotides, siRNA, shRNA, miRNA, EGSs, ribozymes, and aptamers are known in the art.

B. Nanoparticles

30 The cancer cell metabolism modulator can be administered to a subject with or without the aid of a delivery-vehicle. Appropriate delivery vehicles for the compounds are known in the art and can be selected to suit the particular active agent. For example, in some embodiments, the active
agent(s) is incorporated into or encapsulated by, or bound to, a nanoparticle, microparticle, microsphere, micelle, synthetic lipoprotein particle, or carbon nanotube. For example, the compositions can be incorporated into a vehicle such as polymeric microparticles which provide controlled release of the active agent(s). In some embodiments, release of the drag(s) is controlled by diffusion of the active agent(s) out of the microparticles and/or degradation of the polymeric particles by hydrolysis and/or enzymatic degradation.

In some embodiments, two agents are incorporated into the same particles and are formulated for release at different times and/or over different time periods. For example, in some embodiments, one of the agents is released entirely from the particles before release of the second agent begins. In other embodiments, release of the first agent begins followed by release of the second agent before the all of the first agent is released. In still other embodiments, both agents are released at the same time over the same period of time or over different periods of time.

1. Particle Core

The particle core can be a polymeric particle, a lipid particle, a solid lipid particle, an inorganic particle, or combinations thereof. For example, the particle core can be a lipid-stabilized polymeric particle. In preferred embodiments the particle core is a polymeric particle, a solid lipid particle, or a lipid-stabilized polymeric particle, preferably a polymeric particle.

The particle core may have any diameter. The particle core can have a diameter of about 10 nm to about 10 microns, about 10 nm to about 1 micron, about 10 nm to about 500 nm, about 20 nm to about 500 nm, or about 25 nm to about 250 nm. In preferred embodiments the particle core is a nanoparticle core having a diameter from about 25 nm to about 250 nm. In the most preferred embodiment the particles have a diameter of three to 150 nm.

The particle core may have any zeta potential particle core can have a zeta potential from -300 mV to +300 mV, -100 mV to +100 mV, from -50 mV to +50 mV, from -40 mV to +40 mV, from -30 mV to +30 mV, from -20 mV to +20 mV, from -10 mV to +10 mV, or from -5 mV to +5 mV. The particle core can have a negative zeta potential. The particle core can have a
positive zeta potential. In some embodiments the particle core has a substantially neutral zeta potential, i.e. the zeta potential is approximately 0 mV. In some embodiments the particle core has a zeta potential of approximately -20 mV to +20 mV, more preferably -10 mV to +10 mV. In some embodiments, the zeta greater than 0, e.g., 0 mV to +40 mV to enhance mitochondrial targeting.

**Polymeric Particle Core**

The particle core can be a polymeric particle core. The polymeric particle core can be formed from biodegradable polymers, non-biodegradable polymers, or a combination thereof. The polymeric particle core can be a biodegradable polymeric core in whole or in part. For example, an imaging agent or diagnostic agent that needs to be retained in the particles and cleared from the body can be encapsulated in a non-biodegradable polymer matrix.

Biodegradable polymers can include polymers that are insoluble or sparingly soluble in water that are converted chemically or enzymatically in the body into water-soluble materials. Biodegradable polymers can include soluble polymers crosslinked by hydolyzable cross-linking groups to render the crosslinked polymer insoluble or sparingly soluble in water.

Representative biodegradable polymers include polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene oxides, polyalkylene terephthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, polyvinylpyrrolidione, polyglycolides, polysiloxanes, polyurethanes and copolymers thereof, alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, polymers of acrylic and methacrylic esters, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy -propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, cellulose sulphate sodium salt, poly (methyl methacrylate), poly(ethylmethacrylate), poly(butylmethacrylate), poly(isobutylmethacrylate), poly(hexylmethacrylate), poly(isodecylmethacrylate), poly(lauryl methacrylate), poly (phenyl
methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate), polyethylene, polypropylene poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), polyvinyl alcohols), poly(vinyl acetate, poly vinyl chloride polystyrene and polyvinylpyrrolidone, derivatives thereof, linear and branched copolymers and block copolymers thereof, and blends thereof. Exemplary biodegradable polymers include polyesters, poly(ortho esters), polyethylene imines), poly(caprolactones), poly(hydroxybutyrates), poly(hydroxyvalerates), polyanhydrides, poly(acrylic acids), polyglycoiides, poly(urethanes), polycarbonates, polyphosphate esters, polyphosphazenes, derivatives thereof, linear and branched copolymers and block copolymers thereof, and blends thereof. Non-biodegradable polymers can include ethylene vinyl acetate, poly(meth) acrylic acid, polyamides, copolymers and mixtures thereof.

Excipients may also be added to the core polymer to alter its porosity, permeability, and or degradation profile.

The polymeric core can contain one or more hydrophilic polymers. Hydrophilic polymers include cellulosic polymers such as starch and polysaccharides; hydrophilic polypeptides; poly(amine acids) such as poly-L-glutamic acid (PGS), gamma-polyglutamic acid, poly-L-aspartic acid, poly-L-serine, or poly-L-lysine; poiyalkylene glycols and polyalkylene oxides such as polyethylene glycol (PEG), polypropylene glycol (PPG), and poly(ethylene oxide) (PEO); poly(oxyethylated polyol); poly(olefinic alcohol); polynvinlypyrrolidone; poly(hydroxyalkylmethacrylate); poly(saccharides); poiy(hydroxy acids); poly(vinyl alcohol), and copolymers thereof.

Examples of suitable hydrophobic polymers include polyhydroxyacids such as poly(lactic acid), poly(glycolic acid), and poly(lactic acid-co-glycolic acids); polyhydroxyalkanoates such as poly3-hydroxybutyrate or poly4-hydroxybutyrate; polycaprolactones; poly(orthoesters); polyanhydrides; poly(phosphazenes); poly(lactide-co-caprolactones); polycarbonates such as tyrosine polycarbonates; polynamides (including synthetic and natural polynamides), polypeptides, and poly(amine acids); polynesteramides; polyesters; poly(dioxanones); polynalkylene
alkylates); hydrophobic polyethers; polyurethanes; polyethers; polyacetals; polycyanoacrylates; polyacrylates; polymethylmethacrylates; polysiloxanes; poly(oxyethylene)/poly(oxypropylene) copolymers; polyketals; polyphosphates; polyhydroxyvalerates; polyalkylene oxalates; polyalkylene succinates; poly(maleic acids), as well as copolymers thereof.

In certain embodiments, the hydrophobic polymer is an aliphatic polyester. In preferred embodiments, the polymeric core contains biodegradable polyesters or polyanhydrides such as poly(lactic acid), poly(glycolic acid), and poly(lactic-co-glycolic acid).

The molecular weight of the hydrophobic polymer can be varied to tailor the properties of polymeric particle core. For example, the molecular weight of the hydrophobic polymer segment can be varied to engineer nanoparticles possessing the required average particle size and degradation profile. The hydrophobic polymer segment has a molecular weight of between about 150 Da and about 100 kDa, more preferably between about 1 kDa and about 75 kDa, most preferably between about 5 kDa and about 50 kDa.

The polymeric particle core can contain an amphiphilic polymer. Amphiphilic polymers can include block copolymers of any of the hydrophobic and hydrophilic polymers described above. In some embodiments the amphiphilic polymer is a copolymer containing a hydrophobic polyhydroxyacid block and a hydrophilic polyalkylene glycol block. The amphiphilic polymer can be a PLGA-PEG block copolymer, and PGA-PEG block copolymer, or a PLGA-PEG block copolymer.

PEGylation may also be used, in some cases, to decrease charge interaction between a polymer and a biological moiety, e.g., by creating a hydrophilic layer on the surface of the polymer, which may shield the polymer from interacting with the biological moiety. In some cases, the addition of poly(ethylene glycol) repeat units may increase plasma half-life of the polymer (e.g., copolymer, e.g., block copolymer), for instance, by decreasing the uptake of the polymer by the phagocytic system while decreasing transfection/uptake efficiency by cells. Those of ordinary skill in the art will know of methods and techniques for PEGylating a polymer, for
example, by using EDC (l-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) and NHS (N-hydroxysuccinimide) to react a polymer to a PEG group terminating in an amine, or by ring opening polymerization techniques (ROMP).

Copolymers containing poly(ester-ether)s, e.g., polymers having repeat units joined by ester bonds (e.g., R-C-O-R bonds) and ether bonds (e.g., R-O-R' bonds) may be formed as a hydrolyzable polymer, containing carboxylic acid groups, conjugated with poly(ethylene glycol) repeat units to form a poly(ester-ether).

The polymeric particle core can contain any of the above polymers or blends or copolymers thereof. The polymeric particle core can contain one, two, three, or more different polymers.

Amphiphilic compounds include, but are not limited to, phospholipids, such as 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), diarachidoylphosphatidylcholine (DAPC), dibehenoylphosphatidylcholine (DBPC), ditricosanoylphosphatidylcholine (DTPC), and dilignocerylphosphatidylcholine (DLPC), incorporated at a ratio of between 0.01-60 (weight lipid/w polymer), most preferably between 0.1-30 (weight lipid/w polymer).

Phospholipids which may be used include, but are not limited to, phosphatide acids, phosphatidyl cholines with both saturated and unsaturated lipids, phosphatidyl ethanolamines, phosphatidyglycerols, phosphatidylserines, phosphatidylinositol, lysophosphatidyl derivatives, cardiolipin, and β-acetyl-alkyl phospholipids. Examples of phospholipids include, but are not limited to, phosphatidylcholines such as dioleoylphosphatidylcholine, dimyristoylphosphatidylcholine, dipentadecanoylphosphatidylcholine, dilauroylphosphatidylcholine, dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), diarachidoylphosphatidylcholine (DAPC), dibehenoylphosphatidylcholine (DBPC), ditricosanoylphosphatidylcholine (DTPC), dilignocerylphosphatidylcholine (DLPC); and phosphatidylethanolamines such as dioleoylphosphatidylethanolamine or 1-
hexadecyl-2-palmitoylglycerophosphoethanolamine. Synthetic phospholipids with asymmetric acyl chains (e.g., with one acyl chain of 6 carbons and another acyl chain of 12 carbons) may also be used.

The amphiphilic lipid can have a molecular weight of 200 to 1000, e.g., 700-900. By containing a relatively small amount of lipid, the nanoparticles avoid the negative impact that a tri, tetra or higher layer of lipid could have on a nanoparticle, such as an adverse effect on drug release. Thus, in one embodiment, the nanoparticles comprise approximately 10% to 40% lipid (by weight), and will have a size of about 90nm to about 40nm in diameter.

In a particular embodiment, an amphiphilic component that can be used to form an amphiphilic layer is lecithin, and, in particular, phosphatidylcholine. Lecithin forms a phospholipid bilayer having the hydrophilic (polar) heads facing aqueous solutions, and the hydrophobic tails facing each other. Lecithin has an advantage of being a natural lipid that is available from, e.g., soybean, and already has FDA approval for use in other delivery devices.

The particle core can be a lipid particle core. In some embodiments the particle core is a lipid nanoparticle. Lipid particles and lipid nanoparticles are known in the art. The lipid particles and lipid nanoparticles can be lipid micelles, liposomes, or solid lipid particles. The lipid particle can be made from one or a mixture of different lipids. Lipid particles are formed from one or more lipids, which can be neutral, anionic, or cationic at physiologic pH. The lipid particle is preferably made from one or more biocompatible lipids.

