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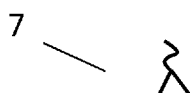
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(54) Title: PRECISION CANCER TREATMENT USING CUSTOM DESIGNED PEPTIDE-COATED DELIVERY VEHICLES AND INTERCHANGEABLE MODULAR ANTIBODIES



Cancer-targeting antibody (e.g., anti-CD20, anti-Her2, anti-PD-1, etc.)



NPD with cancer drug (e.g. vincristine or doxorubicin) with non-specific antibody binding protein (e.g. protein G/A/C) attached

FIG. 1

(57) Abstract: Disclosed herein are methods and novel complexes and compositions that allow for the precise delivery of cancer therapeutic agent. Exemplified complexes include an agent-loaded delivery vehicle that is coated with a secondary targeting molecule that binds to a primary targeting molecule (e.g. an antibody that binds to a cancer antigen). Agent-loaded delivery vehicles exemplified herein include nanoparticles and liposomes coated with an antigen binding protein.



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PRECISION CANCER TREATMENT USING CUSTOM DESIGNED PEPTIDE-COATED DELIVERY VEHICLES AND INTERCHANGEABLE MODULAR ANTIBODIES

BACKGROUND

[0001] Cancer specific antibodies, antibody-drug conjugates (ADCs), and nanoparticles have developed with the intended purpose of treating cancer. However, for various reasons, these technologies have failed to progress beyond their initial success.

[0002] First, antibodies against cancer specific antigens have been successfully developed, most notably the anti-CD20 antibodies (rituximab, ofatumumab, and obinutuzumab) and anti-Her2/neu antibodies (herceptin). These antibodies act through stimulating the body's immune response, for example, ADCC. For reasons that remain poorly understood, most antibodies do not cause enough immune based killing of cancer cells. A notably example is the anti-CD30 antibody, the failure of which subsequently led to the development of brentuximab, an anti-CD30 ADC. The antibodies are very expensive, and each of them can be used in only specific and often rare histological types of cancer.

[0003] ADC is another related technology, which has witnessed the success of drugs like brentuximab (anti-CD30 ADC) and ado-trastuzumab emtansine (anti-Her2 ADC). These drugs must be taken up by the cancer cells through a process called endocytosis. Unfortunately, most antigen-antibody binding do not lead to endocytosis. Moreover, ADCs are expensive and hard to develop. As a result, very few ADCs have been approved.

[0004] Nanoparticle delivery systems have also produced a modest success. A few drugs, such as liposomal doxorubicin and taxol, have been developed using liposome nanoparticles. However, liposome nanoparticles are not designed to reach tumors selectively or preferentially. The success of liposomal doxorubicin and taxol, compared to their naked counterparts, is mainly due to improved stability and pharmacokinetic profiles of the liposomal drugs. The liposomal drugs are associated with a very broad array of toxicities in patients.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] The present invention is illustrated by way of example, and not by way of limitation, in the figures of the accompanying drawings.

[0006] FIG. 1 is a diagram representing an embodiment of a nano-peptide drug (NPD) having an antibody binding protein bound thereto and loaded with a cancer drug; and a cancer-targeting antibody embodiment.

[0007] FIG. 2 is a diagram representing an embodiment of an NPD having an antibody that binds to digoxin (Dig) and a cancer targeting antibody that has Dig bound thereto.

[0008] FIG. 3 is a diagram representing a method embodiment using an NPD for treating B-lymphoma.

[0009] FIG. 4 is a diagram representing a method embodiment for treating B-lymphoma using an NPD that is coated with an antibody against Dig and a cancer targeting antibody that has Dig associated therewith.

[0010] FIG. 5 is a diagram representing a method embodiment for treating +Her2 breast cancer using an NPD that includes an antibody binding protein coated thereon and a primary targeting molecule that binds to +Her2 cancer cells.

[0011] FIG. 6 is a diagram representing a method embodiment for treating +Her2 breast cancer using an NPD that includes an antibody against Dig and a cancer targeting antibody that has Dig associated therewith.

[0012] FIG. 7 is a diagram representing a method embodiment for treating B-lymphoma using an NPD that includes a secondary antibody as a secondary targeting molecule, where the secondary antibody is bound to an antibody binding protein coated on a nanoparticle.

[0013] FIG. 8 is a diagram representing a method embodiment for treating B-lymphoma that involves pretreatment with a primary targeting molecule (e.g., primary antibody, anti-CD20 antibody) and co-administering a delivery vehicle bound to a secondary targeting molecule (e.g., secondary antibody).

[0014] FIG. 9 is a diagram representing a method embodiment for treating B-lymphoma using an NPD that is pre-incubated with the primary antibody.

[0015] FIG. 10 is a diagram representing a method embodiment for treating B-lymphoma using an NPD that includes an antibody binding protein coated thereon and a primary targeting molecule that binds to +Her2 cancer cells.

[0016] FIG. 11 is a diagram representing a method embodiment for inducing a targeted immunotherapeutic response that involves a primary targeting molecule that binds to a cancer

antigen and a secondary targeting molecule that has a portion that binds to the primary targeting molecule and another portion that binds to an immune cell antigen.

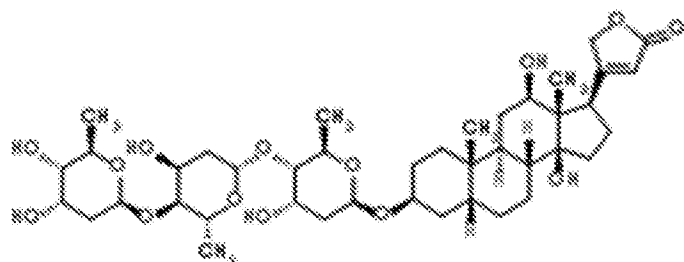
[0017] FIG.12 is a diagram representing a method embodiment for inducing a targeted immunotherapeutic response that involves a primary targeting molecule that binds to a cancer antigen and a CAR cell that is engineered to express a CAR molecule that binds to the primary targeting molecule and a second CAR molecule that binds to a cancer associated antigen.

DETAILED DESCRIPTION

1. Introduction

[0018] Provided herein are methods and complexes for precision targeting of cancer cells. Certain embodiments involve a primary targeting molecule that binds to a cancer antigen and a secondary targeting molecule that binds to the primary targeting molecule. The secondary targeting molecule may be associated with a delivery vehicle that is loaded with a cancer therapeutic agent (agent-loaded delivery vehicle). In a specific embodiment, the secondary targeting molecule coats the agent-loaded delivery vehicle.

[0019] The primary targeting molecule may be in the form of an antibody or aptamer that binds to a specific cancer antigen. FIG. 1 shows an example of a primary targeting molecule **7**, which is an antibody specific to a cancer antigen. In addition, the primary targeting molecule may be an antibody or aptamer engineered to include digoxin (Dig) bound thereto. FIG. 2 shows an example of primary targeting molecule **8** that is a digoxin associated antibody that is specific to a cancer antigen.



Digoxin

[0020] The secondary targeting molecule may be a non-specific antibody binding protein or may be a specific antibody or aptamer that specifically binds to the primary targeting molecule. When the primary targeting molecule includes digoxin, the secondary targeting molecule may be specific to digoxin (e.g. an antibody or aptamer that specifically binds to digoxin).

[0021] In another embodiment, provided is a method that involves administering the primary targeting molecule such as an antibody (primary antibody) and co-administering the agent-loaded delivery vehicle having the secondary targeting molecule bound thereto. The primary targeting molecule binds to cancer antigen on cancer cells and then the agent-loaded delivery vehicle targets the primary targeting molecule to thereby precisely deliver the “payload” to the cancer cells.

[0022] In a more specific embodiment, the secondary targeting molecule is a non-specific antibody-binding protein, such as protein A, protein G, protein C, protein A/G, or protein L, or a secondary antibody that binds to the primary targeting molecule, and optionally, to another antigen. In a specific example, the secondary targeting molecule is an antibody or aptamer that specifically binds to digoxin associated with the primary targeting molecule. In another specific embodiment, the secondary targeting molecule may be a bi-specific or tri-specific antibody that

has a portion that binds to the primary targeting molecule and a portion that binds to a separate antigen (e.g. an antigen on an immune cell FIG.11).

[0023] Associating a nonspecific antibody-binding protein, or an antibody specific to the primary targeting molecule with the delivery vehicle provides for a versatile, universal delivery vehicle complex that can be used in conjunction with any number of primary antibodies. This in turn alleviates the need for the expensive development of multiple different complexes that are specific to only one type of cancer or cancer antigen. The embodiments herein also revitalize currently used cancer therapeutic antibodies, since they can be used as primary targeting molecules and not just as a cancer therapy.

[0024] Another embodiment pertains to a method embodiment for inducing a targeted immunotherapeutic response that involves a primary targeting molecule that binds to a cancer antigen and a CAR cell that is engineered to express a CAR molecule that binds to the primary targeting molecule and a second CAR molecule that binds to a cancer associated antigen.

[0025] Other embodiments relate to a delivery vehicle loaded with a cancer therapeutic agent and coated with an antibody-binding protein or digoxin specific binding molecule to form a complex referred to herein as a nano-peptide drug complex (NPD). There are multiple versions of NPDs contemplated herein. A NPD version **5** shown in FIG. 1 relates to the antibody-binding protein coated delivery vehicle discussed above. In this first version **5**, the antibody-binding protein serves as a secondary targeting molecule that non-specifically binds to the primary antibody. A specific embodiment related to this first version **5**, directed to treating cancer cells expressing CD20 **11** is shown in FIG. 3, for illustration purposes only.

-Step 1 (13): Patient is injected with the anti-CD20 antibody **12** (e.g. rituximab) that binds to a CD20 antigen **11**. CD20 positive lymphoma cells **17** bind the Fab domain **12'** of anti-CD20 antibodies **12**;

-Step 2 (15): Patient is then injected with the NPD **5** that includes a nanoparticle **14** coated with a protein G/A peptide **16** and loaded with a cancer drug **18**. The Fc **12''** domain of the antibody **12** bound to the CD20+ lymphoma cells **17** bind the protein G/A peptides **16** coated on the NPD **5**;

-Step 3: (Not shown) Nanoparticles **14** release cancer drugs **18** inside the cells and/or tumor. Steps 1 and 2 can occur sequentially or simultaneously.

[0026] Another embodiment related to NPD version **5** is provided in FIG. 5 which is directed to treating breast cancer. This embodiment involves the following steps:

-**Step 1 (33)**: Subject is injected with anti-Her2 Ab **32**. Her2+ breast cancer cells **37** bind the Fab domain **32''** of anti-Her2 antibodies **32**;

-**Step 2 (35)**: Patient is injected with the NPD **5**. The Fc domain **32'** of the antibody **32** bound to the Her2+ breast cancer cells **37** bind the protein G/A peptides **16** coated on the NPD **5**;

-**Step 3**: (Not shown) Nanoparticles **14** release cancer drugs **18** inside the cells and/or tumor. Steps 1 and 2 may occur sequentially or simultaneously.

[0027] Another NPD version **10** is shown in FIG. 2. NPD version **10** includes an antibody or aptamer that is specific for Dig. Dig is associated with antibody or aptamer to form primary targeting molecule **8**. The NPD **10** is loaded with cancer drug and is coated with an antibody or aptamer specific to Dig. A specific embodiment related to NPD version **10**, directed to treating cancer cells expressing CD20 **11** is shown in FIG. 4, for illustration purposes only.

-**Step 1 (23)**: Patient is injected with the digoxin conjugated anti-CD20 antibody **8**. CD20 positive lymphoma cells **17** bind the Fab domain **12''** of anti-CD20 antibodies **12**. Dig **19** on the Fc fragment **12'** of the antibody **12** is exposed.

-**Step 2 (25)**: Patient is then injected with the NPD **10**. The digoxin molecules **19** on CD20+ lymphoma cells **17** bind the digoxin-specific Fab or aptamer **42** on the NPD **10**;

-**Step 3**: (Not shown) Nanoparticles **14** release cancer drugs **18** inside the cells and/or tumor. Steps 1 and 2 may occur sequentially or simultaneously.

[0028] Another implementation of NPD version **10** is shown in FIG. 6 directed to treating cancer cells expressing Her2 **21**. This implementation involves the following:

-**Step 1 (43)**: Subject is injected with the digoxin conjugated anti-Her2 antibody **8'**. Her2 positive breast cancer cells **37** bind the Fab domain **32''** of anti-Her2 antibodies **32**. Digoxin **19** on the Fc fragment **32'** of the antibody **32** is exposed.

-**Step 2 (45)**: Patient is then injected with the NPD **10**. The digoxin molecules **19** on Her2+ breast cancer cells **37** bind the digoxin-specific Fab **42** on the NPD **10**;

-**Step 3**: (Not shown) Nanoparticles **14** release cancer drugs **18** inside the cell and/or tumor. Steps 1 and 2 can occur sequentially or simultaneously.

[0029] Importantly, as illustrated in FIGs 3-6, the same NPD can be used for the treatment of many different cancers, which is realized through pre-treating a patient with a select antibody

that recognizes the subject's cancer specific antigen. For example, subjects with lymphoma or breast cancer can be pre-treated with rituximab or trastuzumab, respectively; and the same NPD is expected to be effective for both malignancies. Other examples of primary targeting molecules are described herein.

[0030] It should be pointed out that the pretreatment antibodies do *not* need to possess any anti-tumor activity through conventional immune mechanisms such as antibody-dependent cell-mediated cytotoxicity (ADCC). As a result, many antibodies previously found to be safe but ineffective in human studies, for example, the anti-CD30 antibody (not to be confused with the FDA approved anti-CD30 antibody-MMAE conjugate drug, namely brentuximab), may be easily repurposed for use in the platform described herein. Similarly, new antibodies can be expeditiously developed to target many known cancer specific antigens, as long as the lack of direct anti-tumor effects does not negatively impact the utility of these antibodies.

[0031] In another embodiment shown in FIG. 7, the NPD comprises a secondary antibody specific to a primary targeting molecule that is bound to the antibody-binding protein. In this Embodiment, the secondary antibody serves as a secondary targeting molecule. As shown in FIG. 7, the subject is treated with the primary targeting molecule **12** (anti-CD20 antibody) and then treated with an NPD **13** having the secondary antibody **26** (anti-anti-CD20 antibody) bound thereto. In a specific example, the secondary antibody **26** may be bound via a protein G/A **16** coated on the nanoparticle **14**. Upon the administration of the NPD **13**, the secondary antibody will bind to the primary antibody **12** and cancer drug **18** in the nanoparticle **14** is released into the cell **17** and/or tumor.

[0032] In an alternative embodiment as shown in FIG. 8, the secondary targeting molecule **26** may be an antibody or aptamer that is bound directly to a delivery vehicle **14** to form an NPD **53** without an intervening antibody-binding protein. In this version, each delivery vehicle bound to the secondary targeting molecule is specific to the particular primary targeting molecule and does not benefit from the universality that an NPD provides. Nevertheless, this alternative embodiment still allows for precise delivery of a cancer therapeutic agent payload using known anti-cancer antigen antibodies as primary targeting molecules for delivery vehicles such as nanoparticles and liposomes.

[0033] In any NPD versions where a non-specific binding molecule like protein G/A is used, the primary targeting molecule may be administered separate to the NPD or may be pre-incubated *in*

vitro with the NPD thereby forming a primary targeting molecule bound NPD that can be administered to the subject as a unified complex. An example of this pre-incubation to form the unified complex is found in FIG. 9. In the illustrated embodiment shown in FIG. 9, step 1 **63** involves incubating an NPD **5** with a primary antibody **12** such that the Fc portion **12'** of the primary antibody **12** binds to the protein G/A **16** coated on the nanoparticle **14** to form a NPD/primary targeting molecule complex **56**. Step 2 **65** involves administering the NPD/primary targeting molecule complex to a subject, where the Fab portion **12''** binds to cancer antigen **11** on cancer cells **17** and releases the cancer drug **18**. Moreover, it is noted that alternatively a second antibody can be implemented either directly bound to the nanoparticle or indirectly bound via a non-specific binding molecule.

[0034] When using a secondary antibody or aptamer that is either directly bound to the nanoparticle such as in the embodiments shown in FIGs 4, 6 or 8 or indirectly bound to the nanoparticle such as in the embodiments shown in FIGs 7 or 9, the primary targeting molecule that binds to the cells can be either an antibody or aptamer since the secondary antibody or aptamer bound to the nanoparticle can be developed for either type of molecule.

[0035] It is noted that NPDs that involve multiple antibody linkages, especially embodiments where there is a secondary antibody bound to a nonspecific binding protein, there are potential risks of clumping and/or poor penetration into the microvasculature due to its size. Accordingly, ratio of the antibody, peptide, and size of the nanoparticle delivery vehicle is adjusted to minimize those risks.

[0036] Another embodiment is shown in FIG. 10 that involves administration of NPD having a nonspecific binding molecule coated thereon. At step 1 **73**, the subject undergoes plasmapheresis to deplete the blood of immunoglobulins. At Step 2 **75**, the subject is injected with a primary targeting antibody (e.g. anti-CD20 antibody). Cancer cells (e.g. CD20 positive lymphoma cells) bind to the primary targeting antibody, which may optionally be followed by a brief plasmapheresis treatment. At step 3 **77**, the subject is administered the NPD which binds to the primary targeting antibody bound to the cancer cells. At step 4 (not shown), as cancer drug is released to the cells and/or tumor.

[0037] As mentioned above, it has been found that drug conjugates are not always efficiently endocytosed to deliver the payload to the cancer cells. The embodiments described herein avoid the necessity of endocytosis. For example, the delivery vehicle can be tailored to allow for the

release of cancer therapeutic agent based on factors present in the tumor tissue such as pH or can be tailored for time released. In this sense, the delivery vehicle can be precisely targeted to the tumor and can release the cancer therapeutic agent in the tumor microenvironment. Thus, the embodiments described herein allow for precise delivery of cancer therapeutic agent to the tumor, overcoming one of the drawbacks of the convention drug conjugates.

[0038] Other embodiments relate to methods of making NPDs and other delivery vehicles described herein. In one embodiment, the method involves loading a delivery vehicle with a cancer therapeutic agent; and coating the delivery vehicle with a secondary targeting molecule that binds to a primary targeting molecule, wherein said loading and coating steps may occur in either order. Various delivery vehicles that can be used in accord with the teachings herein are described below.

[0039] Some of the advantages of NPD include:

- A single NPD can be used in many types of cancer.
- A number of antibodies recognizing cancer surface markers have been evaluated in clinical trials. These antibodies are safe and are discontinued due to inadequate anti-tumor activity. However, these antibodies will work effectively with the NPDs.
- NBD based on digoxin recognition does not introduce bacterial proteins.
- NBD based on Fc recognition requires no modification of the labeling antibodies.

2. Definitions

[0040] Unless otherwise defined, all technical and scientific terms used herein are intended to have the same meaning as commonly understood in the art to which this invention pertains and at the time of its filing. Although various methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. However, the skilled should understand that the methods and materials used and described are examples and may not be the only ones suitable for use in the invention. Moreover, it should also be understood that as measurements are subject to inherent variability, any temperature, weight, volume, time interval, pH, salinity, molarity or molality, range, concentration and any other measurements, quantities or numerical expressions given herein are intended to be approximate and not exact or critical figures unless expressly stated to the contrary. Hence, where appropriate to the invention and as understood by those of skill in the art, it is proper to describe the various aspects of the invention using approximate or relative

terms and terms of degree commonly employed in patent applications, such as: so dimensioned, about, approximately, substantially, essentially, consisting essentially of, comprising, and effective amount.

[0041] Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics, protein, and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present invention generally are performed according to conventional methods well known in the art and as described in various general and more specific references, unless otherwise indicated. *See, e.g.*, Sambrook *et al.* *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989); Ausubel *et al.*, *Current Protocols in Molecular Biology*, Greene Publishing Associates (1992, and Supplements to 2002); Harlow and Lan, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990); Principles of Neural Science, 4th ed., Eric R. Kandel, James H. Schwartz, Thomas M. Jessell editors. McGraw-Hill/Appleton & Lange: New York, N. Y. (2000). Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

[0042] As used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural references unless the content clearly dictates otherwise.

[0043] The term “about” as used herein means approximately, roughly, around, or in the region of. When the term “about” is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term “about” is used herein to modify a numerical value above and below the stated value by a variance of 20 percent up or down (higher or lower).

[0044] The term “agent-loaded delivery vehicle” refers to a delivery vehicle loaded with a cancer therapeutic agent. Examples of delivery vehicles include nanoparticles, liposomes, microemulsions and dendrimers.

[0045] The term “antibody” is used here in its broadest sense refers to an immunoglobulin, or derivative or fragment or active fragment thereof, having an area on the surface or in a cavity which specifically binds to and is thereby defined as complementary with a particular spatial and polar organization of another molecule. The term “antibody,” as used herein, also includes antibody substitutes or any natural, recombinant or synthetic molecule that specifically binds

with high affinity to a particular target. Thus, the term “antibody” includes such synthetic antibodies or antibody substitutes such as aptamers, affibodies, affimers, avimers, aptides, and the like. Therefore, when describing the assay systems, devices and methods according to embodiments of the invention here, use of the term “antibody” for use as, for example, a reagent in the assay, indicates any of these alternatives also can be used.

[0046] The term “aptamer” refers to a nucleic acid or peptide molecule that specifically binds to a molecule of interest (target) with high affinity. Generally, aptamers are engineered through repeated rounds of *in vitro* selection or equivalently, SELEX (systematic evolution of ligands by exponential enrichment) to bind to various molecular targets such as small molecules, proteins, nucleic acids, and even cells, tissues and organisms. The aptamer may be prepared by any known method, including synthetic, recombinant, and purification methods, and may be used alone or in combination with other aptamers specific for the same target.

[0047] The terms “bind” or “bound” when referring to the binding by an antibody or aptamer to means binding through weak and noncovalent interactions such as electrostatic interactions, hydrogen bonds, Van der Waals forces, and hydrophobic interactions.

[0048] As used herein, a “cancer therapeutic agent” pertains to an agent that possesses selectively cytotoxic or cytostatic effects to cancer cells over normal cells.

[0049] The term “cancer cell” as used herein means a cell that shows aberrant cell growth, such as increased cell growth. A cancer cell may be a hyperplastic cell, a cell that shows a lack of contact inhibition of growth *in vitro*, a tumor cell that is incapable of metastasis *in vivo*, or a metastatic cell that is capable of metastasis *in vivo*.

[0050] The term “cancer” or “tumor” as used herein means is intended to include any neoplastic growth in a patient, including an initial tumor and any metastases. The cancer can be of the liquid or solid tumor type. Liquid tumors include tumors of hematological origin (hematological cancer), including, e.g., myelomas (e.g., multiple myeloma), leukemias (e.g., Waldenstrom's syndrome, chronic lymphocytic leukemia, other leukemias), and lymphomas (e.g., B-cell lymphomas, non-Hodgkins lymphoma). Solid tumors can originate in organs, and include cancers such as lung, breast, prostate, ovary, colon, kidney, and liver.