The lipid particles may be formed from a combination of more than one lipid, for example, a charged lipid may be combined with a lipid that is non-ionic or uncharged at physiological pH.

The particle core can be a lipid micelle. Lipid micelles for drug delivery are known in the art. Lipid micelles can be formed, for instance, as a water-in-oil emulsion with a lipid surfactant. An emulsion is a blend of two immiscible phases wherein a surfactant is added to stabilize the dispersed droplets. In some embodiments the lipid micelle is a microemulsion. A microemulsion is athermodynamically stable system composed of at least
water, oil and a lipid surfactant producing a transparent and
thermodynamically stable system whose droplet size is less than 1 micron,
from about 10 nm to about 500 nm, or from about 10 nm to about 250 nm.
Lipid micelles are generally useful for encapsulating hydrophobic active
agents, including hydrophobic therapeutic agents, hydrophobic prophylactic
agents, or hydrophobic diagnostic agents. The particle core can be a
liposome. Liposomes are small vesicles composed of an aqueous medium
surrounded by lipids arranged in spherical bilayers. Liposomes can be
classified as small unilamellar vesicles, large unilamellar vesicles, or multi-
lamellar vesicles. Multi-lamellar liposomes contain multiple concentric lipid
bilayers. Liposomes can be used to encapsulate agents, by trapping
hydrophilic agents in the aqueous interior or between bilayers, or by trapping
hydrophobic agents within the bilayer.

The lipid micelles and liposomes typically have an aqueous center.
The aqueous center can contain water or a mixture of water and alcohol.
Suitable alcohols include, but are not limited to, methanol, ethanol, propanol,
(such as isopropanol), butanol (such as -butanol, isobutanol, sec-butanol,
tert-butanol, pentanol (such as amy alcohol, isobutyl carbinol), hexanol
(such as 1-hexanol, 2-hexanol, 3-hexanol), heptanol (such as 1-heptanol, 2-
heptanol, 3-heptanol and 4-heptanol) or octanol (such as 1-octanol) or a
combination thereof.

The particle core can be a solid lipid particle. Solid lipid particles
present an alternative to the colloidal micelles and liposomes. Solid lipid
particles are typically submicron in size, i.e. from about 10 nm to about 1
micron, from 10 nm to about 500 nm, or from 10 nm to about 250 nm. Solid
lipid particles are formed of lipids that are solids at room temperature. They
are derived from oil-in-water emulsions, by replacing the liquid oil by a solid
lipid.

Suitable neutral and anionic lipids include, but are not limited to,
sterols and lipids such as cholesterol, phospholipids, lysolipids,
lysophospholipids, sphingolipids or pegyated lipids. Neutral and anionic
lipids include, but are not limited to, phosphatidylcholine (PC) (such as egg
PC, soy PC), including 1,2-diacyl-glycerol-3-phosphochoHnes;
phosphatidylserine (PS), phosphatidylglycerol, phosphatidylinositol (Pi); glycolipids; sphingophospholipids such as sphingomyelin and sphingoglycolipids (also known as 1-ceramidyl glucosides) such as ceramide galactopyranoside, gangliosides and cerebrosides; fatty acids, sterols, containing a carboxylic acid group for example, cholesterol; 1,2-diacyl-sn-glycero-3-phosphoethanolamine, including, but not limited to, 1,2-dioleoylphosphoethanolamine (DOPE), 1,2-dihexadecylphosphoethanolamine (DUPE), 1,2-distearoylphosphatidylcholine (DSPC), 1,2-dipalmitoyl phosphatidylcholine (DPPC), and 1,2-dimyristoylphosphatidylcholine (DMPC). The lipids can also include various natural (e.g., tissue derived L-a-phosphatidyl: egg yolk, heart, brain, liver, soybean) and/or synthetic (e.g., saturated and unsaturated 1,2-diacyl-3-glycero-3-phosphocholines, 1-acyl-2-acyi-3-glycero-3-phosphocholines, 1,2-diheptanoyl-SN-glycero-3-phosphocholine) derivatives of the lipids.

Suitable cationic lipids include, but are not limited to, N-[l-(2,3-dioleoyloxy)propyiJ-N,N,N-trimethyi ammonium salts, also references as TAP lipids, for example methylsulfate salt. Suitable TAP lipids include, but are not limited to, DOTAP (dioleoyl-), DMTAP (dimyristoyl-), DPTAP (dipalmitoyl-), and DSTAP (distearoyl-). Suitable cationic lipids in the liposomes include, but are not limited to, dimethyldioctadecyl ammonium bromide (DDAB), 1,2-diacyloxy-3-trimethylammonium propanes, N-[l-(2,3-diacyloxypropyl)-N,N-dimethyl amine (DODAP), 1,2-diacyloxy-3-dimethylammonium propanes, N-fl-(2,3-diacyloxypropyl]-N,N,N-trimethylammonium chloride (DOTMA), 1,2-dialkyloxy-3-dimethylammonium propanes, dioctadecylamido-lyspermine (DOGS), 3-[N-(N',N'-dimethylaminomethane)carbamoyl]cholesterol (DC-Choi); 2,3-dioleolol-3-(2-sperminecarboxamido)-ethyl]-N,N -dimethyl-1-propanaminium trifluoroacetate (DOSPA), β-aianyl cholesterol, cetyl trimethyl ammonium bromide (CTAB), diC4-amidine, N-ferf-butyl-N'-tetradecyl-3-tetraecylamino-propionarnidine, N-(alpha-trimethylarrimonoacetyl)didodecyl-D-glutamate chloride (TMAG), ditetradecaiiyl-N-(trimethylammonio-acetyl)diethanolamine chloride, 1,3-dioleolox-2-(6-carboxy-spenny)-propylamid (DOSPER), and N,N,N',N'-tetramethyl- bis(2-hydroxylethyl)-2,3-dioleoloxyl-1,4-
butanediammonium iodide. In one embodiment, the cationic lipids can be 1-[2-(acyloxy)ethyl]2-alkyl(alkenyl)-3-(2-hydroxyethyl)imidazolinium chloride derivatives, for example, 1-[2-(9(Z)-octadecenoyloxy)ethyl]-2-(8(Z)-heptadecenyl)-3-(2-hydroxyethyl)imidazolinium chloride (DOTIM), and 1-[2-(hexadecanoykixy)ediyl]-2-pentadecyl-3-(24iydroxyethyl)imidazolinium chloride (DPTIM). In one embodiment, the cationic lipids can be 2,3-dialkyloxypropyl quaternary ammonium compound derivatives containing a hydroxyalkyl moiety on the quaternary amine, for example, 1,2-dioleoyl-3-dimethyl-hydroxyethyl ammonium bromide (DORI), 1,2-dioleyloxypropyl-3-dimethyl-hydroxy ethyl ammonium bromide (DORJE), 1,2-dioleyloxypropyl-3-dimethyl-hydroxypropyl ammonium bromide (DORIE-HP), 1,2-dioleyl-oxypropyl-3-dimethyl-hydroxybutyl ammonium bromide (DORIE-HB), 1,2-dioleyloxypropy 1-3-dimethyl-hydroxypentyl ammonium bromide (DORIE-Hpe), 1,2-dimyristyl-oxypropyl-3-dimethy3-hydroxylethyl ammonium bromide (DMRIE), 1,2-dipalmityloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DPRIE), and 1,2-disterpropyl-3-dimethyl-hydroxyetyl ammonium bromide (DSRIE).

Suitable solid lipids include, but are not limited to, higher saturated alcohols, higher fatty acids, sphingolipids, synthetic esters, and mono-, di-, and triglycerides of higher saturated fatty acids. Solid lipids can include aliphatic alcohols having 10-40, preferably 12-30 carbon atoms, such as cetostearyl alcohol. Solid lipids can include higher fatty acids of 10-40, preferably 12-30 carbon atoms, such as stearic acid, palmitic acid, decanoic acid, and behenic acid. Solid lipids can include glycerides, including monoglycerides, diglycerides, and triglycerides, of higher saturated fatty acids having 10-40, preferably 12-30 carbon atoms, such as glyceryl monoistearte, glycerol behenate, glycerol palmitostearate, glycerol trilaurate, tricaprin, trilaurin, trimyristin, tripalmitm, tristearin, and hydrogenated castor oil. Suitable solid lipids can include cetyl palmitate, beeswax, or cyclodextrin.

The particle core can be an inorganic particle such as metal or semiconductor particles. The particle core can be a metal nanoparticle, a semiconductor nanoparticle, or a core-shell nanoparticle. Inorganic particles
and inorganic nanoparticles can be formulated into a variety of shapes such as rods, shells, spheres, and cones. The inorganic particle may have any dimension. The inorganic particle can have a greatest dimension less than 1 micron, from about 10 nm to about 1 micron, from about 10 nm to about 500 nm, or from 10 nm to about 250 nm.

The inorganic particle core can contain a metal. Suitable metals can include alkali metals such as lithium, sodium, potassium, rubidium, cesium and francium; alkaline earth metals such as beryllium, magnesium, calcium, strontium, barium and radium; transition metals such as zinc, molybdenum, cadmium, scandium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, yttrium, zirconium, niobium, technetium, ruthenium, rhodium, palladium, silver, tungsten, iridium, and platinum; post-transition metals such as aluminum, gallium, indium, tin, thallium, lead, and bismuth; lanthanoids such as lanthanum, cerium, neodymium, and europium; and actinoids such as actinium, thorium, protactinium, uranium, neptunium, and plutonium. The metal can be biodegradable or non-biodegradable. Biodegradable metals can include alloys of iron or magnesium with the above metals, including alloys of magnesium, aluminum, and zinc.

The inorganic particle core can contain a metal oxide. Metal oxides of any of the above metals are contemplated. Suitable metal oxides can include metal oxides that contain one or more of the following metals: titanium, scandium, iron, tantalum, cobalt, chromium, manganese, platinum, iridium, niobium, vanadium, zirconium, tungsten, rhodium, ruthenium, copper, zinc, yttrium, molybdenum, technetium, palladium, cadmium, hafnium, rhenium and combinations thereof. Suitable metal oxides can include cerium oxides, platinum oxides, yttrium oxides, tantalum oxides, titanium oxides, zinc oxides, iron oxides, magnesium oxides, aluminum oxides, iridium oxides, niobium oxides, zirconium oxides, tungsten oxides, rhodium oxides, ruthenium oxides, alumina, zirconia, silicone oxides such as silica based glasses and silicon dioxide, or combinations thereof. The metal oxide can be non-biodegradable. The metal oxide can be a biodegradable metal oxide. Biodegradable metal oxides can include silicon oxide, aluminum oxide and zinc oxide.
The particle core can be a hybrid particle. Hybrid particle, as used herein, refers to a particle that combines the features of two or more of polymeric particles, lipid particles, and inorganic particles. Examples of hybrid particles can include polymer-stabilized liposomes, polymer-coated inorganic particles, or lipid-coated polymeric particles. The hybrid particle can contain a polymeric inner region, a lipid inner region, or an inorganic inner region. The hybrid particle can contain a polymer outer layer, a lipid outer layer, or an inorganic outer layer.