[0051] The term “co-administration” or “co-administering” as used herein refers to the administration of an active agent before, concurrently, or after the administration of another active agent such that the biological effects of either agents overlap. The combination of agents

as taught herein can act synergistically to treat or prevent the various diseases, disorders or conditions described herein. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects. Concurrent administration includes administration of two different molecules bound together prior to administration. For example, administering a primary targeting molecule and co-administering an agent-loaded delivery vehicle coated with a secondary targeting molecule includes the situation of concurrent administration where the agent-loaded delivery vehicle is bound to the primary targeting molecule.

[0052] The term “coat” as used herein refers to a material that is present on an outer region of a delivery vehicle such that the material is accessible to molecules outside the delivery vehicle. The term coat includes coats formed from substantially one material as well as a plurality of materials that can, for example, be arranged as multi-layer coats. A coat may be “complete”, indicating that the coat substantially or completely surrounds the outer surface of the delivery vehicle (e.g., substantially all surface atoms of the core are covered with coat material). Alternatively, the coat may be “incomplete” such that the coat partially surrounds the outer surface of the delivery vehicle (e.g., partial coverage of the surface atoms is achieved). In addition, it is possible to create coats of a variety of thicknesses, which can be defined in terms of the number of “monolayers” of coat material that are bound to each core. A “monolayer” is a term known in the art referring to a single complete coating of a material (with no additional material added beyond complete coverage). For certain applications, coats may be of a thickness between about 1 and 10 monolayers, where it is understood that this range includes non-integer numbers of monolayers. Non-integer numbers of monolayers can correspond to the state in which incomplete monolayers exist. Incomplete monolayers may be either homogeneous or inhomogeneous, forming islands or clumps of coat material on the surface of the delivery vehicle. Coats may be either uniform or non-uniform in thickness. In the case of a coat having non-uniform thickness, it is possible to have an “incomplete coat” that contains more than one monolayer of coat material. A coat may optionally comprise multiple layers of a plurality of materials in an onion-like structure, such that each material acts as a coat for the next-most inner layer. Between each layer there is optionally an interface region. The coat material may be physically associated with the delivery vehicle and/or chemically bound to atoms of the delivery vehicle including covalent binding, ionic binding, hydrogen binding or Van Der Waal forces.

[0053] The term “cancer antigen” refers to a biomolecule that appears or increases in amount as an indicator of or marker of a particular disease or condition. In particular, this term includes any naturally occurring and detectable biological molecule, the presence of which, or the presence of which at or above a certain concentration or amount, on cancer cells is increased.

[0054] The term “primary targeting molecule” refers to a molecule that preferentially binds to a cancer antigen. Examples of primary targeting molecules include antibodies and aptamers with specific affinity to a cancer antigen.

[0055] The term “secondary targeting molecule” is a molecule or complex of molecules that binds to a primary targeting molecule. The secondary targeting molecule may include a bi-specific molecule or tri-specific molecule. Examples of secondary targeting molecules include antibody binding proteins, antibody binding nucleotides, antibodies and aptamers. Antibody binding proteins and nucleotides generally bind to any human antibody. Antibodies and aptamers have specific, preferential binding to a primary targeting molecule. A complex of molecules that binds to a primary targeting molecule includes an antigen binding protein bound to a secondary antibody.

[0056] A “therapeutically effective amount” refers to an amount of the primary targeting molecule, secondary targeting molecule, CAR cell and/or agent-loaded delivery vehicle, which, when administered or co-administered in a proper dosing regimen, is sufficient to reduce or ameliorate the severity, duration, or progression of the disorder being treated (e.g., cancer), prevent the advancement of the disorder being treated (e.g., cancer), cause the regression of the disorder being treated (e.g., cancer), or enhance or improve the prophylactic or therapeutic effects(s) of another therapy. The full therapeutic effect does not necessarily occur by administration of one dose and may occur only after administration of a series of doses. Thus, a therapeutically effective amount may be administered in one or more administrations per day for successive days.

3. Detailed Description of Embodiments

Delivery vehicles

[0057] In some embodiments, provided are unique delivery vehicles designed for delivering of an agent to cancer cells, tumor or tumor microenvironment. In these embodiments, typically a cancer therapeutic agent is encapsulated in a suitable vehicle to either aid in the delivery of the compound to target cells, to increase the stability of the composition, or to minimize potential toxicity of the composition. As will be appreciated by a skilled artisan, a variety of vehicles are suitable for delivering an agent. Non-limiting examples of suitable structured fluid delivery systems may include nanoparticles, liposomes, microemulsions, micelles, dendrimers and other phospholipid-containing systems. Methods of incorporating compositions into delivery vehicles are known in the art.

Nanoparticles

[0058] As used herein, “nanoparticle” is used to refer to a nanostructure that is typically between about 5 nm and 400 nm across the largest dimension of the structure. A nanoparticle used as a delivery vehicle may be spherical, tubular, cylindrical, cubic, hexagonal, dumbbell or any other shape that may be envisaged or built in a laboratory setting. A nanoparticle may typically be between about 5 nm and 400 nm across the largest dimension, but in some instances, may be bigger or smaller. In another embodiment, the average size of a plurality of nanoparticles in a composition may typically be between about 5 nm and 400 nm across the largest dimension. In one embodiment, the largest dimension of a nanoparticle of the invention may be between about 100 nm and about 300 nm. In another embodiment, the largest dimension of a nanoparticle may be between about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, or 300 nm. In some embodiments, the largest dimension may be greater than 400 nm, for instance, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, or greater than 10,000 nm.

[0059] The nanoparticles release their agent “payload” over a number of days as a function of their degradation profile *in vivo*. The nanoparticles may be biodegradable in nature and thus may gradually degrade in an aqueous environment such as occurs *in vivo*. If the agents are dispersed throughout the nanoparticles, then their release will occur as the outermost layers of the nanoparticle degrade or as the pores within the nanoparticle enlarge. Agents can be released from such nanoparticles over time-courses ranging from 1 day to at least 2 weeks.

The nanoparticles are preferably not engulfed by either their carrier cells or other cells at the target site. They function rather by gradually releasing their payload into the environment of the target site(s).

[0060] As used in the context of nanoparticle diameters, the term “about” means $\pm 5\%$ of the absolute value stated. Thus, it is to be understood that although these particles are referred to herein as nanoparticles, the invention intends to embrace microparticles as well.

[0061] In other embodiments, the nanoparticle is a natural or artificial polymer.

Polymer nanoparticles may be biodegradable, bioresorbable, or bioerodable polymers.

Preferably, the nanoparticle is bio-compatible. Non-limiting examples of polymers that are considered to be biodegradable, bioresorbable, or bioerodable include, but are not limited to, albumin, collagen, gelatin and prolamines such as zein, polysaccharides such as alginate, cellulose derivatives and polyhydroxyalkanoates such as polyhydroxybutyrate aliphatic polyesters; poly(glycolic acid) and/or copolymers thereof (e.g., poly(glycolide trimethylene carbonate); poly(caprolactone glycolide); poly(lactic acid) and/or isomers thereof (e.g., poly-L(lactic acid) and/or poly-D(lactic acid) and/or copolymers thereof (e.g. DL-PLA), with and without additives (e.g. calcium phosphate glass), and/or other copolymers (e.g.

poly(caprolactone lactide), poly(lactide glycolide), poly(lactic acid ethylene glycol); poly(ethylene glycol) (in its various weights, i.e. 2000 D, 4000 D, 6000 D, 8000 D, etc.); poly(ethylene glycol) diacrylate; poly(lactide); polyalkylene succinate; polybutylene diglycolate; polyhydroxybutyrate (PHB); polyhydroxyvalerate (PHV); polyhydroxybutyrate/polyhydroxyvalerate copolymer (PHB/PHV); poly(hydroxybutyrate-co-valerate); polyhydroxyalkanoates (PHA); polycaprolactone; poly(caprolactone-polyethylene glycol) copolymer; poly(valerolactone); polyanhydrides; poly(orthoesters) and/or blends with polyanhydrides; poly(anhydride-co-imide); polycarbonates (aliphatic); poly(hydroxyl-esters); polydioxanone; polyanhydrides; polyanhydride esters; polycyanoacrylates; poly(alkyl 2-cyanoacrylates); poly(amino acids); poly(phosphazenes); poly(propylene fumarate); poly(propylene fumarate-co-ethylene glycol); poly(fumarate anhydrides); fibrinogen; fibrin; gelatin; cellulose and/or cellulose derivatives and/or cellulosic polymers (e.g., cellulose acetate, cellulose acetate butyrate, cellulose butyrate, cellulose ethers, cellulose nitrate, cellulose propionate, cellophane); chitosan and/or chitosan derivatives (e.g., chitosan NOCC, chitosan NOOC-G); alginate; polysaccharides; starch; amylase; collagen; polycarboxylic acids; poly(ethyl

ester-co-carboxylate carbonate) (and/or other tyrosine derived polycarbonates); poly(iminocarbonate); poly(BPA-iminocarbonate); poly(trimethylene carbonate); poly(iminocarbonate-amide) copolymers and/or other pseudo-poly(amino acids); poly(ethylene glycol); poly(ethylene oxide); poly(ethylene oxide)/poly(butylene terephthalate) copolymer; poly(epsilon-caprolactone-dimethyltrimethylene carbonate); poly(ester amide); poly(amino acids) and conventional synthetic polymers thereof; poly(alkylene oxalates); poly(alkylcarbonate); poly(adipic anhydride); nylon copolyamides; NO-carboxymethyl chitosan NOCC); carboxymethyl cellulose; copoly(ether-esters) (e.g., PEO/PLA dextrans); polyketals; biodegradable polyethers; biodegradable polyesters; polydihydropyrans; polydepsipeptides; polyarylates (L-tyrosine-derived) and/or free acid polyarylates; polyamides (e.g., Nylon 66, polycaprolactam); poly(propylene fumarate-co-ethylene glycol) (e.g., fumarate anhydrides); hyaluronates; poly-p-dioxanone; polypeptides and proteins; polyphosphoester; polyphosphoester urethane; polysaccharides; pseudo-poly(amino acids); starch; terpolymer; (copolymers of glycolide, lactide, or dimethyltrimethylene carbonate); rayon; rayon triacetate; latex; and/pr copolymers, blends, and/or composites of above.

[0062] Non-limiting examples of polymers that are considered to be biostable include, but are not limited to, parylene; parylene c; parylene f; parylene n; parylene derivatives; maleic anhydride polymers; phosphorylcholine; poly n-butyl methacrylate (PBMA); polyethylene-co-vinyl acetate (PEVA); PBMA/PEVA blend or copolymer; polytetrafluoroethene (Teflon®) and derivatives; poly-paraphenylene terephthalamide (Kevlar®); poly(ether ether ketone) (PEEK); poly(styrene-b-isobutylene-b-styrene) (Translute™); tetramethyldisiloxane (side chain or copolymer); polyimides polysulfides; poly(ethylene terephthalate); poly(methyl methacrylate); poly(ethylene-co-methyl methacrylate); styrene-ethylene/butylene-styrene block copolymers; ABS; SAN; acrylic polymers and/or copolymers (e.g., n-butyl-acrylate, n-butyl methacrylate, 2-ethylhexyl acrylate, lauryl-acrylate, 2-hydroxy-propyl acrylate, polyhydroxyethyl, methacrylate/methylmethacrylate copolymers); glycosaminoglycans; alkyd resins; elastin; polyether sulfones; epoxy resin; poly(oxymethylene); polyolefins; polymers of silicone; polymers of methane; polyisobutylene; ethylene-alphaolefin copolymers; polyethylene; polyacrylonitrile; fluorosilicones; poly(propylene oxide); polyvinyl aromatics (e.g. polystyrene); poly(vinyl ethers) (e.g. polyvinyl methyl ether); poly(vinyl ketones); poly(vinylidene halides) (e.g. polyvinylidene fluoride, polyvinylidene chloride); poly(vinylpyrrolidone);

poly(vinylpyrrolidone)/vinyl acetate copolymer; polyvinylpyrrolidone prolactin or silk-elastin polymers (SELP); silicone; silicone rubber; polyurethanes (polycarbonate polyurethanes, silicone urethane polymer) (e.g., chronoflex varieties, bionate varieties); vinyl halide polymers and/or copolymers (e.g. polyvinyl chloride); polyacrylic acid; ethylene acrylic acid copolymer; ethylene vinyl acetate copolymer; polyvinyl alcohol; poly(hydroxyl alkylmethacrylate); Polyvinyl esters (e.g. polyvinyl acetate); and/or copolymers, blends, and/or composites of above. Non-limiting examples of polymers that can be made to be biodegradable and/or bioresorbable with modification include, but are not limited to, hyaluronic acid (hyaluron); polycarbonates; polyorthocarbonates; copolymers of vinyl monomers; polyacetals; biodegradable polyurethanes; polyacrylamide; polyisocyanates; polyamide; and/or copolymers, blends, and/or composites of above. As can be appreciated, other and/or additional polymers and/or derivatives of one or more of the above listed polymers may be used.

[0063] In a specific embodiment, the nanoparticles are carboxylate functionalized mesoporous silica nanoparticles (MSNs). MSNs can be made and loaded in accord with techniques known in the art. See for example, U.S. Patent No. 9,271,936 and U.S. Pat. Pub. 20100255103. In a specific embodiment, MSNs are loaded with a cancer therapeutic agent and coated with an antibody-binding protein.

Liposomes

[0064] Embodiments may implement liposomes in place of nanoparticles as delivery vehicles in the various embodiments described herein. Liposomes are small closed vesicles comprising at least one lipid bilayer and an internal aqueous compartment. As used herein, liposomes are not nanoparticles. Liposomes may be anionic, neutral or cationic. They may be unilamellar or multilamellar. Liposome may comprise without limitation unilamellar vesicle lipids, multilamellar vesicle lipids and extruded lipids including DOTMA, DOTAP, DOTIM, DDAB, alone or together with cholesterol to yield DOTMA and cholesterol, DOTAP and cholesterol, DOTIM and cholesterol, and DDAB and cholesterol. Methods for preparation of multilamellar vesicle lipids are known in the art (see for example U.S. Pat. No. 6,693,086, the teachings of which relating to multilamellar vesicle lipid preparation are incorporated herein by reference). Extruded lipids are prepared in a similar manner but are then extruded through filters of decreasing size, as described in Templeton et al., Nature Biotech, 15:647-652, 1997, the teachings of which relating to extruded lipid preparation are incorporated herein by reference.

[0065] Liposomes can be coated with an antibody binding protein using techniques known in the art. See for example, Su et al. *Langmuir*, **2014**, *30* (21), pp 6219–6227. In specific embodiments, liposomes are coated with protein A, protein G, protein L, or combinations thereof.

[0066] In certain embodiments, liposomes may be comprised of a variety of different types of phospholipids having varying hydrocarbon chain lengths. Phospholipids generally comprise two fatty acids linked through glycerol phosphate to one of a variety of polar groups. Suitable phospholipids include phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylcholine (PC), and phosphatidylethanolamine (PE). The fatty acid chains comprising the phospholipids may range from about 6 to about 26 carbon atoms in length, and the lipid chains may be saturated or unsaturated. Suitable fatty acid chains include (common name presented in parentheses) n-dodecanoate (laurate), n-tetradecanoate (myristate), n-hexadecanoate (palmitate), n-octadecanoate (stearate), n-eicosanoate (arachidate), n-docosanoate (behenate), n-tetracosanoate (lignocerate), cis-9-hexadecenoate (palmitoleate), cis-9-octadecanoate (oleate), cis,cis-9,12-octadecadienoate (linoleate), all cis-9, 12, 15-octadecatrienoate (linolenate), and all cis-5,8,11,14-eicosatetraenoate (arachidonate). The two fatty acid chains of a phospholipid may be identical or different. Acceptable phospholipids include dioleoyl PS, dioleoyl PC, distearoyl PS, distearoyl PC, dimyristoyl PS, dimyristoyl PC, dipalmitoyl PG, stearyl,oleoyl PS, palmitoyl,linolenyl PS, and the like.

[0067] The phospholipids may come from any natural source, and, as such, may comprise a mixture of phospholipids. For example, egg yolk is rich in PC, PG, and PE, soybeans contain PC, PE, PI, and PA, and animal brain or spinal cord is enriched in PS. Phospholipids may come from synthetic sources too. Mixtures of phospholipids having a varied ratio of individual phospholipids may be used. Mixtures of different phospholipids may result in liposome compositions having advantageous activity or stability of activity properties. The above mentioned phospholipids may be mixed, in optimal ratios with cationic lipids, such as N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethyl ammonium chloride, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate, 3,3'-deheptyloxacarbocyanine iodide, 1,1'-dedodecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate, 1,1'-dioleoyl-3,3,3',3'-tetramethylindo

carbocyanine methanesulfonate, N-4-(delinoleylaminostyryl)-N-methylpyridinium iodide, or 1,1,-dilinoylel-3,3,3',3'-tetramethylindocarbocyanine perchloarate.

[0068] Liposomes may optionally comprise sphingolipids, in which spingosine is the structural counterpart of glycerol and one of the one fatty acids of a phosphoglyceride, or cholesterol, a major component of animal cell membranes. Liposomes may optionally, contain pegylated lipids, which are lipids covalently linked to polymers of polyethylene glycol (PEG). PEGs may range in size from about 500 to about 10,000 daltons.

[0069] Liposomes may further comprise a suitable solvent. The solvent may be an organic solvent or an inorganic solvent. Suitable solvents include, but are not limited to, dimethylsulfoxide (DMSO), methylpyrrolidone, N-methylpyrrolidone, acetronitrile, alcohols, dimethylformamide, tetrahydrofuran, or combinations thereof.

[0070] Liposomes loaded with an agent may be prepared by any known method of preparing liposomes for drug delivery, such as, for example, detailed in U.S. Pat. Nos. 4,241,046, 4,394,448, 4,529,561, 4,755,388, 4,828,837, 4,925,661, 4,954,345, 4,957,735, 5,043,164, 5,064,655, 5,077,211 and 5,264,618, the disclosures of which are hereby incorporated by reference in their entirety. For example, liposomes may be prepared by sonicating lipids in an aqueous solution, solvent injection, lipid hydration, reverse evaporation, or freeze drying by repeated freezing and thawing. In a preferred embodiment the liposomes are formed by sonication. The liposomes may be multilamellar, which have many layers like an onion, or unilamellar. The liposomes may be large or small. Continued high-shear sonication tends to form smaller unilamellar liposomes.

[0071] As would be apparent to one of ordinary skill, all of the parameters that govern liposome formation may be varied. These parameters include, but are not limited to, temperature, pH, concentration of methionine compound, concentration and composition of lipid, concentration of multivalent cations, rate of mixing, presence of and concentration of solvent.

Other delivery vehicles

[0072] In another embodiment, the delivery vehicle is a microemulsion. Microemulsions are generally clear, thermodynamically stable solutions comprising an aqueous solution, a surfactant, and "oil." The "oil" in this case, is the supercritical fluid phase. The surfactant rests at the oil-water interface. Any of a variety of surfactants are suitable for use in microemulsion formulations including those described herein or otherwise known in the art. The aqueous

microdomains suitable for use in the invention generally will have characteristic structural dimensions from about 5 nm to about 100 nm. Aggregates of this size are poor scatterers of visible light and hence, these solutions are optically clear. As will be appreciated by a skilled artisan, microemulsions can and will have a multitude of different microscopic structures including sphere, rod, or disc shaped aggregates. In one embodiment, the structure may be micelles, which are the simplest microemulsion structures that are generally spherical or cylindrical objects. Micelles are like drops of oil in water, and reverse micelles are like drops of water in oil. In an alternative embodiment, the microemulsion structure is the lamellae. It comprises consecutive layers of water and oil separated by layers of surfactant. The “oil” of microemulsions optimally comprises phospholipids. Any of the phospholipids detailed above for liposomes are suitable for embodiments directed to microemulsions. The cancer therapeutic agent may be encapsulated in a microemulsion by any method generally known in the art. Methods of coating microemulsions is found, for example, in Kumari et al., *Pharm Dev Technol.* 2017 Jun;22(4):617-626.

[0073] In yet another embodiment, the delivery vehicle may be a dendritic macromolecule, or a dendrimer. Generally speaking, a dendrimer is a branched tree-like molecule, in which each branch is an interlinked chain of molecules that divides into two new branches (molecules) after a certain length. This branching continues until the branches (molecules) become so densely packed that the canopy forms a globe. Generally, the properties of dendrimers are determined by the functional groups at their surface. For example, hydrophilic end groups, such as carboxyl groups, would typically make a water-soluble dendrimer. Any method generally known in the art may be utilized to make dendrimers and to encapsulate cancer therapeutic agents therein. For example, dendrimers may be produced by an iterative sequence of reaction steps, in which each additional iteration leads to a higher order dendrimer. Consequently, they have a regular, highly branched 3D structure, with nearly uniform size and shape. Furthermore, the final size of a dendrimer is typically controlled by the number of iterative steps used during synthesis. A variety of dendrimer sizes are suitable for use in the invention. Generally, the size of dendrimers may range from about 1 nm to about 100 nm.

Cancer

[0074] The methods taught herein may be used to treat a neoplasm or a cancer. The neoplasm may be malignant or benign, the cancer may be primary or metastatic; the neoplasm

or cancer may be early stage or late stage. Non-limiting examples of neoplasms or cancers that may be treated with a composition of the invention may include acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, appendix cancer, astrocytomas (childhood cerebellar or cerebral), B-cell lymphoma; basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, brainstem glioma, brain tumors (cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic gliomas), breast cancer, bronchial adenomas/carcinoids, Burkitt lymphoma, carcinoid tumors (childhood, gastrointestinal), carcinoma of unknown primary, central nervous system lymphoma (primary), cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous T-cell lymphoma, desmoplastic small round cell tumor, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma in the Ewing family of tumors, extracranial germ cell tumor (childhood), extragonadal germ cell tumor, extrahepatic bile duct cancer, eye cancers (intraocular melanoma, retinoblastoma), gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, germ cell tumors (childhood extracranial, extragonadal, ovarian), gestational trophoblastic tumor, gliomas (adult, childhood brain stem, childhood cerebral astrocytoma, childhood visual pathway and hypothalamic), gastric carcinoid, hairy cell leukemia, head and neck cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, hypopharyngeal cancer, hypothalamic and visual pathway glioma (childhood), intraocular melanoma, islet cell carcinoma, Kaposi sarcoma, kidney cancer (renal cell cancer), laryngeal cancer, leukemias (acute lymphoblastic, acute myeloid, chronic lymphocytic, chronic myelogenous, hairy cell), lip and oral cavity cancer, liver cancer (primary), lung cancers (non-small cell, small cell), lymphomas (AIDS-related, Burkitt, cutaneous T-cell, Hodgkin, non-Hodgkin, primary central nervous system), macroglobulinemia (Waldenström), malignant fibrous histiocytoma of bone/osteosarcoma, medulloblastoma (childhood), melanoma, intraocular melanoma, Merkel cell carcinoma, mesotheliomas (adult malignant, childhood), metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia syndrome (childhood), multiple myeloma/plasma cell neoplasm, mycosis fungoides, myelodysplastic syndromes,