The particle core can be a polymer-stabilized lipid particle. The particle core can be a polymer-stabilized liposome. Polymer-stabilized liposomes are described, for example, in WO 2008/082721 by Dominguez et al. The particle core can be a polymer-stabilized solid lipid particle. Solid lipid particles have been coated with polymers to impart stability (see Nahire et al., Biomacromolecules, 14:841-853 (2013)) or to impart stealth properties (see Uner and Yener, Int. J. Nanomedicine, 2:289-300 (2007)). The polymer-stabilized liposomes and polymer-stabilized solid lipid particles include a lipid particle core stabilized by the presence of a coating polymer. The coating polymer can be covalently or non-covalently bound to the lipid particle. The coating polymer can be a lipophilic polymer, a biodegradable polymer, a polymer decreasing uptake by the RES, or a combination thereof.

The particle core can be a polymer-stabilized inorganic particle such as a polymer-coated metal nanoparticle. WO 2013/070653 by Alocilja et al. described metal nanoparticle stabilized by a polysaccharide coating polymer.

Suitable lipophilic polymers can include aliphatic polyesters, such as polylactic acid, polyglycolic acid and their copolymers; poly(ε-caprolactone), poly(ω-valerolactone), polyesters with longer (i.e., C5 to C25) hydrocarbon chains; dendritic polymers of polyesters containing a modified terminal hydroxyl; aliphatic and aromatic polycarbonates; aliphatic polyamides, polypeptides; polyesteramides; polyurethanes; silicones, such as poly(dimethylsiloxanes); lipophilic poly(phosphazenes); poly(methacrylic acid), poly(styrene) and hydrophobic polyacrylic, polyvinyl and polystyrene carriers.
2. Particle Properties

Particles may be microparticles or nanoparticles. Nanoparticles are preferred for intertissue application, penetration of cells, and certain routes of administration. The nanoparticles may have any desired size for the intended use. The nanoparticles may have any diameter from 10 nm to 1,000 nm. The nanoparticle can have a diameter from 10 nm to 900 nm, from 10 nm to 800 nm, from 10 nm to 700 nm, from 10 nm to 600 nm, from 10 nm to 500 nm, from 20 nm to 500 nm, from 30 nm to 500 nm, from 40 nm to 500 nm, from 50 nm to 500 nm, from 60 nm to 400 nm, from 50 nm to 350 nm, from 50 nm to 300 nm, or from 50 nm to 200 nm. In preferred embodiments the nanoparticles can have a diameter less than 400 nm, less than 300 nm, or less than 200 nm. The preferred range is between 50 nm and 300 nm, or 25 nm and 250 nm, or 80 nm and 150 nm.

Each of the components of the nanoparticles is discussed in more detail below.

One embodiment provides nanoparticles that are engineered to maximize half-life and targeting of the nanoparticles to WAT or WAT vasculature by adjusting the amount of PEG and the density of targeting moieties of the nanoparticles.

C. Targeting Moieties

The composition can include one or more targeting moieties associated with, linked, conjugated, or otherwise attached directly or indirectly to the modulator of cancer cell metabolism, or to a nanoparticle or oilier delivery vehicle thereof. In some embodiments, the composition includes a targeting moiety can, for example, target the composition to cancer cells or a tumor microenvironment, target the mitochondria, or a combination thereof. Accordingly, the modulators or the nanoparticles can include binding moieties or targeting moieties that specifically bind to the target agent. A positive feedback loop is created when the nanoparticles release an inducing agent that causes a targeted cell, tissue or organ to increase the expression or bioavailability of a target agent specifically recognized by the nanoparticle.
Representative targeting moieties include, but are not limited to, antibodies and antigen binding fragments thereof, aptamers, peptides, and small molecules. The binding moiety can be conjugated to a polymer that forms the nanoparticle. Typically the binding moiety is displayed on the outer shell of the nanoparticle. The outer shell serves as a shield to prevent the nanoparticles from being recognized by a subject's immune system thereby increasing the half-life of the nanoparticles in the subject. The nanoparticles also contain a hydrophobic core. In preferred embodiments, the hydrophobic core is made of a biodegradable polymeric material. The inner core carries therapeutic payloads and releases the therapeutic payloads at a sustained rate after systemic, intraperitoneal, oral, pulmonary, or topical administration. The nanoparticles also optionally include a detectable label, for example a fluorophore or NMR contrast agent that allows visualization of nanoparticles within plaques.

The targeting moiety can be an antibody or antigen binding fragment thereof. The targeting moieties should have an affinity for a cell-surface receptor or cell-surface antigen on the target cells. The targeting moieties may result in internalization of the particle within the target cell.

The targeting moiety can specifically recognize and bind to a target molecule specific for a cell type, a tissue type, or an organ. The target molecule can be a cell surface polypeptide, lipid, or glycolipid. The target molecule can be a receptor that is selectively expressed on a specific cell surface, a tissue or an organ. Cell specific markers can be for specific types of cells including, but not limited to stem cells, skin cells, blood cells, immune cells, muscle cells, nerve cells, cancer cells, virally infected cells, and organ specific cells. The cell markers can be specific for endothelial, ectodermal, or mesenchymal cells. Representative cell specific markers include, but are not limited to cancer specific markers.
1. Exemplary Types of Targeting Moieties
   
a. Peptide Targeting Moieties

   In a preferred embodiment, the targeting moiety is a peptide. Specifically, the plaque targeted peptide can be, but is not limited to, one or more of the following: RGD, iRGD(CRGDK/RGPD/EC), LyP-1, P3(CKGGRAKDC), or their combinations at various molar ratios. The targeting peptides can be covalently associated with the polymer and the covalent association can be mediated by a linker. The peptides target to actively growing (angiogenic) vascular endothelial cells. Those angiogenic endothelial cells frequently appear in metabolic tissues such as adipose tissues.

b. Antibody Targeting Moieties

   The targeting moiety can be an antibody or an antigen-binding fragment thereof. The antibody can be any type of immunoglobulin that is known in the art. For instance, the antibody can be of any isotype, e.g., IgA, IgD, IgE, IgG, IgM, etc. The antibody can be monoclonal or polyclonal. The antibody can be a naturally-occurring antibody, e.g., an antibody isolated and/or purified from a mammal, e.g., mouse, rabbit, goat, horse, chicken, hamster, human, etc. Alternatively, the antibody can be a genetically-engineered antibody, e.g., a humanized antibody or a chimeric antibody. The antibody can be in monomeric or polymeric form. The antigen binding portion of the antibody can be any portion that has at least one antigen binding site, such as Fab, F(ab')2, dsFv, sFv, diabodies, and triabodies. In certain embodiments, the antibody is a single chain antibody.

e. Aptamer Targeting Moieties

   Aptamers are oligonucleotide or peptide sequences with the capacity to recognize virtually any class of target molecules with high affinity and specificity. Aptamers bind to targets such as small organics, peptides, proteins, cells, and tissues. Unlike antibodies, some aptamers exhibit stereoselectivity. The aptamers can be designed to bind to specific targets expressed on cells, tissues or organs.
2. Exemplary Targets and Embodiments

The targeting moiety can be specific for a host, tissue, organ, cell, organelle, non-nuclear organelle, or cellular compartment. The targeting moiety can bind to its ligand or receptor, which is located on the surface of a target cell such as to bring the composition and cell membranes sufficiently close to each other to allow penetration of the composition into the cell.

The targeting moiety can be, for example, an antibody or antigen binding fragment thereof an antibody domain, an antigen, a cell surface receptor, a cell surface adhesion molecule, or a peptide selected by phage display that binds specifically to a defined cell. In some embodiments, the target moiety binds to the target cell through traditional receptor:ligand interactions. In some embodiments the interaction of the targeting signal with the cell does not occur through a traditional receptor:ligand interaction. The eukaryotic cell has a number of distinct cell surface molecules. The structure and function of each molecule can be specific to the origin, expression, character and structure of the cell. Determining the unique cell surface complement of molecules of a specific cell type can be determined using techniques well known in the art.

The target cell specificity of the composition can be altered by merely changing the targeting moiety. It is known in the art that nearly every cell type in a tissue in a mammalian organism possesses some unique cell surface receptor or antigen. Thus, it is possible to incorporate nearly any ligand for the cell surface receptor or antigen as a targeting moiety. For example, peptidyl hormones can be used a targeting moieties to target delivery to those cells which possess receptors for such hormones. Chemokines and cytokines can similarly be employed as targeting signals to target delivery of the complex to their target cells. A variety of technologies have been developed to identify genes that are preferentially expressed in certain cells or cell states and one of skill in the art can employ such technology to identify targeting signals which are preferentially or uniquely expressed on the target tissue of interest.

In some embodiments, the targeting moiety is an antibody or an antigen-binding fragment thereof, for example, an antibody that is specific to
a tumor antigen such as a tumor cell surface marker. In some embodiments, the targeting moiety includes an antibody binding domain, for example from proteins known to bind antibodies such as Protein A and Protein G from Staphylococcus aureus. Other domains known to bind antibodies are known in the art and can be substituted. Such domains can be used to link a targeting antibody to the composition. In certain embodiments, the antibody is polyclonal, monoclonal, linear, humanized, chimeric or a fragment thereof. Representative antibody fragments are those fragments include, but are not limited to, Fab, Fab', F(ab')₂, Fv diabodies, linear antibodies, single chain antibodies and bispecific antibodies known in the art. Antibodies can be derived from human genes and produced to reduce potential immunogenicity to a human host as is known in the art. For example, transgenic mice which contain the entire human immunoglobulin gene cluster are capable of producing "human" antibodies can be utilized. In some embodiments, single chain antibodies modeled on human antibodies are prepared in prokaryotic culture.

In some embodiments, the targeting moiety is a ligand that binds to cell surface antigens or receptors that are specifically expressed on tumor cells or tumor-associated neovascularure or are overexpressed on tumor cells or tumor-associated neovascularure as compared to normal tissue. Tumors also secrete a large number of ligands into the tumor microenvironment that affect tumor growth and development. Receptors that bind to ligands secreted by tumors, including, but not limited to growth factors, cytokines and chemokines, including the chemokines provided below, are suitable for use as targeting moieties. Ligands secreted by tumors can be targeted using soluble fragments of receptors that bind to the secreted ligands. Soluble receptor fragments are fragments polypeptides that may be shed, secreted or otherwise extracted from the producing cells and include the entire extracellular domain, or fragments thereof.

In some embodiments, the targeting moiety is an Fc domain of immunoglobulin heavy chains that bind to Fc receptors expressed on tumor cells or on tumor-associated neovascularure. The Fc region as used herein includes the polypeptides containing the constant region of an antibody
excluding the first constant region immunoglobulin domain. Thus Fc refers
to the last two constant region immunoglobulin domains of IgA, IgD, and
IgG, and the last three constant region immunoglobulin domains of IgE and
IgM. In a preferred embodiment, the Fc domain is derived from a human or
murine immunoglobulin. In a more preferred embodiment, the Fc domain is
derived from human IgG1 or murine IgG2a including the CH2 and CH3
regions.

Preferably, the targeting moiety selectively targets tumor cells or the
tumor microenvironment. Tumor cells express cell surface markers which
may only be expressed in the tumor, or present in non-tumor cells but
preferentially presented in tumor cells. In some embodiments, the targeting
domains bind to antigens, ligands or receptors that are specific to tumor cells
or tumor-associated neovasculature, or are upregulated in tumor cells or
tumor-associated neovasculature compared to normal tissue. Exemplary
cancer and tumor specific target molecules are discussed in more detail
below.

a. Tumor-specific and tumor-associated
antigens

In some embodiments, the targeting moiety binds to an antigen that is
expressed by tumor cells. The antigen expressed by the tumor may be
specific to the tumor, or may be expressed at a higher level on the tumor
cells as compared to non-tumor cells. Antigenic markers such as
serologically defined markers known as tumor associated antigens, which are either uniquely expressed by cancer cells or are present at markedly higher
levels (e.g., elevated in a statistically significant manner) in subjects having a
malignant condition relative to appropriate controls, are contemplated for use
in certain embodiments.

Tumor-associated antigens include, for example, cellular oncogene-
encoded products or aberrantly expressed proto-oncogene-encoded products
(e.g., products encoded by the neu, ras, trk, and kit genes), or mutated forms
of growth factor receptor or receptor-like cell surface molecules (e.g., surface
receptor encoded by the c-erb B gene). Other tumor-associated antigens
include molecules that may be directly involved in transformation events, or
molecules that may not be directly involved in oncogenic transformation events but are expressed by tumor cells (e.g., carcinoembryonic antigen, CA-125, melanoma associated antigens, etc.) (see, e.g., U.S. Pat. No. 6,699,475; Jager, et al., Int. J. Cancer, 106:8 17-20 (2003); Kennedy, et al.,/nr. Rev. Immunol., 22: 14 1-72 (2003); Scanlan, et al. Cancer Immun., 4:1 (2004)).

Genes that encode cellular tumor associated antigens include cellular oncogenes and proto-oncogenes that are aberrantly expressed. In general, cellular oncogenes encode products that are directly relevant to the transformation of the cell, and because of this, these antigens are particularly preferred targets for immunotherapy. An example is the tumorigenic neu gene that encodes a cell surface molecule involved in oncogenic transformation. Other examples include the ras, kit, and trk genes. The products of proto-oncogenes (the normal genes which are mutated to form oncogenes) may be aberrantly expressed (e.g., overexpressed), and this aberrant expression can be related to cellular transformation. Thus, the product encoded by proto-oncogenes can be targeted. Some oncogenes encode growth factor receptor molecules or growth factor receptor-like molecules that are expressed on the tumor cell surface. An example is the cell surface receptor encoded by the c-erbB gene. Other tumor-associated antigens may or may not be directly involved in malignant transformation. These antigens, however, are expressed by certain tumor cells and may therefore provide effective targets. Some examples are carcinoembryonic antigen (CEA), CA 125 (associated with ovarian carcinoma), and melanoma specific antigens.

In ovarian and other carcinomas, for example, tumor associated antigens are detectable in samples of readily obtained biological fluids such as serum or mucosal secretions. One such marker is CA125, a carcinoma associated antigen that is also shed into the bloodstream, where it is detectable in serum (e.g., Bast, et al., N Eng. J. Med., 309:883 (1983);

Lloyd, et al., Int. J. Canc., 71:842 (1997). CA1 25 levels in serum and other biological fluids have been measured along with levels of other markers, for example, carcinoembryonic antigen (CEA), squamous cell carcinoma antigen (SCC), tissue polypeptide specific antigen (TPS), sialyl TN mucin
(STN), and placental alkaline phosphatase (FLAP), in efforts to provide
diagnostic and/or prognostic profiles of ovarian and other carcinomas (e.g.,
(1997); Kudoh, et al., Gynecol OhsieL Invest., 47:52 (1999)). Elevated
serum CA125 may also accompany neuroblastoma (e.g., Hirokawa, et al.,
Surg. Today, 28:349 (1998), while elevated CEA and SCC, among others,
may accompany colorectal cancer (Gebauer, et al., Anticancer Res.,
17(4B):2939 (1997)).

The tumor associated antigen, mesothelin, defined by reactivity with
monoclonal antibody K-1, is present on a majority of squamous cell
carcinomas including epithelial ovarian, cervical, and esophageal tumors,
and on mesotheliomas (Chang, et al., Cancer Res., 52:181 (1992); Chang, et
K-1, mesothelin is detectable only as a cell-associated tumor marker and has
not been found in soluble form in serum from ovarian cancer patients, or in
medium conditioned by OVCAR-3 cells (Chang, et al., Int. J. Cancer,
50:373 (1992)). Structurally related human mesothelin polypeptides,
however, also include tumor-associated antigen polypeptides such as the
distinct mesothelin related antigen (MRA) polypeptide, which is detectable
as a naturally occurring soluble antigen in biological fluids from patients
having malignancies (see WO 00/50900).

A tumor antigen may include a cell surface molecule. Tumor
antigens of known structure and having a known or described function,
include the following cell surface receptors: FIERI (GenBank Accession No.
U48722), HER2 (Yoshmo, et al., J. Immunol., 152:2393 (1994); Disis, et al.,
Cane, Res., 54:16 (1994); GenBank Acc. Nos. X03363 and M17730), HER3
(GenBank Acc. Nos. U29339 and M34309), HER4 (Plowman, et al., Nature,
366:473 (1993); GenBank Acc. Nos. L07868 and T64105), epidermal
growth factor receptor (EGFR) (GenBank Acc. Nos. U48722, and K03193),
vascular endothelial cell growth factor (GenBank No. M32977), vascular

Tumor antigens of interest include antigens regarded in the art as "cancer/testis" (CT) antigens that are immunogenic in subjects having a malignant condition (Scanlan, et al. Cancer Immun., 4:1 (2004)). CT antigens include at least 19 different families of antigens that contain one or more members and that are capable of inducing an immune response, including but not limited to MAGEA (CT1); BAGE (CT2); MAGEB (CT3); GAGE (CT4); SSX (CT5); NY-ESO-1 (CT6); MAGEC (CT7); SYCP1 (C8); SPANXB1 (CT1 1.2); NA88 (CT18); CTAGE (CT21); SPA17 (CT22); OY-TES-1 (CT23); CAGE (CT26); HOM-TES-85 (CT28); HCA661 (CT30); NY-SAR-35 (CT38); FATE (CT43); and TPTE (CT44).

Additional tumor antigens that can be targeted, including a tumor-associated or tumor-specific antigen, include, but not limited to, alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dek-can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A1, hsp70-2, KIAA0205, Mart2, Mum-1, 2, and 3, neo-PAP, myosin class I, OS-9, pml-
A fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomeras, Bage-1, Gage 3,4,5,6,7, GnTV, Herv-K-mel, Lage-1, Mage-Al2,3,4,6,10,12, Mage-C2, NA-88, NY-Eso-I/Lage-2, SP17, SSX-2, and TRP2-M2, MeianA (MART-I), gplOO (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p53, CEA, RAGE, NY-ESO (LAGE), SCP-1, Hom/Mel-40, FRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstem Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p53erbB2, p580erbB-3, c-mei, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β-Catenin, CDK4, Mum-1, pl6, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, a-fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29VBCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NBY70K, NY-CO-1, RCASl, SIX’CA\R16, TA-90 (Mac-2 binding protein\Acyclophilin C-associated protein), TAAL6, TAG72, TLP, and TPS. Other tumor-associated and tumor-specific antigens are known to those of skill in the art and are suitable for targeting by the disclosed compositions.

In some embodiments, the targeting moiety targets PD-L1, or another tumor-specific checkpoint proteins, or SLAMF7.

b. Antigens associated with tumor neovasculature

Therapeutics can be ineffective in treating tumors because they are inefficient at tumor penetration. Tumor-associated neovasculature provides a readily accessible route through which therapeutic agents can access the tumor. In another embodiment the composition contains a domain that specifically binds to an antigen that is expressed by neovasculature associated with a tumor.

The antigen may be specific to tumor neovasculature or may be expressed at a higher level in tumor neovasculature when compared to normal vasculature. Exemplary antigens that are over-expressed by tumor-associated neovasculature as compared to normal vasculature include, but are
not limited to, VEGF/KDR, Tie2, vascular cell adhesion molecule (VCAM), endoglin and α5β3 integrin/vitronectin. Other antigens that are overexpressed by tumor-associated neovascularization as compared to normal vasculature are known to those of skill in the art and are suitable for targeting by the disclosed compositions.

c. Chemokines/chemokine receptors

In another embodiment, the compositions contain a domain that specifically binds to a chemokine or a chemokine receptor. Chemokines are soluble, small molecular weight (8—14 kDa) proteins that bind to their cognate G-protein coupled receptors (GPCRs) to elicit a cellular response, usually directional migration or chemotaxis. Tumor cells secrete and respond to chemokines, which facilitate growth that is achieved by increased endothelial cell recruitment and angiogenesis, subversion of immunological surveillance and maneuvering of the tumoral leukocyte profile to skew it such that the chemokine release enables the tumor growth and metastasis to distant sites. Thus, chemokines are vital for tumor progression.

Based on the positioning of the conserved two N-terminal cysteine residues of the chemokines, they are classified into four groups namely CXC, CC, CX3C and C chemokines. The CXC chemokines can be further classified into ELR+ and ELR- chemokines based on the presence or absence of the motif 'glu-leu-arg (ELR motif)' preceding the CXC sequence. The CXC chemokines bind to and activate their cognate chemokine receptors on neutrophils, lymphocytes, endothelial and epithelial cells. The CC chemokines act on several subsets of dendritic cells, lymphocytes, macrophages, eosinophils, natural killer cells but do not stimulate neutrophils as they lack CC chemokine receptors except murine neutrophils. There are approximately 50 chemokines and only 20 chemokine receptors, thus there is considerable redundancy in this system of ligand/receptor interaction.

Chemokines elaborated from the tumor and the stromal cells bind to the chemokine receptors present on the tumor and the stromal cells. The autocrine loop of the tumor cells and the paracrine stimulator loop between the tumor and the stromal cells facilitate the progression of the tumor.
Notably, CXCR2, CXCR4, CCR2 and CCR7 play major roles in tumorigenesis and metastasis. CXCR2 plays a vital role in angiogenesis and CCR2 plays a role in the recruitment of macrophages into the tumor microenvironment. CCR7 is involved in metastasis of the tumor cells into the sentinel lymph nodes as the lymph nodes have the ligand for CCR7, CCL21. CXCR4 is mainly involved in the metastatic spread of a wide variety of tumors.

d. Mitochondrial Targeting

The modulator or delivery vehicle can be targeted to the mitochondria. This can be carried out using a mitochondrial targeting moiety, or by designing the modulator or delivery to preferentially accumulate in the mitochondria based on mitochondrial physiology (e.g., membrane potential, etc.). For example, cationic ligands, mitochondrial targeting moieties and peptides and polymeric combinations with cationic proteins are all ways to target via the mitochondrial membrane potential. In some embodiments, the composition include a cationic delivery vehicle, for example nanoparticles having co-polymers with proteins that are positively charged.

Mitochondrial targeting agents can be peptides, for example, those that consist of or include a leader sequence of highly positively charged amino acids. This allows the protein to be targeted to the highly negatively charged mitochondria. Unlike receptor-ligand approaches that rely upon stochastic Brownian motion for the ligand to approach the receptor, the mitochondrial localization signal of some embodiments is drawn to mitochondria because of charge.

In some embodiments, the mitochondrial targeting agent is a protein transduction domain. Mitochondrial targeting agents also include short peptide sequences (Yousif, et al., Chembiochem., 10(13):2131 (2009), for example mitochondrial transporters-synthetic cell-permeable peptides, also known as mitochondria-penetrating peptides (MPPs), that are able to enter mitochondria. MPPs are typically cationic, but also lipophilic: this combination of characteristics facilitates permeation of the hydrophobic mitochondrial membrane. For example, MPPs can include alternating

Mitochondrial targeting agents also include mitochondrial localization signals or mitochondrial targeting signals. Many mitochondrial proteins are synthesized as cytosolic precursor proteins containing a leader sequence, also known as a presequence, or peptide signal sequence. The identification of the specific sequences necessary for translocation of a linked compound into a mitochondrion can be determined using predictive software known to those skilled in the art.