myelodysplastic/myeloproliferative diseases, myelogenous leukemia (chronic), myeloid leukemias (adult acute, childhood acute), multiple myeloma, myeloproliferative disorders (chronic), nasal cavity and paranasal sinus cancer, nasopharyngeal carcinoma, neuroblastoma, non-Hodgkin lymphoma, non-small cell lung cancer, oral cancer, oropharyngeal cancer, osteosarcoma/malignant fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial cancer (surface epithelial-stromal tumor), ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, pancreatic cancer (islet cell), paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal astrocytoma, pineal germinoma, pineoblastoma and supratentorial primitive neuroectodermal tumors (childhood), pituitary adenoma, plasma cell neoplasia, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell carcinoma (kidney cancer), renal pelvis and ureter transitional cell cancer, retinoblastoma, rhabdomyosarcoma (childhood), salivary gland cancer, sarcoma (Ewing family of tumors, Kaposi, soft tissue, uterine), Sezary syndrome, skin cancers (nonmelanoma, melanoma), skin carcinoma (Merkel cell), small cell lung cancer, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer with occult primary (metastatic), stomach cancer, supratentorial primitive neuroectodermal tumor (childhood), T-Cell lymphoma (cutaneous), testicular cancer, throat cancer, thymoma (childhood), thymoma and thymic carcinoma, thyroid cancer, thyroid cancer (childhood), transitional cell cancer of the renal pelvis and ureter, trophoblastic tumor (gestational), unknown primary site (adult, childhood), ureter and renal pelvis transitional cell cancer, urethral cancer, uterine cancer (endometrial), uterine sarcoma, vaginal cancer, visual pathway and hypothalamic glioma (childhood), vulvar cancer, Waldenström macroglobulinemia, and Wilms tumor (childhood).

Primary and Secondary Targeting Molecules

[0075] As taught herein, certain embodiments involve the use of an antibody as the primary targeting molecule that binds to a cancer antigen. In this sense, the antibody is a primary antibody. The primary antibody may be administered separately to a nano-peptide drug complex, or may be bound with a nano-peptide drug complex prior to administration. In certain alternative embodiments, the nano-peptide drug complex is associated with a second targeting molecule. The second targeting molecule can be an antibody binding protein, such as protein A, protein G, protein A/G, or protein L, which has non-specific binding to antibodies, such as the

primary antibody. Alternatively, the nano-peptide drug complex can include a intermediary antibody, or “secondary antibody” that is bound to the antibody binding protein on the delivery vehicle. This secondary antibody will typically be specific to the primary antibody.

[0076] In other alternative embodiments, the primary targeting molecule can be an aptamer, that targets a cancer antigen, and the secondary antibody is specific to the aptamer. As with the primary antibodies, the aptamer can be administered separate to the nano-peptide drug complex or can be bound with the nano-peptide drug complex prior to administration.

[0077] In other alternative embodiments, a secondary antibody or aptamer is bound directly to a delivery vehicle without an antibody binding protein. In this instance, the secondary antibody or aptamer will be specific to the primary targeting molecule that is administered separate to the delivery vehicle or bound with the secondary antibody or aptamer prior to administration.

In further embodiments, the secondary molecule is a bi-specific or tri-specific that includes two or more portions, wherein one portion binds to a primary targeting molecule, and another portion that binds to an antigen such as an antigen on an immune cell.

[0078] Antibodies used in accord with the teachings herein can be monoclonal or polyclonal and can be prepared by techniques that are well known in the art such as, for example, immunization of a host and collection of sera or hybrid cell line technology, or recombinant technology. The term includes monoclonal antibodies, polyclonal antibodies, anti-idiotypic antibodies, synthetic antibodies, multispecific antibodies (e.g., bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired binding activity. The antibodies can be chimeric antibodies, including humanized antibodies as described in Jones et al., *Nature* 321:522-525, 1986, Riechmann et al., *Nature* 332:323-329, 1988, Presta, *Curr. Opin. Struct. Biol.* 2:593-596, 1992, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115, 1998, Harris, *Biochem. Soc. Transactions* 23:1035-1038, 1995, and Hurle and Gross, *Curr. Opin. Biotech.* 5:428-433, 1994. Antibodies of any class or isotype (e.g., IgA, IgA1, IgA2, IgD, IgE, IgG, IgG1, IgG2, IgG3, IgG4, and IgM) can be used.

[0079] As noted above, the term “antibody” also includes any antibody fragment(s) that retain a functional antigen binding region. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments, all of which are known in the art. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab"

fragments, each with a single antigen-binding site. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-combining sites and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. F(ab')₂ antibody fragments are pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known. "Fv" is the minimum antibody fragment which contains a complete antigen-binding site. Diabodies are described more fully in, for example, European Patent No. 404,097, International Patent Application WO 1993/01161, Hudson et al., Nat. Med. 9:129-134, 2003, and Hollinger et al., PNAS USA 90: 6444-6448, 1993. Triabodies and tetrabodies also are described in Hudson et al., Nat. Med. 9:129-134, 2003.

[0080] Examples of known antibodies that can be used as primary targeting molecules include the following:

anti-estrogen receptor antibody, anti-progesterone receptor antibody, anti-p53 antibody, anti-HER-2/neu antibody, anti-EGFR antibody, anti-cathepsin D antibody, anti-Bcl-2 antibody, anti-E-cadherin antibody, anti-CA125 antibody, anti-CA15-3 antibody, anti-CA19-9 antibody, anti-c-erbB-2 antibody, anti-P-glycoprotein antibody, anti-CEA antibody, anti-retinoblastoma protein antibody, anti-ras oncoprotein antibody, anti-Lewis X antibody, anti-Ki-67 antibody, anti-PCNA antibody, anti-CD3 antibody, anti-CD4 antibody, anti-CD5 antibody, anti-CD7 antibody, anti-CD8 antibody, anti-CD9/p24 antibody, anti-CD10 antibody, anti-CD11c antibody, anti-CD13 antibody, anti-CD14 antibody, anti-CD15 antibody, anti-CD19 antibody, anti-CD20 antibody, anti-CD22 antibody, anti-CD23 antibody, anti-CD30 antibody, anti-CD31 antibody, anti-CD33 antibody, anti-CD34 antibody, anti-CD35 antibody, anti-CD38 antibody, anti-CD41 antibody, anti-LCA/CD45 antibody, anti-CD45RO antibody, anti-CD45RA antibody, anti-CD39 antibody, anti-CD44 antibody, anti-CD51 antibody, anti-CD71 antibody, anti-CD100 antibody, anti-CD117 antibody, anti-CD95/Fas antibody, anti-CD99 antibody, anti-CD106 antibody, anti-CD71 antibody, anti-c-myc antibody, anti-cytokeratins antibody, anti-vimentins antibody, anti-melanosomes antibody, and anti-prostate specific antigen antibody.

[0081] The primary targeting molecule may also be in the form of an aptamer designed for targeting a cancer antigen. In certain embodiments, the primary targeting molecule is an antibody or aptamer specific to a cancer cell or cancer antigen that has been engineered to include digoxin. Digoxin can be associated (or conjugated) with an primary targeting molecule

through a number of techniques including chemical manipulation to generate a covalent bond between the antibody or aptamer, typically at the Fc domain, and digoxin. Then a secondary targeting molecule used has specific binding to digoxin. Digoxin is a FDA approved drug for cardiology. Digoxin toxicity is treated by high affinity antibodies for digoxin. A few drugs in the form of the Fab fragment have been approved, including digibind. Digoxin has been conjugated to a number of proteins, including alkaline phosphatase, horse radish peroxidase (HRP), glucose oxidase, albumin. In these cases, digoxin does not interfere with the function of its binding partner. High affinity aptamer for digoxin has been developed, with the affinity (K_d) at 0.05 nM (Aptagen.com). In a specific example, the primary targeting molecule is a cancer-specific antibody, e.g. rituximab, with Digoxin (Dig) conjugated. And the NPD is a cancer agent-loaded delivery vehicle (loaded with vincristine or doxorubicin, for example), with digoxin-specific Fab or aptamer (Apt) attached.

Antibody-Binding Molecules

[0082] The antibody-binding molecule for coating an agent-loaded delivery vehicle includes protein A, protein G, protein A/G, protein L, and any combination thereof. These proteins are known to bind with high affinity to human and mouse antibodies. The antibody-binding protein can be synthesized or commercially available antibody-binding protein can be implemented. In a specific embodiment, the antibody-binding protein is a synthesized protein A/G. In an alternative embodiment, the antibody-binding molecule is not a protein but an antibody binding nucleotide which can bind selectively to the Fc-domain of antibodies. However, reference to antibody-binding protein as used herein is construed to include such antibody binding nucleotides, unless specified otherwise.

Cancer Antigens

[0083] A cancer antigen is an antigen that is expressed preferentially by cancer cells (i.e., it is expressed at higher levels in cancer cells than on non-cancer cells) and in some instances it is expressed solely by cancer cells. The cancer antigen may be expressed within a cancer cell or on the surface of the cancer cell. Examples of cancer antigen include but are not limited to MART-1/Melan-A, gp100, adenosine deaminase-binding protein (ADAbp), FAP, cyclophilin b, colorectal associated antigen (CRC)—C017-1A/GA733, carcinoembryonic antigen (CEA), CAP-1, CAP-2, etv6, AML1, prostate specific antigen (PSA), PSA-1, PSA-2, PSA-3, prostate-specific membrane antigen (PSMA), T cell receptor/CD3-zeta chain, and CD20. Examples of other

cancer antigens include but are not limited to MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1, MAGE-C2, MAGE-C3, MAGE-C4, MAGE-05). Examples of other the cancer antigens include but are not limited to GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9. Examples of other cancer antigens include but are not limited to BAGE, RAGE, LAGE-1, NAG, GnT-V, MUM-1, CDK4, tyrosinase, p53, MUC family, HER2/neu, p21ras, RCAS1, α -fetoprotein, E-cadherin, α -catenin, β -catenin, γ -catenin, p120ctn, gp100^{Pmel117}, PRAME, NY-ESO-1, cdc27, adenomatous polyposis coli protein (APC), fodrin, Connexin 37, Ig-idiotype, p15, gp75, GM2 ganglioside, GD2 ganglioside, human papilloma virus proteins, Smad family of tumor antigens, Imp-1, PIA, EBV-encoded nuclear antigen (EBNA)-1, brain glycogen phosphorylase, SSX-1, SSX-2 (HOM-MEL-40), SSX-1, SSX-4, SSX-5, SCP-1 and CT-7, and c-erbB-2. Examples of other cancer antigens include but are not limited to CD20, CD52, CD33, CD22, CD38, CD40, mucin, P21, and MPG.