In some embodiments, the composition is encapsulated, coupled to, or otherwise associated with mitochondriotropic liposomes. Mitochondriotropic liposomes are cationic liposomes that can be used to deliver an encapsulated agent to the mitochondria of a cell. Mitochondriotropic liposomes are known in the art. See, for example, U.S. Patent Application Publication No. US 2008/0095834 to Weissig, et al.

Mitochondriotropic liposomes are liposomes which contain a hydrophobized amphiphilic dieiocalized cation, such as atri phenylphosphonium or a quinolinium moiety, incorporated into or conjugate to the lipid membrane of the liposome. As a result, the liposomes can be used to deliver compounds incorporated within them to the mitochondria.

D. Additional Moieties

The modulator itself, or nanoparticles or another vehicle carrying the modulator can be associated with, linked, conjugated, or otherwise attached directly or indirectly to one or more additional moieties. For example, nanoparticles can contain one or more polymer conjugates containing end-to-end linkages between the polymer and a moiety. The moiety can be a targeting moiety, a detectable label, or a therapeutic, prophylactic, or diagnostic agent. For example, a polymer conjugate can be a PLGA-PEG-
phosphonate. The additional targeting elements may refer to elements that bind to or otherwise localize the nanoparticles to a specific locale. The locale may be a tissue, a particular cell type, or a subcellular compartment. The targeting element of the nanoparticle can be an antibody or antigen binding fragment thereof, an aptamer, or a small molecule (less than 500 Daltons). The additional targeting elements may have an affinity for a cell-surface receptor or cell-surface antigen on a target cell and result in internalization of the particle within the target cell.

In some embodiments, the moiety is pH sensitive, lactate sensitive, acid sensitive, or mitochondrial membrane potential sensitive. In some embodiments, the moiety is one that keeps the composition away from the immune cells.

E. Imaging Agents

The nanoparticles can also contain a detectable label, such as a radioisotope, a fluorophore (e.g., fluorescein isothiocyanate (FTTC), phycoerythrin (PE)), an enzyme (e.g., alkaline phosphatase, horseradish peroxidase), element particles (e.g., gold particles) or a contrast agent. These may be encapsulated within, dispersed within, or conjugated to the polymer.

For example, a fluorescent label can be chemically conjugated to a polymer of the nanoparticle to yield a fluorescently labeled polymer. In other embodiments the label is a contrast agent. A contrast agent refers to a substance used to enhance the contrast of structures or fluids within the body-in medical imaging. Contrast agents are known in the art and include, but are not limited to agents that work based on X-ray attenuation and magnetic resonance signal enhancement. Suitable contrast agents include iodine and barium.

III. Methods of Making Particles

A. Polymer Conjugates

Methods of polymer synthesis are described, for instance, in Braun et al. (2005) Polymer Synthesis: Theory and Practice. New York, NY: Springer. The polymers may be synthesized via step-growth polymerization, chain-growth polymerization, or plasma polymerization. In most case they can be purchased from commercial sources.
In some embodiments an amphiphilic polymer is synthesized starting from a hydrophobic polymer terminated with a first reactive coupling group and a hydrophilic polymer terminated with a second reactive coupling group capable of reacting with the first reactive coupling group to form a covalent bond. One of either the first reactive coupling group or the second reactive coupling group can be a primary amine, where the other reactive coupling group can be an amine-reactive Sinking group such as isothiocyanates, isocyanates, acyl azides, NHS esters, sulfonyl chlorides, aldehydes, glyoxals, epoxides, oxiranes, carbonates, ary1 halides, imidoesters, carbodiimides, anhydrides, and fluorophenyl esters. One of either the first reactive coupling group or the second reactive coupling group can be an aldehyde, where the other reactive coupling group can be an aldehyde reactive linking group such as hydrazides, alkoxyamines, and primary amines. One of either the first reactive coupling group or the second reactive coupling group can be a thiol, where the other reactive coupling group can be a sulfhydryl reactive group such as maleimides, haloacetyl5s, and pyridyl disulfides.

In preferred embodiments a hydrophobic polymer terminated with an amine or an amine-reactive Sinking group is coupled to a hydrophilic polymer terminated with complimentary reactive linking group. For example, an NHS ester activated PLGA can be formed by reacting PLGA-CO(OH) with NHS and a coupling reagent such as dicyclohexylcarbodiimide (DCC) or ethyl(dimethySammopropyl) carbodiimide (EDC). The NHS ester activated PLGA can be reacted with a hydrophilic polymer terminated with a primary amine, such as a PEG-NH₂ to form an amphiphilic PLGA-6-PEG block copolymer.

In some embodiments a conjugate of an amphiphilic polymer with a targeting moiety is formed using the same or similar coupling reactions. In some embodiments the conjugate is made starting from a hydrophilic polymer terminated on one end with a first reactive coupling group and terminated on a second end with a protective group. The hydrophilic polymer is reacted with a targeting moiety having a reactive group that is complimentary to the first reactive group to form a covalent bond between the hydrophilic polymer and the targeting moiety. The protective group can
then be removed to provide a second reactive coupling group, for example to allow coupling of a hydrophobic polymer block to the conjugate of the hydrophilic polymer with the targeting moiety. A hydrophobic polymer terminated with a reactive coupling group complimentary to the second reactive coupling group can then be covalently coupled to form the conjugate. Of course, the steps could also be performed in reverse order, i.e. a conjugate of a hydrophobic polymer and a hydrophilic polymer could be formed first followed by deprotection and coupling of the targeting moiety to the hydrophilic polymer block.

In some embodiments a conjugate is formed having a moiety conjugated to both ends of the amphiphilic polymer. For example, an amphiphilic polymer having a hydrophobic polymer block and a hydrophilic polymer block may have targeting moiety conjugated to the hydrophilic polymer block and an additional moiety conjugated to the hydrophobic polymer block. In some embodiments the additional moiety can be a detectable label. In some embodiments the additional moiety is a therapeutic, prophylactic, or diagnostic agent. For example, the additional moiety could be a moiety used for radiotherapy. The conjugate can be prepared starting from a hydrophobic polymer having on one end a first reactive coupling group and a another end first protective group and a hydrophilic polymer having on one end a second reactive coupling group and on another end a second protective group. The hydrophobic polymer can be reacted with the additional moiety having a reactive coupling group complimentary to the first reactive coupling group, thereby forming a conjugate of the hydrophobic polymer to the additional moiety. The hydrophilic polymer can be reacted with a targeting moiety having a reactive coupling group complimentary to the second reactive coupling group, thereby forming a conjugate of the hydrophilic polymer to the targeting moiety. The first protective group and the second protective group can be removed to yield a pair of complimentary reactive coupling groups that can be reacted to covalently link the hydrophobic polymer block to the hydrophilic polymer block.
B. Emulsion Methods

In some embodiments, a multimodal nanoparticle is prepared using an emulsion solvent evaporation method. For example, a polymeric material is dissolved in a water immiscible organic solvent and mixed with a drug solution or a combination of drug solutions. In some embodiments a solution of a therapeutic, prophylactic, or diagnostic agent to be encapsulated is mixed with the polymer solution. The polymer can be, but is not limited to, one or more of the following: PLA, PGA, PCL, their copolymers, polyacrylates, the aforementioned PEGylated polymers, the aforementioned Polymer-drug conjugates, the aforementioned polymer-peptide conjugates, or the aforementioned fluorescently labeled polymers, or various forms of their combinations. The drug molecules can be, but are not limited to, one or a more of the following: PPARgamma activators (e.g. Rosiglitazone, (RS)-5-(4-(2-[methyl(pyridin-2-yl)amino]ethoxy)benzyl)[thiazolidine-2,4-dione, Pioglitazone, (RS)-5-(4-[2-(5-ethylpyridin-2-yl]ethoxy)[-benzyl]thiazolindine-2,4-dione, Troglitazone, (RS)-5-(4-[((6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)methoxy][benzyl]thiazolidine-2,4-dione etc.), prostaglandin E2 analog (PGE2, (5Z,11a,13E,15S)-7-[(4-hydroxy-2-(3-hydroxyOCt-1-enyl) 5-oxocyclopentyl] hept-5-enoic acid etc.), beta3 adrenoceptor agonist (CL 316243, Disodium 5-[(2R)-2-[[2R]-2-(3-Chlorophenyl)-2-hydroxyethyl]amino][propyl]-1,3-benzodioxole-2,2-dicarboxylate hydrate, etc.), Fibroblast Growth Factor 21 (FGF-21), Irisin, RNA, DNA, chemotherapeutic compounds, nuclear magnetic resonance (NMR) contrast agents, or combinations thereof. The water immiscible organic solvent, can be, but is not limited to, one or more of the following: chloroform, dichloromethane, and acyl acetate. The drug can be dissolved in, but is not limited to, one or more of the following: acetone, ethanol, methanol, isopropyl alcohol, acetonitrile and Dimethyl sulfoxide (DMSO).

In some embodiments the polymer solution contains one or more polymer conjugates as described above. The polymer solution can contain a first amphiphilic polymer conjugate having a hydrophobic polymer block, a hydrophilic polymer block, and a targeting moiety conjugated to the hydrophilic end. In preferred embodiments the polymer solution contains...
one or more additional polymers or amphiphilic polymer conjugates. For example the polymer solution may contain, in addition to the first amphiphilic polymer conjugate, one or more hydrophobic polymers, hydrophilic polymers, lipids, amphiphilic polymers, polymer-drug conjugates, or conjugates containing other targeting moieties. By controlling the ratio of the first amphiphilic polymer to the additional polymers or amphiphilic polymer conjugates, the density of the targeting moieties can be controlled. The first amphiphilic polymer may be present from 1% to 100% by weight of the polymers in the polymer solution. For example, the first amphiphilic polymer can be present at 10%, 20%, 30%, 40%, 50%, or 60% by weight of the polymers in the polymer solution.

An aqueous solution is then added into the resulting mixture solution to yield emulsion solution by emulsification. The emulsification technique can be, but not limited to, probe sonication or homogenization through a homogenizer. The plaque-targeted peptides or fluorophores or drugs may be associated with the surface of, encapsulated within, surrounded by, and/or distributed throughout the polymeric matrix of this inventive particle.

C. Nanoprecipitation Method

In another embodiment, a multimodal nanoparticle is prepared using nanoprecipitation methods or microfluidic devices. A polymeric material is mixed with a drag or drag combinations in a water miscible organic solvent. The polymer can be, but is not limited to, one or more of the following: PLA, PGA, PCL, their copolymers, polyacrylates, the aforementioned PEGylated polymers, the aforementioned Polymer-drug conjugates, the aforementioned polymer-peptide conjugates, or the aforementioned fluorescently labeled polymers, or various forms of their combinations. Tire drag molecules can be, but are not limited to, one or more of the following: PPARgamma activators (e.g. Rosiglitazone, (RS)-5-[4-(2-[methyl(pyridin-2-yl)amino]ethoxy)benzyl]thiazolidine-2,4-dione, Pioglitazone, (RS)-5-(4-[2-(5-ethylypyridin-2-yl)ethoxy]benzyl)thiazolidine-2,4-dione, Troglitazone, (RS)-5-(4-[6-[3-hydroxy-2-(3-hydroxyoct-1-enyl)oxy]benzyl]thiazolidine-2,4-dione etc.), prostaglandin E2 analog (PGE2, (5Z, 11a, 13E, 15S)-7-[3-hydroxy-2-(3-hydroxyoct-1-enyl)-5-oxo-
cyclopentyl] hept-5-enoic acid etc.), beta.3 adrenoceptor agonist (CL 3 16243, Disodium 5-[(2R)-2-][(2R)-2-(3-Chlorophenyl)-2-
hydroxyethy]a]mio[proply]-l,3-benzodioxole-2,2-dicarboxylate hydrate, etc.), RNA, DNA, chemotherapeutic compounds, nuclear magnetic
resonance (NMR) contrast agents, or combinations thereof. The water
miscible organic solvent, can be, but is not limited to, one or more of the
following: acetone, ethanol, methanol, isopropyl alcohol, acetonitrile and
Dimethyl sulfoxide (DMSO). The resulting mixture solution is then added to
a polymer non-solvent, such as an aqueous solution, to yield nanoparticle
solution. The plaque-targeted peptides or fluorophores or drugs may be
associated with the surface of, encapsulated within, surrounded by, and/or
distributed throughout the polymeric matrix of this inventive particle.

D. Microfluidics

Methods of making nanoparticles using microfluidics are known in
the art. Suitable methods include those described in U.S. Patent Application
Publication No. 2010/0022680 A1 by Karnik et al. In general, the
microfluidic device comprises at least two channels that converge into a
mixing apparatus. The channels are typically formed by lithography, etching,
embossing, or molding of a polymeric surface. A source of fluid is attached
to each channel, and the application of pressure to the source causes the flow
of the fluid in the channel. The pressure may be applied by a syringe, a
pump, and/or gravity. The inlet streams of solutions with polymer, targeting
moieties, lipids, drug, payload, etc. converge and mix, and the resulting
mixture is combined with a polymer non-solvent solution to form the
nanoparticles having the desired size and density of moieties on the surface.
By varying the pressure and flow rate in the inlet channels and the nature and
composition of the fluid sources nanoparticles can be produced having
reproducible size and structure.

E. Other Methodologies

1. Solvent Evaporation

In this method the polymer is dissolved in a volatile organic solvent,
such as methylene chloride. The drug (either soluble or dispersed as fine
particles) is added to the solution, and the mixture is suspended in an
aqueous solution that contains a surface active agent such as poly(vinyl alcohol). The resulting emulsion is stirred until most of the organic solvent evaporated, leaving solid microparticles. The resulting microparticles are washed with water and dried overnight in a lyophihzer. Microparticles with different sizes (0.5-1000 microns) and morphologies can be obtained by this method. This method is useful for relatively stable polymers like polyesters and polystyrene.

However, labile polymers, such as polyanhydrides, may degrade during the fabrication process due to the presence of water. For these polymers, the following two methods, which are performed in completely anhydrous organic solvents, are more useful.

2. **Hot Melt Microencapsulation**

In this method, the polymer is first melted and then mixed with the solid particles. The mixture is suspended in a non-miscible solvent (like silicon oil), and, with continuous stirring, heated to 5°C above the melting point of the polymer. Once the emulsion is stabilized, it is cooled until the polymer particles solidify. The resulting microparticles are washed by decantation with petroleum ether to give a free-flowing powder. Microparticles with sizes between 0.5 to 1000 microns are obtained with this method. The external surfaces of spheres prepared with this technique are usually smooth and dense. This procedure is used to prepare microparticles made of polyesters and polyanhydrides. However, this method is limited to polymers with molecular weights between 1,000-50,000.

3. **Solvent Removal**

This technique is primarily designed for polyanhydrides. In this method, the drag is dispersed or dissolved in a solution of the selected polymer in a volatile organic solvent like methylene chloride. This mixture is suspended by stirring in an organic oil (such as silicon oil) to form an emulsion. Unlike solvent evaporation, this method can be used to make microparticles from polymers with high melting points and different molecular weights. Microparticles that range between 1-300 microns can be obtained by this procedure. The external morphology of spheres produced with this technique is highly dependent on the type of polymer used.
4. Spray-Drying

In this method, the polymer is dissolved in organic solvent. A known amount of the active drug is suspended (insoluble drags) or co-dissolved (soluble drags) in the polymer solution. The solution or the dispersion is then spray-dried. Typical process parameters for a mini-spray drier (Buchi) are as follows: polymer concentration = 0.04 g/mL, inlet temperature = -24°C, outlet temperature = 13-15 °C, aspirator setting = 15, pump setting = 10 mL/minute, spray flow = 600 Nl/hr, and nozzle diameter = 0.5 mm. Microparticles ranging between 1-10 microns are obtained with a morphology which depends on the type of polymer used.

5. HydrogeS Microparticles

Microparticles made of gel-type polymers, such as alginate, are produced through traditional ionic gelation techniques. The polymers are first dissolved in an aqueous solution, mixed with barium sulfate or some bioactive agent, and then extruded through a microdroplet forming device, which in some instances employs a flow of nitrogen gas to break off the droplet. A slowly stirred (approximately 100-170 RPM) ionic hardening bath is positioned below the extruding device to catch the forming microdroplets. The microparticles are left to incubate in the bath for twenty to thirty minutes in order to allow sufficient time for gelation to occur. Microparticle particle size is controlled by using various size extruders or varying either the nitrogen gas or polymer solution flow rates. Chitosan microparticles can be prepared by dissolving the polymer in acidic solution and crosslinking it with tripolyphosphate. Carboxymethyl cellulose (CMC) microparticles can be prepared by dissolving the polymer in acid solution and precipitating the microparticle with lead ions. In the case of negatively charged polymers (e.g., alginate, CMC), positively charged ligands (e.g., polylysine, polyethyleneimine) of different molecular weights can be ionically attached.

F. Molecules to be Encapsulated or Attached to the surface of the particles

There are two principle groups of molecules to be encapsulated or attached to the polymer, either directly or via a coupling molecule: targeting
and/or attachment molecules and therapeutic, nutritional, diagnostic or prophylactic agents. These can be coupled using standard techniques. The targeting molecule or therapeutic molecule to be delivered can be coupled directly to the polymer or to a material such as a fatty acid which is incorporated into the polymer.

Functionality refers to conjugation of a ligand to the surface of the particle via a functional chemical group (carboxylic acids, aldehydes, amines, sulfhydryls and hydroxyls) present on the surface of the particle and present on the ligand to be attached. Functionality may be introduced into the particles in two ways.

The first is during the preparation of the microparticles, for example during the emulsion preparation of microparticles by incorporation of stabilizers with functional chemical groups.

A second is post-particle preparation, by direct crosslinking particles and ligands with homo- or heterobifunctional crosslinkers. This second procedure may use a suitable chemistry and a class of crosslinkers (CDI, EDAC, glutaraldehydes, etc. as discussed in more detail below) or any other crosslinker that couples ligands to the particle surface via chemical modification of the particle surface after preparation. This second class also includes a process whereby amphiphilic molecules such as fatty acids, lipids or functional stabilizers may be passively adsorbed and adhered to the particle surface, thereby introducing functional end groups for tethering to ligands.

IV. Formulations

The formulations and pharmaceutical compositions containing an effective amount of the disclosed composition in a pharmaceutical carrier appropriate for administration to an individual in need thereof to treat one or more symptoms of a disease or disorder are also provided. The formulations can be administered parenterally (e.g., by intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection or infusion). It may also be possible to administer topically (e.g., to a mucosal surface such as the mouth, lungs, intranasal, intravaginally, etc.). The compositions can be administered locally or systemically.
Drugs can be formulated for immediate release, extended release, or modified release. A delayed release dosage form is one that releases a drug (or drugs) at a time other than promptly after administration. An extended release dosage form is one that allows at least a twofold reduction in dosing frequency as compared to that drug presented as a conventional dosage form (e.g. as a solution or prompt drug-releasing, conventional solid dosage form). A modified release dosage form is one for which the drug release characteristics of time course and/or location are chosen to accomplish therapeutic or convenience objectives not offered by conventional dosage forms such as solutions, ointments, or promptly dissolving dosage forms. Delayed release and extended release dosage forms and their combinations are types of modified release dosage forms.

Formulations are prepared using a pharmaceutically acceptable "carrier" composed of materials that are considered safe and effective and may be administered to an individual without causing undesirable biological side effects or unwanted interactions. The "carrier" is all components present in the pharmaceutical formulation other than the active ingredient or ingredients.

**Parenteral Formulations.**

The composition, for example, a modulator of cancer cell metabolism having a cancer cell-targeting moiety associated with, linked, conjugated, or otherwise attached directly or indirectly to the modulator of cancer cell metabolism, or to a nanoparticle or other deliver" vehicle thereof can be formulated for parenteral delivery, such as injection or infusion, in the form of a solution or suspension, or a powder. The formulation can be administered via any route, such as, the blood stream or directly to the organ or tissue to be treated. The particles may be provided in a lyophilized or dried form in a unit dosage form, for suspension at the time of injection. These may be provided in a kit with an appropriate amount of diluent such as sterile water or buffered solution.

Parenteral formulations can be prepared as aqueous compositions using techniques known in the art. Typically, such compositions can be prepared as injectable formulations, for example, solutions or suspensions;
solid forms suitable for using to prepare solutions or suspensions upon the addition of a reconstitution medium prior to injection; emulsions, such as water-in-oil (w/o) emulsions, oil-in-water (o/w) emulsions, and microemulsions thereof, liposomes, or emulsomes.

The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, one or more polyols (e.g., glycerol, propylene glycol, and liquid polyethylene glycol), oils, such as vegetable oils (e.g., peanut oil, corn oil, sesame oil, etc.), and combinations 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/or by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride.

Solutions and dispersions of the compounds or nanoparticles can be prepared in water or another solvent or dispersing medium suitably mixed with one or more pharmaceutically acceptable excipients including, but not limited to, surfactants, dispersants, emulsifiers, pH modifying agents, and combination thereof.

Suitable surfactants may be anionic, cationic, amphoteric or nonionic surface active agents. Suitable anionic surfactants include, but are not limited to, those containing carboxylate, sulfonate and sulfate ions.

Examples of anionic surfactants include sodium, potassium, ammonium of long chain alkyl sulfonates and alkyl aryl sulfonates such as sodium dodecylebenzene sulfonate; dialkyl sodium sulfosuccinates, such as sodium dodecylebenzene sulfonate; dialkyl sodium sulfosuccinates, such as sodium bis-(2-ethylthioxyl)-sulfosuccinate; and alkyl sulfates such as sodium lauryl sulfate. Cationic surfactants include, but are not limited to, quaternary ammonium compounds such as benzalkonium chloride, benzethonium chloride, cetrimonium bromide, steaeryl dimethylbenzyl ammonium chloride, polyoxyethylene and coconut amine. Examples of nonionic surfactants include ethylene glycol monostearate, propylene glycol myristate, glyceryl monostearate, glyceryl stearate, poiyglyceryl-4-oleate, sorbitan acylate, sucrose acylate, PEG-150 laurate, PEG-400 monolaurate, polyoxyethylene monolaurate, polysorbates, polyoxyethylene octylphenylether, PEG-1000.
cetyl ether, polyoxyethylene tridecyl ether, polypropylene glycol butyl ether, Polyoxamer® 401, stearoyl monoisopropanolamide, and polyoxyethylene hydrogenated tallow amide. Examples of amphoteric surfactants include sodium N-dodecyl-p-alanine, sodium N-lauryl -P-iminodipropionate, myristoamphoacetate, lauryl betaine and lauryl sulfobetaine.