Cancer Therapeutic Agents

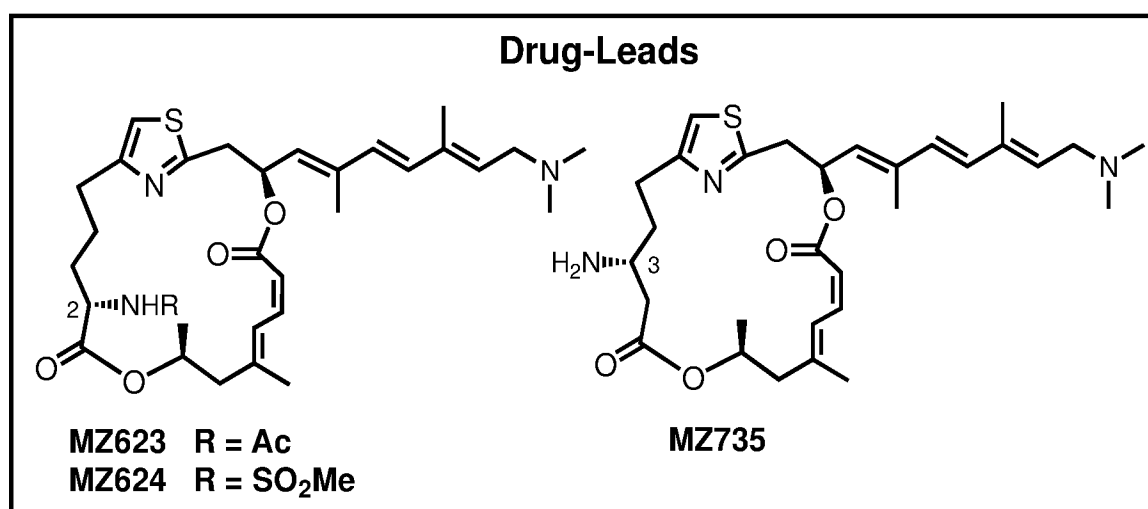
[0084] Cancer therapeutic agents (also referred herein as “Cancer drugs”) that can be loaded into a delivery vehicle and used in accord with the teachings herein include including radioisotopes, cytostatic and chemotherapeutic agents. Radioisotopes that may be utilized with the present invention include for example, ²¹¹At, ¹³¹I, ¹²⁵I, ⁹⁰Y, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁵³Sm, ²¹²Bi, ³²P and ⁶⁰C, Examples of chemotherapeutic agents include for example, Erlotinib (TARCEVA™, Genentech, Oceanside, Calif.), Bortezomib (VELCADE™, Millenium Pharmaceuticals, Cambridge, Mass.), Fulvestrant (FASLODEX™, AstraZeneca, Wilmington, Del.), Sutent (SU11248, Pfizer, New York, N.Y.), Letrozole (FEMARA™, Novartis, Basal Switzerland), Imatinib mesylate (GLEEVEC™, Novartis, Basal Switzerland), PTK787/ZK 222584 (Novartis, Basal Switzerland), Oxaliplatin (Eloxatin™, Sanofi, Bridgewater, N.J.), Leucovorin, Rapamycin (Sirolimus, RAPAMUNE™, Pfizer New York, N.Y.), Lapatinib (GSK572016, GlaxoSmithKline, Philadelphia, Pa.), Lonaferinib (SCH 66336), Sorafenib (BAY43-9006, Bayer Labs. Liversen, Germany), and Gefitinib (IRESSA™, AstraZeneca, Wilmington, Del.), AG1478, AG1571 (SU 5271; Sugen), alkylating agents such as thiotepa and CYTOXAN™ cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines

including altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate and trimethylmelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB 1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammall and calicheamicin omegall (Angew Chem Intl. Ed. Engl. (1994) 33:183-186); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, anthramycin, azaserine, bleomycins, cactinomycin, carabacin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN™ doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitioestanol, mepitioestane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins;

mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSKTM polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., TAXOLTM paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANETM Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumburg, Ill.), and TAXOTERETM doxorubicin (Rhone-Poulenc Rorer, Antony, France); chloranbucil; GEMZARTM gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINETM vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); and retinoids such as retinoic acid; capecitabine.

[0085] In addition, cancer therapeutic agents include those agents described and claimed in WO 2017/079558, which is incorporated herein by reference. These include CK-1 inhibitors, proteasome inhibitors, PI3 inhibitors, or dual CK-1/PI3 inhibitors.

[0086] Moreover, certain investigational drugs can be loaded into the NPDs as described herein including the following:



Effective Amounts, Regimens, Formulations

[0087] The primary targeting molecule and/or nano-peptide drug complex are administered in effective amounts. An effective amount is a dosage of the agent sufficient to provide a medically desirable result. The effective amount will vary with the particular condition being treated, the age and physical condition of the subject being treated, the severity of the condition, the duration of the treatment, the nature of the concurrent or combination therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. It is preferred generally that a maximum dose be used, that is, the highest safe dose according to sound medical judgment.

[0088] For example, if the subject has a tumor, an effective amount may be that amount that reduces the tumor volume or load (as for example determined by imaging the tumor). Effective amounts may also be assessed by the presence and/or frequency of cancer cells in the blood or other body fluid or tissue (e.g., a biopsy). If the tumor is impacting the normal functioning of a tissue or organ, then the effective amount may be assessed by measuring the normal functioning of the tissue or organ.

[0089] The primary targeting molecules, secondary targeting molecules, NPDs, CAR cells and/or agent-loaded delivery vehicles may be provided as pharmaceutical compositions.

Pharmaceutical compositions are sterile compositions that typically include a pharmaceutically-acceptable carrier. The term “pharmaceutically-acceptable carrier” means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration to a human or other subject contemplated by the invention. The term “carrier” denotes an organic or inorganic ingredient, natural or synthetic, with which the cells, nanoparticles and agent(s) are combined to facilitate administration. The components of the pharmaceutical compositions are commingled in a manner that precludes interaction that would substantially impair their desired pharmaceutical efficiency.

[0090] The primary targeting molecules, secondary targeting molecules, CAR cells, NPDs and/or agent-loaded delivery vehicles may be administered by one of multiple modes of administration. Modes of administering include, but are not limited to oral administration, parenteral administration such as intravenous, subcutaneous, intramuscular or intraperitoneal injections, rectal administration by way of suppositories, transdermal administration, intraocular administration or administration by any route or method that delivers a therapeutically effective

amount of the drug or composition to the cells or tissue to which it is targeted. Alternatively, routine experimentation will determine other acceptable routes of administration. When it is desirable to deliver them systemically, may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers. Pharmaceutical parenteral formulations include aqueous solutions of the ingredients. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Alternatively, suspensions of ingredients may be prepared as oil-based suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides.

Immunotherapeutic Methods

[0091] Immunotherapy using bi- or tri-specific therapeutic antibodies and Chimeric Antigen Receptor- (CAR-) T cells represent the latest breakthroughs in cancer treatment, and drugs using these technologies have been approved by the Food and Drug Administration (FDA). However, the current products are excessively expensive, and surprisingly, lacking adequate specificity for cancer cells. As a result, bi- or tri-specific therapeutic antibodies and CAR-T cells generate many severe adverse events (SAEs). Collectively, these limitations severely restrict the availability of latest immunotherapy for patients who suffer from various cancers.

[0092] According to further embodiments as illustrated in FIGs 11 and 12, disclosed is the use of interchangeable modular antibodies (imABs) that overcome the limitations of current bi- or tri-specific therapeutic antibodies and CAR-T cells described above.

[0093] FIG. 11 shows embodiment 105 that does not necessarily involve the implementation of an NPD but rather is able to facilitate an immune response by the targeting of a cancer cell with endogenous immune cell (e.g. CD3+ T-Cell). Examples of cancer cells include CD20+ B-cell lymphoma or Her2+ breast cancer cells. According to a first step 102 of this embodiment, a digoxin associated antibody 107 (primary targeting molecule) is administered which binds to a target antigen 108 on a cancer cell 106 (e.g. CD20+ on a lymphoma cell, as shown). The antibody 107 together with digoxin acts as a “handle” molecule that binds with high affinity and specificity to the target antigen. In a second step 103, an immunoglobulin 109 (second targeting molecule) is administered. As shown, the immunoglobulin 109 is bispecific in that it includes a first portion 112 that recognizes digoxin and a second portion 114 that recognizes an antigen of

the immune cell. The first and second portions 112, 114 can be joined using standard methods known in the art.

[0094] In the Example shown in FIG. 11, the second portion 114 recognizes CD3 115 on the surface of CD3+ T-cell 113, however as explained below, the immunoglobulin 109 could be engineered to recognize any of a number of different antigens. The first portion 112 binds to digoxin on the antibody 107 bound to the cancer cell. The second portion 114 binds to CD3 (115) on the CD3+ T-cell 113, which causes the T-Cell to recognize the cancer cell, thereby generating a T-cell mediated immune response by the host against the cancer cell 106. It is noted that the immunoglobulin 109 could be engineered such that the second portion 114 includes a portion that binds to one antigen and another portion that binds to a different antigen, thereby providing a tri-specific recognition.

[0095] FIG. 12 shows another embodiment 120 that involves implementation of CAR T-cells. This embodiment involves engineering a CAR T-cell 129 to express two CAR molecules, such as immunoglobulin chains 132 and 134. The first chain 132 recognizes digoxin. The second chain 134 recognizes a surface antigen of a cancer cell 128 (e.g. CD19+ CD20+ lymphoma cell, as shown). In a first step 121, the digoxin associated antibody 107 is administered and binds to surface antigen (CD20, 129) on the cancer cell 128. The CAR T-Cell 129 is administered, wherein the first chain 132 binds to digoxin on the digoxin associated antibody 107 and the second chain binds to a different surface antigen (CD19, 130) on the cancer cell 128. The binding of the first and second chains 132,134 to their respective recognized antigens on the cancer cell 128 generates an immune response against the cancer cell 128. The second chain 134 could include a portion that binds to one antigen and another portion that binds to a different antigen. The two different portions could bind to two different antigens on the cancer cell, which would increase the immune response efficiency.

[0096] Because the immunoglobulin 109 can be used in any cancer, the cost of the therapeutic antibody is expected to be substantially lower than the current bi- or tri-specific therapeutic antibodies. Because antibody 107 can be made using standard chemical conjugation of digoxin to a large repertoire of antibodies that are already approved or safely tested in humans with different cancer, the cost of making antibody 107 is expected to be low. Because a large series of dig-conjugated antibodies can be readily produced, this technology will bring antibody based immunotherapy to many cancers. Other handles in addition to digoxin can be introduced to

further increase the safety, efficacy, and broad applicability of this technology. In contrast, Blinatumomab and Catumaxomab are the only approved bi- or tri-specific therapeutic antibodies, for use in acute lymphoblastic leukemia and malignant ascites, respectively. The impact of the new technology described herein is expected to be very high.

[0097] The CAR cell described above and illustrated in FIG. 12 may be engineered according to techniques known in the art. See for example, U.S. Patent Pub No. US20170137783; US20180161369; and 20170137515 whose teachings are incorporated herein. Engineering the Car cell includes introducing one or more CAR molecules (e.g., nucleic acids encoding one or more CAR molecules, e.g. the two immunoglobulin chains 132 and 134) into an immune cell population, under conditions suitable for of the CAR molecule thereby producing a CAR-expressing cell population.

[0098] In certain embodiments, the CAR molecule comprises an antigen binding domain (e.g., an antigen binding domain of an antibody molecule). The method includes contacting the first or transient CAR-expressing cell population with a ligand of the CAR molecule, e.g., a ligand of the CAR antigen binding domain (e.g., a cognate antigen molecule (e.g., a recombinant antigen) or an anti-idiotypic antibody molecule), under conditions such that immune cell expansion and/or activation occurs, thereby producing an “expanded and/or activated immune cell population.” In embodiments, the ligand of the CAR molecule is present in/on (e.g., immobilized or attached to) a substrate, e.g., a non-naturally occurring substrate. The method can further include culturing the population of immune cells in the presence of the ligand of the CAR molecule.