The formulation can contain a preservative to prevent the growth of microorganisms. Suitable preservatives include, but are not limited to, parabens, chlorobutanol, phenol, sorbic acid, and thimerosal. The formulation may also contain an antioxidant to prevent degradation of the active agent(s) or nanoparticles.

The formulation is typically buffered to a pH of 3-8 for parenteral administration upon reconstitution. Suitable buffers include, but are not limited to, phosphate buffers, acetate buffers, and citrate buffers.

Water soluble polymers are often used in formulations for parenteral administration. Suitable water-soluble polymers include, but are not limited to, polyvinylpyrrolidone, dextran, carboxymethylcellulose, and polyethylene glycol.

Sterile injectable solutions can be prepared by incorporating the compound or nanoparticles in the required amount in the appropriate solvent or dispersion medium with one or more of the excipients listed above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized compositions into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those listed above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the compound or nanoparticle plus any additional desired ingredient from a previously sterile-filtered solution thereof. The powders can be prepared in such a manner that the particles are porous in nature, which can increase dissolution of the particles. Methods for making porous particles are well known in the art.

Pharmaceutical formulations for parenteral administration are preferably in the form of a sterile aqueous solution or suspension of particles.
formed from one or more polymer-drug conjugates. Acceptable solvents include, for example, water, Ringer’s solution, phosphate buffered saline (PBS), and isotonic sodium chloride solution. The formulation may also be a sterile solution, suspension, or emulsion in a nontoxic, parenterally acceptable diluent or solvent such as 1,3-butanediol.

In some instances, the formulation is distributed or packaged in a liquid form. Alternatively, formulations for parenteral administration can be packed as a solid, obtained, for example by lyophilization of a suitable liquid formulation. The solid can be reconstituted with an appropriate carrier or diluent prior to administration.

Solutions, suspensions, or emulsions for parenteral administration may be buffered with an effective amount of buffer necessary to maintain a pH suitable for ocular administration. Suitable buffers are well known by those skilled in the art and some examples of useful buffers are acetate, borate, carbonate, citrate, and phosphate buffers.

Solutions, suspensions, or emulsions for parenteral administration may also contain one or more tonicity agents to adjust the isotonic range of the formulation. Suitable tonicity agents are well known in the art. Examples include glycerin, mannitol, sorbitol, sodium chloride, and other electrolytes.

Solutions, suspensions, or emulsions for parenteral administration may also contain one or more preservatives to prevent bacterial contamination of the ophthalmic preparations. Suitable preservatives are known in the art, and include polyhexamethylenebiguanidine (PHMB), benzalkonium chloride (BAK), stabilized oxychioro complexes (otherwise known as Purite®), phenylmercuric acetate, chlorobutanol, sorbic acid, chlorhexidine, benzyl alcohol, parabens, thimerosal, and mixtures thereof.

Solutions, suspensions, or emulsions for parenteral administration may also contain one or more excipients known art, such as dispersing agents, wetting agents, and suspending agents.
V. Methods of Use

A. Treatment Regimen

1. Dosage and Effective Amounts

Methods of using the disclosed compositions to treat cancer are provided. The methods typically include administering a subject in a need thereof an effective amount of a composition including a modulator of cancer metabolism. A therapeutically effective amounts of modulator used in the treatment of cancer are typically sufficient to reduce or alleviate one or more symptoms of cancer. Symptoms of cancer may be physical, such as tumor burden, or biological such as proliferation of cancer cells.

Accordingly, the amount of modulator can be effective to, for example, kill tumor cells or inhibit proliferation or metastasis of the tumor cells. Preferably the modulator, for example via the targeting moiety, is preferentially delivered cancer cells. Preferably the modulator does not target or otherwise modulate the metabolism of non-cancer cells, particular immune cells such as tumor infiltrating lymphocytes, or does so at a reduced level compared to cancer (e.g., tumor) cells. In this way, by-products and other effects associated with aberrant metabolism in cancer cells are reduced, preferably leading directly or indirectly to cancer cell death. In some embodiments, the modulator reduces cancer cell migration, angiogenesis, immune escape, radioresistance, or a combination thereof. In some embodiments, the modulator induces a change in the cancer cell itself or its microenvironment that reduces suppression or induces activation of an immune response against the cancer cell. For example, in some embodiments, the composition is administered in an effective amount to enhance and/or prolonging the activation of T cells (i.e., increasing antigen-specific proliferation of T cells, enhance cytokine production by T cells, stimulate differentiation, stimulate effector functions of T cells and/or promote T cell survival) or overcome T cell exhaustion and/or anergy.

The actual effective amounts of modulator can vary according to factors including the specific modulator administered, the particular composition formulated, the mode of administration, and the age, weight,
condition of the subject being treated, as well as the route of administration and the disease or disorder.

The therapeutic result of the modulator can be compared to a control. Suitable controls are known in the art. A typical control is a comparison of a condition or symptom of a subject prior to and after administration of the modulator. The condition or symptom can be a biochemical, molecular, physiological, or pathological readout. For example, the effect of the composition on a particular symptom, pharmacologic, or physiologic indicator can be compared to an untreated subject, or the condition of the subject prior to treatment. In some embodiments, the symptom, pharmacologic, or physiologic indicator is measured in a subject prior to treatment, and again one or more times after treatment is initiated. In some embodiments, the control is a reference level, or average determined based on measuring the symptom, pharmacologic, or physiologic indicator in one or more subjects that do not have the disease or condition to be treated (e.g., healthy subjects). In some embodiments, the effect of the treatment is compared to a conventional treatment that is known the art, such as one of those discussed herein.

In some embodiments, the effective amount of modulator causes little or no killing of non-cancerous cells, and preferably little or no inhibition of metabolism in non-cancer cells. It is particularly preferred that the composition have little or no effect on immune cells such as TIL.

In some embodiments, dosages are administered once, twice, or three times daily, or every other day, two days, three days, four days, five days, or six days to a human. In some embodiments, dosages are administered about once or twice every week, every two weeks, every three weeks, or every four weeks. In some embodiments, dosages are administered about once or twice every month, every two months, every three months, every four months, every five months, or every six months.

In some embodiments, the regimen includes one or more cycles of a round of therapy followed by a daig holiday (e.g., no drug). The round of the therapy can be, for example, and of the administrations discussed above.
Likewise, the drag holiday can be 1, 2, 3, 4, 5, 6, or 7 days; or 1, 2, 3, 4 weeks, or 1, 2, 3, 4, 5, or 6 months.

In particular embodiments, the subject is administered a dosage of between about 6 mg/kg and 18 mg/kg. Particular dosage regimens include, for example, one or more cycles in which the subject is administered the drag each of five days in a row, followed by a two-day drag holiday.

The disclosed compositions can be administered alone or in combination with one or more conventional therapies, for example, a conventional cancer therapy. In some embodiments, the conventional therapy includes administration of one or more of the disclosed compositions in combination with one or more additional active agents. The combination therapies can include administration of the active agents together in the same admixture, or in separate admixtures. Therefore, in some embodiments, the pharmaceutical composition includes two, three, or more active agents. Such formulations typically include an effective amount of a modulator of cancer cell metabolism. The additional active agent(s) can have the same, or different mechanisms of action. In some embodiments, the combination results in an additive effect on the treatment of the cancer. In some embodiments, the combinations result in a more than additive effect on the treatment of the disease or disorder.

Additional therapeutic agents include conventional cancer therapeutics such as chemotherapeutic agents, cytokines, chemokines, and radiation therapy. The majority of chemotherapeutic drugs can be divided into: alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, and other antitumour agents. All of these drugs affect cell division or DNA synthesis and function in some way. Additional therapeutics include monoclonal antibodies and the tyrosine kinase inhibitors e.g., imatinib mesylate (GLEEVEC® or GLIVEC®), which directly targets a molecular abnormality in certain types of cancer (chronic myelogenous leukemia, gastrointestinal stromal tumors).

Representative chemotherapeutic agents include, but are not limited to, amsacrine, bleomycin, busulfan, capecitabine, carboplatin, cannustine, chlorambucil, cisplatin, cladribine, clofarabine, crisantaspase,
cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, docetaxel, doxorubicin, epipodophyllotoxins, epirubicin, etoposide, etoposide phosphate, fludarabine, fluorouracil, gemcitabine, hydroxycarbamide, idarubicin, ifosfamide, infotecan, leucovorin, liposomal doxorubicin, liposomal daunorubicin, lomustine, mechlorethamine, melphalan, mercaptopurine, mesna, methotrexate, mitomycin, mitoxantrone, oxaliplatin, paclitaxel, pemetrexed, pentostatin, procarbazine, raltitrexed, satraplatin, streptozocin, teniposide, tegafur-uracil, temozolomide, teniposide, thiopeta, thiotepa, tioguanine, topotecan, vinorelbine, taxol and derivatives thereof, trastuzumab (HERCEPTIN®), cetuximab, and rituximab (RITUXAN® or MABTHERA®), bevacizumab (AVASTIN®), and combinations thereof. Representative pro-apoptotic agents include, but are not limited to, fludarabine, cytosine arabinoside, actinomycin D, lactosylceramide, 15d-PGJ(2) and combinations thereof.

In some embodiments, the disclosed compositions and methods are used prior to or in conjunction with an immunotherapy such inhibition of checkpoint proteins such as PD-1 or CTLA-4, adoptive T cell therapy, and/or a cancer vaccine. Methods of adoptive T cell therapy are known in the art and used in clinical practice. Generally adoptive T cell therapy involves the isolation and ex vivo expansion of tumor specific T cells to achieve greater number of T cells than what could be obtained by vaccination alone. The tumor specific T cells are then infused into patients with cancer in an attempt to give their immune system the ability to overwhelm remaining tumor via T cells, which can attack and kill the cancer. Several forms of adoptive T cell therapy can be used for cancer treatment including, but not limited to, culturing tumor infiltrating lymphocytes or TIL; isolating and expanding one particular T cell or clone; and using T cells that have been engineered to recognize and attack tumors. In some embodiments, the T cells are taken directly from the patient's blood. Methods of priming and activating T cells in vitro for adoptive T cell cancer therapy are known in the art. See, for example, Wang, et al. Blood, 109(1 1):4865-4872 (2007) and Hervas-Stubbs, et al, J Immunol., 189(7):3299-30 (2012).
Historically, adoptive T cell therapy strategies have largely focused on the infusion of tumor antigen specific cytotoxic T cells (CTL) which can directly kill tumor cells. However, CD4+ T helper (Th) cells can also be used. Th can activate antigen-specific effector cells and recruit cells of the innate immune system such as macrophages and dendritic cells to assist in antigen presentation (APC), and antigen primed Th cells can directly activate tumor antigen-specific CTL. As a result of activating APC, antigen specific Th1 have been implicated as the initiators of epitope or determinant spreading which is a broadening of immunity to other antigens in the tumor.

The ability to elicit epitope spreading broadens the immune response to many potential antigens in the tumor and can lead to more efficient tumor cell kill due to the ability to mount a heterogeneic response. In this way, adoptive T cell therapy can used to stimulate endogenous immunity.

In some embodiments, the T cells express a chimeric antigen receptor (CARs, CAR T cells, or CARTs). Artificial T cell receptors are engineered receptors, which graft a particular specificity onto an immune effector cell. Typically, these receptors are used to graft the specificity of a monoclonal antibody onto a T cell and can be engineered to target virtually any tumor associated antigen. First generation CARs typically had the intracellular domain from the CDS ζ-chain, which is the primary transmitter of signals from endogenous TCRs. Second generation CARs add intracellular signaling domains from various costimulatory protein receptors (e.g., CD28, 41BB, ICOS) to the cytoplasmic tail of the CAR to provide additional signals to the T cell, and third generation CARs combine multiple signaling domains, such as CD3ζ-CD28-41BB or CD3ζ-CD28-OX40, to further enhance effectiveness.

In some embodiments, the disclosed compositions and methods are used prior to or in conjunction with a cancer vaccine, for example a dendritic cell cancer vaccine. Vaccination typically includes administering a subject an antigen (e.g., a cancer antigen) together with an adjuvant to elicit therapeutic T cells in vivo. In some embodiments, the cancer vaccine is a dendritic cell cancer vaccine in which the antigen delivered by dendritic cells primed ex vivo to present the cancer antigen. Examples include, for

**B. Subjects to be Treated**

In general, the disclosed compositions and methods of treatment thereof are useful in the context of cancer, including tumor therapy. In a mature animal, a balance usually is maintained between cell renewal and cell death in most organs and tissues. The various types of mature cells in the body have a given life span; as these cells die, new cells are generated by the proliferation and differentiation of various types of stem cells. Under normal circumstances, the production of new cells is so regulated that the numbers of any particular type of cell remain constant. Occasionally, though, cells arise that are no longer responsive to normal growth-control mechanisms. These cells give rise to clones of cells that can expand to a considerable size, producing a tumor or neoplasm. A tumor that is not capable of indefinite growth and does not invade the healthy surrounding tissue extensively is benign. A tumor that continues to grow and becomes progressively invasive is malignant. The term cancer refers specifically to a malignant tumor. In addition to uncontrolled growth, malignant tumors exhibit metastasis. In this process, small clusters of cancerous cells dislodge from a tumor, invade the blood or lymphatic vessels, and are carried to other tissues, where they continue to proliferate. In this way a primary tumor at one site can give rise to a secondary tumor at another site.

The compositions and methods described herein are useful for treating subjects having benign or malignant tumors by delaying or inhibiting the growth of a tumor in a subject, reducing the growth or size of the tumor, inhibiting or reducing metastasis of the tumor, and/or inhibiting or reducing symptoms associated with tumor development or growth.

Malignant tumors which may be treated are classified herein according to the embryonic origin of the tissue from which the tumor is derived. Carcinomas are tumors arising from endodermal or ectodermal
tissues such as skin or the epithelial lining of internal organs and glands. The disclosed compositions are particularly effective in treating carcinomas. Sarcomas, which arise less frequently, are derived from mesodermal connective tissues such as bone, fat, and cartilage. The leukemias and lymphomas are malignant tumors of hematopoietic cells of the bone marrow. Leukemias proliferate as single cells, whereas lymphomas tend to grow as tumor masses. Malignant tumors may show up at numerous organs or tissues of the body to establish a cancer.

The types of cancer that can be treated with the provided compositions and methods include, but are not limited to, cancers such as vascular cancer such as multiple myeloma, adenocarcinomas and sarcomas, of bone, bladder, brain, breast, cervical, colo-rectal, esophageal, kidney, liver, lung, nasopharangeal, pancreatic, prostate, skin, stomach, and uterine. In some embodiments, the disclosed compositions are used to treat multiple cancer types concurrently. The compositions can also be used to treat metastases or tumors at multiple locations.

In some embodiments, the cancers are characterized as being triple negative breast cancer, or having one or more KRAS-mutations, EGFR mutations, ALK mutations, RB1 mutations, HIF mutations, KEAP mutations, NRF mutations, or other metabolic-related mutations, or combinations thereof.

Example 1: Immune modulation within Tumors.

Materials and Methods

* Vehicle is DMSO

* KULA18 is a nanoparticle composition of DCA (test article) (see preferred embodiment described above).

  » DCA = dichloroacetate

All arms were dosed intraperitoneal! with the DCA group getting 50mpk and KULA18 group getting 10mpk, all on a 5 on (dosed) 2 off (not dosed) schedule. After 17 days the mice were sacrificed and the tumors resected for analysis via FACS.

Tissue = tumor
The flow cytometry results are shown in the Figures 3A-5F and Tables 2 and 3 below.

The results in Figures 3A-3B show that targeted DCA nanoparticles (Group 3) increase the amount of CD4 and CD8 cell in the tumor. DCA by itself (Group 7) actually lowers the amount of CD4 and CD8 in the tumors.

The results in Figures 4A-4B and 4D-4E show that DCA (Group 7) does not have much of an effect on PD-1 and CTLA4, however, targeted DCA (KULA18) nanoparticles lower both CD4 and CD8 (Group 3).

- KULA18 increases immune cell infiltration into the tumor
- KULA18 significantly lowers multiple checkpoint proteins including PD-1, CTLA4, LAG3 and TIM3
- KULA18’s unique properties are due to its ability to target the cancer cells specifically. If it was not able to target cancer cells specifically it would also inhibit PDK in immune cells which would in turn inhibit glycolysis in immune cells and not lower checkpoint proteins - see data from dichloroacetate, a non-targeted PDK inhibitor.

Table 1: Cell Populations and Marker Signature:

<table>
<thead>
<tr>
<th>Population</th>
<th>Marker Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ T cells</td>
<td>CD3+CD4+CD8-PD-1&lt;sup&gt;+&lt;/sup&gt;CTLA-4&lt;sup&gt;-&lt;/sup&gt;LAG3&lt;sup&gt;-&lt;/sup&gt;/Tim3&lt;sup&gt;-&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD8&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>CD3+CD4+CD8&lt;sup&gt;-&lt;/sup&gt;PD-1&lt;sup&gt;+&lt;/sup&gt;CTLA-4&lt;sup&gt;-&lt;/sup&gt;LAG3&lt;sup&gt;-&lt;/sup&gt;/Tim3&lt;sup&gt;-&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tregs</td>
<td>CD3+CD4+CD&lt;sub&gt;25&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;FoxP3&lt;sup&gt;-&lt;/sup&gt;PD-1&lt;sup&gt;-&lt;/sup&gt;CTLA-4&lt;sup&gt;-&lt;/sup&gt;LAG3&lt;sup&gt;-&lt;/sup&gt;/Tim3&lt;sup&gt;-&lt;/sup&gt;</td>
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Table 2: Flow Cytometry Results - CB4, CBS, Treg counts

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<thead>
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<th>Group</th>
<th>Animal</th>
<th>% relative to Live</th>
<th>Count</th>
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<tr>
<td></td>
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<td>CB4</td>
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<tr>
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<td>a1</td>
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<td>Group 2</td>
<td>b1</td>
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<td>Group 4</td>
<td>d1</td>
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<tr>
<td>Group 5</td>
<td>e1</td>
<td>3</td>
<td>18.9</td>
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<tr>
<td>Group 6</td>
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<td>3</td>
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<tr>
<td>Group 7</td>
<td>g1</td>
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<td>7.37</td>
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<tr>
<td>Group 8</td>
<td>h1</td>
<td>3</td>
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<tr>
<td>Group 9</td>
<td>i1</td>
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<tr>
<td>Group 15</td>
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<td>18.9</td>
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Table 3: Flow Cytometry Results - PD-1, CTLA4, LAG3, Tim3 expression

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I claim:

1. A method of treating cancer comprising administering a subject an effective amount of a composition to reduce one or more symptoms of cancer, the composition comprising a modulator of cancer cell metabolism and a cancer cell- or a glucose-depleted and/or lactate-rich disease environment- targeting moiety, wherein the targeting moiety is associated with, linked, conjugated, or otherwise attached directly or indirectly to the modulator of cancer cell metabolism, or to a nanoparticle or other delivery vehicle thereof.

2. The method of claim 1, wherein the modulator reduces cancer cell glycolysis.

3. The method of claim 2, wherein the modulator is a glucose transporter (GLUTs) inhibitor.

4. The method of claim 2, wherein the modulator is a hexokinase inhibitor.

5. The method of claim 2, wherein the modulator is a phosphofructokinase inhibitor.

6. The method of claim 2, wherein the modulator is a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) inhibitor.

7. The method of claim 2, wherein the modulator is a phosphoglycerate mutase (PGM) inhibitor.

8. The method of claim 2, wherein the modulator is an enolase (ENO) inhibitor.

9. The method of claim 2, wherein the modulator is a pyruvate kinase (PK) activator.

10. The method of claim 2, wherein the modulator is a lactate dehydrogenase inhibitor.

11. The method of claim 2, wherein the modulator is a pyruvate dehydrogenase kinase (PDK) inhibitor.
12. The method of claim 2, wherein the modulator is a glucose-6-phosphate dehydrogenase (G6PD) inhibitor.

13. The method of claim 1, wherein the modulator inhibits components of the tricarboxylic acid (TCA) cycle in cancer cells.

14. The method of claim 1, wherein the modulator inhibits a monocarboxylate transporter (MCTs) in cancer cells.

15. The method of any one of claims 1-14 wherein the modulator is a small molecule.

16. The method of any one of claims 1-14 wherein the modulator is a functional nucleic acid.

17. The method of any one of claims 1-16, wherein the composition is administered prior to or in conjunction with an immunotherapy.

18. The method of claim 17, wherein the immunotherapy comprises adoptive T cell therapy.

19. The method of claim 17, wherein the immunotherapy comprises inhibitors of checkpoint proteins.

20. The method of any one of claims 1-19 wherein the targeting moiety preferentially targets cancer cells.

21. The method of any one of claims 1-20 wherein the modulator accumulates in cancer cells at an increased amount relative to non-cancer cells.

22. The method of claim 21, wherein the non-cancer cells are immune cells.

23. The method of claim 22, wherein the immune cells are tumor infiltrating lymphocytes.

24. The method of any one of claims 1-23, wherein the targeting moiety binds to a cancer antigen.

25. The method of any one of claims 1-24 wherein the modulator or nanoparticle preferentially accumulates in the mitochondria.
26. The method of any one of claims 1-25 wherein the modulator or nanoparticle includes a mitochondrial target moiety or signal.

27. The method of claim 26, wherein the mitochondrial targeting moiety or signal is selected from the group consisting of cationic ligands, cationic proteins, cationic polymers, cationic polymer-peptide conjugates, mitochondrial localization signals, and protein transduction domains.

28. A pharmaceutical composition comprising a modulator of cancer cell metabolism and a cancer cell- or glucose-depleted and/or lactate-rich disease environment- targeting moiety, wherein the targeting moiety is associated with, linked, conjugated, or otherwise attached directly or indirectly to the modulator of cancer cell metabolism, or to a nanoparticle or other delivery vehicle thereof, in an effective amount to reduce one or more symptoms of cancer when administered to a subject in need thereof.
FIG. 1
**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61K47/54 A61K47/69 A61K31/19 A61P35/00

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, EMBASE, BIOSIS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:
  * **A** document defining the general state of the art which is not considered to be of particular relevance
  * **E** earlier application or patent but published on or after the international filing date
  * **L** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  * **O** document referring to an oral disclosure, use, exhibition or other means
  * **P** document published prior to the international filing date but later than the priority date claimed
  * **T** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle of theory underlying the invention
  * **X** document of particular relevance; the claimed invention cannot be considered new or cannot be considered to involve an inventive step when the document is taken alone
  * **Y** document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  * **A** document member of the same patent family

Date of the actual completion of the international search

24 February 2017

Date of mailing of the international search report

07/03/2017

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk

Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Monami, Amelie
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