[0099] In one embodiment, the nucleic acid encoding the one or more CAR molecules (e.g., the immunoglobulin chains) is an RNA molecule, e.g., an in vitro transcribed (IVT) RNA. In one embodiment, a CAR encoding RNA construct as described herein is introduced into the immune cell population by transfection or electroporation. In a specific embodiment, the CAR molecule is expressed transiently (e.g., the CAR molecule does not, or does not substantially, integrate into the cellular genome). In other embodiments, the nucleic acids encoding the one or more CAR molecules is a DNA vector or an RNA vector. In one embodiment, the vector is selected from the group consisting of a DNA, an RNA, a plasmid, a lentivirus vector, adenoviral vector, or a retrovirus vector. In one embodiment, the vector is a lentivirus. In one embodiment, the nucleic acids are stably integrated into the cellular genome.

In embodiments, the encoded CAR molecule is as described herein, e.g., a tumor antigen-binding CAR (e.g., CD19 CAR) as described herein.

[0100] In another embodiment, the ligand of the one or more CAR molecules is a cancer associated antigen, e.g., a cancer associated antigen recognized by a CAR molecule as described herein, e.g., a CD19 CAR.)

[0101] In some embodiments, the population of immune cells used in the methods described herein is acquired, e.g., obtained, from a blood sample from a subject (e.g., a cancer patient). In one embodiment, the population of immune cells is obtained by apheresis.

In some embodiments, the immune cell population includes immune effector cells, e.g., as described herein. Exemplary immune effector cells include T cells, e.g., alpha/beta T cells and gamma/delta T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, myeloid-derived phagocytes, or a combination thereof.

[0102] In certain embodiments, the immune cell population includes primary T cells or subsets of lymphocytes, including, for example, anergized T cells, naïve T cells, T-regulatory cells, Th-17 cells, stem T cells, or a combination thereof.

[0103] In some embodiments, the immune cell population includes peripheral blood mononucleated cells (PBMCs), or cord blood cells, or a combination thereof.

In certain embodiments, the methods disclosed herein may involve contacting the expanded and/or activated immune cell population with two different nucleic acids encoding a first CAR molecule (e.g. chain recognizing digoxin) and the second CAR molecule (e.g. chain recognizing cancer cell antigen), thereby generating a CAR cell that expresses both the first and second CAR molecules for use in the method described in FIG. 12. As described above, the nucleic acid encoding the second CAR molecule may be selected from the group consisting of a DNA, an RNA, a plasmid, a lentivirus vector, adenoviral vector, or a retrovirus vector. In other embodiments, the nucleic acid encoding a second CAR molecule is an IVT RNA.

In some embodiment, the first and second CAR molecules are directed to the same antigen, e.g., the same tumor cell antigen. In one embodiment, the first and second CAR molecules are the same CAR molecule. In such embodiments, the immune cell population expressing the first CAR is expanded and/or activated in vitro or ex vivo, e.g., by contacting said immune cell population with the tumor cell antigen or an anti-idiotypic antibody against the CAR binding antibody molecule (e.g., a CD19-antigen or anti-CD19 idiotype antibody immobilized onto a

non-cellular or cellular substrate as described herein). Alternatively, or in combination, the immune cell population expressing (e.g., stably expressing) the second CAR is expanded and/or activated in vivo, e.g. by contacting an endogenous tumor cell antigen (e.g., CD19). In one embodiment, the second CAR-expressing immune cell is administered to a subject, e.g., as part of a therapeutic protocol.

[0104] In certain embodiments, the methods further comprise storing the expanded and/or activated immune cell population after the appropriate expansion period. In one embodiment, the expanded and/or activated immune cell population is cryopreserved according to a method described herein. In one embodiment, the expanded and/or activated immune cell population is cryopreserved in an appropriate media, e.g., an infusible media, e.g., as described herein. In some embodiments, production of the CAR cells includes acquiring (e.g., obtaining) the expanded and/or activated immune cell population using one or more of the methods described herein. For example, the expanded and/or activated immune cell population may have been previously obtained by introducing one or more CAR molecules (e.g., one or more nucleic acid molecules encoding the first CAR molecule and a second CAR molecule) under conditions suitable for expression of the one or more CAR molecules; and optionally contacting said CAR-expressing cell population with a ligand of the one or more CAR molecules, e.g., a ligand of the CAR antigen binding domain (e.g., a cognate antigen molecule (e.g., a recombinant antigen) or an anti-idiotypic antibody molecule), under conditions such that immune cell expansion and/or activation occurs. In embodiments, the ligand of the one or more CAR molecules is present in/on (e.g., immobilized or attached to) a substrate, e.g., a non-naturally occurring substrate, as described herein. The expanded and/or activated immune cell population can be stored under suitable conditions, e.g., cryopreservation, as described herein.

CAR Molecules

[0105] In accordance with the methods, preparations, and reaction mixtures described herein, an immune effector cell, e.g., obtained by a method described herein, can be engineered to contain one or more CAR molecules (also referred to herein as “CARs”) that targets one or more cancer associated antigens. In some embodiments, the cancer associated antigen is a tumor antigen described in International Application WO2015/142675, filed Mar. 13, 2015, which is herein incorporated by reference in its entirety.

[0106] In some embodiments, the cancer associated antigen (tumor antigen) which the second CAR molecule (e.g. immunoglobulin chain) recognizes may be one or more of: CD19; CD123; CD22; CD30; CD171; CS-1 (also referred to as CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRvIII); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlcp(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAca-Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms-Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDGalp(1-4)bDGlcp(1-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); Folate receptor beta; tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein-coupled receptor class C group 5, member D (GPCR5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3

(ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostein; surviving; telomerase; prostate carcinoma tumor antigen-1 (PCTA-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B1 (CYP1B1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1).

In some embodiments, the antigen binding domain of the CAR molecule comprises an antibody, an antibody fragment, an scFv, a Fv, a Fab, a (Fab')₂, a single domain antibody (SDAB), a VH or VL domain, or a camelid VHH domain.

Subjects Related to Immunotherapy

[0107] In one embodiment, the subject, e.g., the subject from which immune cells are acquired and/or the subject treated, is a human, e.g., a cancer patient.

In certain embodiments, the subject has a disease associated with expression of a tumor- or cancer associated-antigen, e.g., a disease as described herein. In one embodiment, the subject has a cancer, e.g., a cancer as described herein.

[0108] In one embodiment, the subject has a cancer that is chosen from a hematological cancer, a solid tumor, or a metastatic lesion thereof. Exemplary cancers include, but are not limited to, B-cell acute lymphocytic leukemia (B-ALL), T-cell acute lymphocytic leukemia (T-ALL), acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), B cell promyelocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma (MCL), marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma (HL), plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, and Waldenstrom macroglobulinemia. In one embodiment, the cancer is ALL. In another embodiment, the cancer is CLL.

[0109] In embodiments, the subject does not have a relapsed cancer. In other embodiments, the subject has a relapsed cancer.

[0110] In one embodiment, the immune cell (e.g., the population of immune effector cells) is acquired, e.g., obtained, from a subject having a hematological cancer, e.g., a leukemia, e.g., CLL, ALL, or a lymphoma, e.g., MCL, NHL, or HL.

[0111] As will be apparent to one of ordinary skill in the art from a reading of this disclosure, further embodiments of the present invention can be presented in forms other than those specifically disclosed above. The particular embodiments described above are, therefore, to be considered as illustrative and not restrictive. Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific

embodiments described herein. Such equivalents are considered to be within the scope of this invention. Although the invention has been described and illustrated in the foregoing illustrative embodiments, it is understood that the present disclosure has been made only by way of example, and that numerous changes in the details of implementation of the invention can be made without departing from the spirit and scope of the invention, which is limited only by the claims that follow. Features of the disclosed embodiments can be combined and rearranged in various ways within the scope and spirit of the invention. The scope of the invention is as set forth in the appended claims and equivalents thereof, rather than being limited to the examples contained in the foregoing description. The teachings of the cited references are incorporated herein in their entirety to the extent not inconsistent with the teachings herein.

CLAIMS

What is claimed is:

1. A method of treating cancer in a subject in need comprising administering an amount of a primary targeting molecule that targets cancer cells and co-administering an amount of agent-loaded delivery vehicles, wherein the agent-loaded delivery vehicles are coated with a secondary targeting molecule that targets the primary targeting molecule.
2. The method of claim 1, wherein the primary targeting molecule is an antibody or aptamer.
3. The method of any of claims 1-2, wherein the primary targeting molecule targets a cancer antigen.
4. The method of any of claims 1-3, wherein the secondary targeting molecule is an antibody-binding protein.
5. The method of any of claims 1-4, wherein the antibody-binding protein comprises protein A, protein A/G, protein G, protein L, or any combination thereof.
6. The method of any of claims 1-3, wherein the secondary targeting molecule comprises an aptamer or an antibody.
7. The method of any of claims 1-6, wherein the delivery vehicles comprise nanoparticles.
8. The method of claim 7, wherein the nanoparticles are mesoporous silica nanoparticles.
9. The method of any of claims 1-6, wherein the delivery vehicle comprises liposomes.
10. The method of claim 1, wherein the primary targeting molecule is administered prior to the agent-loaded delivery vehicles.
11. The method of claim 1, wherein the primary targeting molecule is bound to the secondary targeting molecule prior to administration.
12. The method of any of claims 1-10, wherein the agent-loaded delivery vehicles are loaded with cancer therapeutic agent.
13. The method of any of claims 1-12, wherein the delivery vehicle comprises an antibody-binding protein bound to a secondary antibody that binds to the primary targeting molecule.
14. The method of claim 13, wherein the primary targeting molecule is bound to the secondary antibody prior to administration.
15. A nano-peptide drug complex (NPD) comprising an agent-loaded delivery vehicle loaded with a cancer therapeutic agent and coated with a secondary targeting molecule.

16. The NPD of claim 15, wherein the agent-loaded delivery vehicle is a nanoparticle or liposome.
17. The NPD of claim 16, wherein the agent-loaded delivery vehicle is a mesoporous silica nanoparticle.
18. The NPD of any of claims 15-17, wherein the secondary targeting molecule is bound to a primary targeting molecule that binds to a cancer antigen.
19. The NPD of any of claims 15-18, wherein the secondary targeting molecule is an antibody-binding protein.
20. The NPD of claim 19, wherein the antibody-binding protein comprises protein A, protein A/G, protein G, protein L, or any combination thereof.
21. The NPD of any of claims 19-20, wherein the antibody-binding protein is bound to a secondary antibody that binds to a primary targeting molecule.
22. The NPD of claim 21, further comprising a primary targeting molecule bound to the secondary antibody.
23. The NPD of any of claims 15-22, wherein the agent-loaded delivery vehicle is a nanoparticle or liposome.
24. A method of making an NPD comprising loading a delivery vehicle with a cancer therapeutic agent; and coating the delivery vehicle with a secondary targeting molecule that binds to a primary targeting molecule, wherein said loading and coating steps may occur in either order, or concurrently.
25. The method of claim 24, further comprising incubating the delivery vehicle, *in vitro*, with a primary targeting molecule following said coating step such that said primary targeting molecule binds to said secondary targeting molecule.
26. The method of any of claims 24-25, wherein said primary targeting molecule targets a cancer antigen.
27. A method of making an NPD comprising loading a delivery vehicle with a cancer therapeutic agent; and coating the delivery vehicle with an antibody-binding protein, wherein said loading and coating steps may occur in either order, or concurrently.
28. The method of claim 27, further comprising incubating the delivery vehicle with a secondary antibody that binds to a primary targeting molecule.

29. The method of claim 28, further comprising incubating the delivery vehicle, *in vitro*, with a primary targeting molecule such that said primary targeting molecule binds to said secondary antibody.
30. An NPD made by the method of any of claims 23-29.
31. A composition comprising an NPD of any of claims 15-23 and 30, further comprising a pharmaceutically acceptable carrier.
32. The NPD of any of claims 15-23 and 30 wherein the secondary targeting molecule is antibody specific to digoxin.
33. The method of claim 6, wherein the secondary targeting molecule is an antibody or aptamer that is indirectly bound to the agent-loaded delivery vehicle via an antibody binding protein.
34. The method of any of claims 1-3, wherein the primary targeting molecule is a cancer antigen specific antibody with digoxin bound thereto and the secondary targeting molecule is an antibody or aptamer specific to digoxin.
35. A method of treating cancer in a subject in need comprising administering an amount of a primary targeting molecule that targets a cancer cell and co-administering a secondary targeting molecule that comprises a first portion that binds to the primary targeting molecule and a second portion that binds an antigen associated with an immune cell in the subject.
36. The method of claim 35, wherein the primary targeting molecule is an antibody or aptamer.
37. The method of any of claims 35-36, wherein the primary targeting molecule targets a cancer antigen.
38. The method of any of claims 35-37, wherein the immune cell is a CD3+ T-cell.
39. The method of any of claims 35-38, wherein the antigen on the immune cell is CD3.
40. The method of any of claims 35-39, wherein the primary targeting molecule binds to a cancer associated antigen on the surface of cancer cell.
41. The method of any of claim 40, wherein the cancer antigen comprises one or more antigens selected from the group consisting of CD19; CD123; CD22; CD30; CD171; CS-1 (also referred to as CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRvIII);

ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAc-Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms-Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); Folate receptor beta; tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein-coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p

(ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostatein; surviving; telomerase; prostate carcinoma tumor antigen-1 (PCTA-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B1 (CYP1B1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1).

42. The method of any of claims 40 or 41, wherein the one or more cancer associated antigens comprises CD19, CD20, or Her2, or combination thereof.

43. The method of any of claims 35-42, wherein the secondary targeting molecule comprises a bis-specific or tri-specific antibody.

44. The method of any of claims 35-43, wherein the primary targeting molecule comprises an antibody conjugated to digoxin and wherein the first portion of the secondary targeting molecule binds to digoxin.
45. A system comprising a primary targeting molecule that targets a cancer cell and a secondary targeting molecule that comprises a first portion that binds to the primary targeting molecule and a second portion that binds an antigen associated with an immune cell in the subject.
46. The system of claim 45, wherein the primary targeting molecule comprises an antibody conjugated to digoxin.
47. The system of claim 45 or 46, wherein the antigen associated with an immune cell is CD3.
48. The system of any of claims 45-47, wherein the secondary targeting molecule is an antibody and wherein the first portion recognizes digoxin and second portion recognizes CD3.
49. A kit containing a container that holds a primary targeting molecule that targets a cancer cell and a second container that holds a secondary targeting molecule that comprises a first portion that binds to the primary targeting molecule and a second portion that binds an antigen associated with an immune cell in the subject.
50. A method of treating cancer in a subject in need comprising administering an amount of a primary targeting molecule that targets a cancer cell and co-administering a CAR cell engineered to express one or more CAR molecules, wherein the one or more CAR molecules comprise a first CAR molecule that targets the primary targeting molecule and a second CAR molecule that targets a cancer associated antigen.
51. The method of claim 50, wherein the primary targeting molecule targets a first cancer associated antigen and the second CAR molecule targets a second cancer associated antigen.
52. The method of claim 51, wherein the first cancer associated antigen and second cancer associated antigen are the same or different antigens.
53. The method of any of claims 50-52, wherein the primary targeting molecule is an antibody conjugated to digoxin and wherein the first CAR molecule targets to digoxin.

54. The method of any of claims 51-53, wherein the first or second cancer associated antigens is one or more antigens selected from the group consisting of CD19; CD123; CD22; CD30; CD171; CS-1 (also referred to as CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRvIII); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAc-Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms-Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); Folate receptor beta; tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein-coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor

20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostatein; surviving; telomerase; prostate carcinoma tumor antigen-1 (PCTA-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B1 (CYP1B1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1).

55. The method of any of claims 51-54, wherein the first or second cancer associated antigens comprises CD19, CD20, or Her2, or combination thereof.

56. A system comprising a primary targeting molecule that targets a cancer cell and a CAR cell engineered to express one or more CAR molecules, wherein the one or more CAR molecules comprise a first CAR molecule that targets the primary targeting molecule and a second CAR molecule that targets a cancer associated antigen.

57. The system of claim 56, wherein the primary targeting molecule comprises an antibody conjugated to digoxin.

58. The system of claim 57, wherein the primary targeting molecule targets a first cancer associated antigen and the second CAR molecule targets a second cancer associated antigen.

59. The system of any of claims 56-58, wherein the primary targeting molecule comprises an antibody conjugated to digoxin.

60. The method of claim 58 or 59, wherein the first cancer associated antigen and second cancer associated antigen are the same or different antigens.

61. The method of any of claims 58-60, wherein the first or second cancer associated antigens is one or more antigens selected from the group consisting of CD19; CD123; CD22; CD30; CD171; CS-1 (also referred to as CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRvIII); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAc-Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms-Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast

activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); Folate receptor beta; tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein-coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostatein; surviving; telomerase; prostate carcinoma tumor antigen-1 (PCTA-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B1 (CYP1B1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5

(PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1).

62. The method of any of claims 58-61, wherein the first or second cancer associated antigens comprises CD19, CD20, or Her2, or combination thereof.

63. A kit comprising a container that contains a primary targeting molecule that targets a cancer cell and another container that contains a CAR cell engineered to express one or more CAR molecules, wherein the one or more CAR molecules comprise a first CAR molecule that targets the primary targeting molecule and a second CAR molecule that targets a cancer associated antigen.

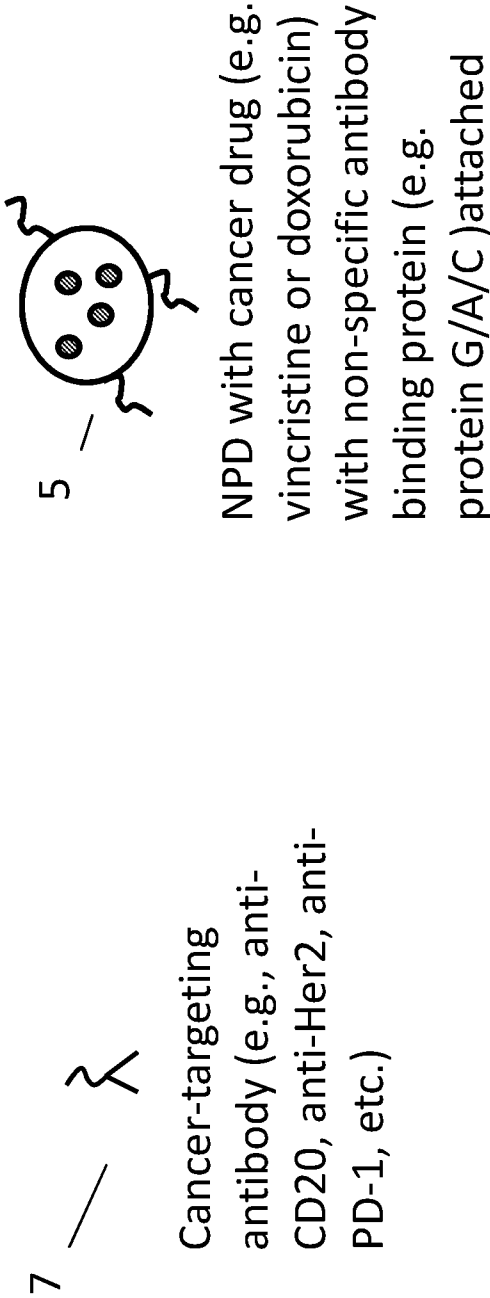


FIG. 1

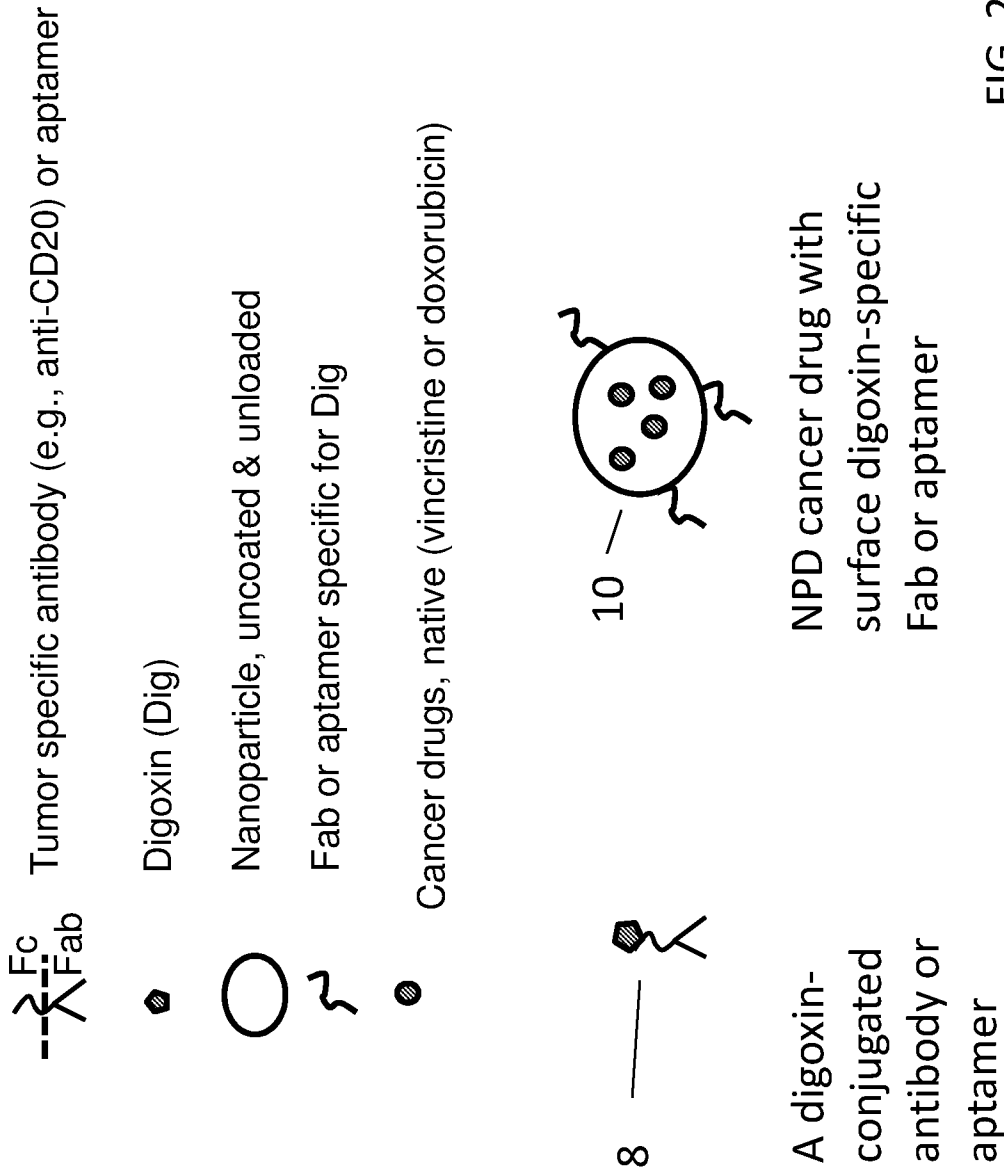


FIG. 2

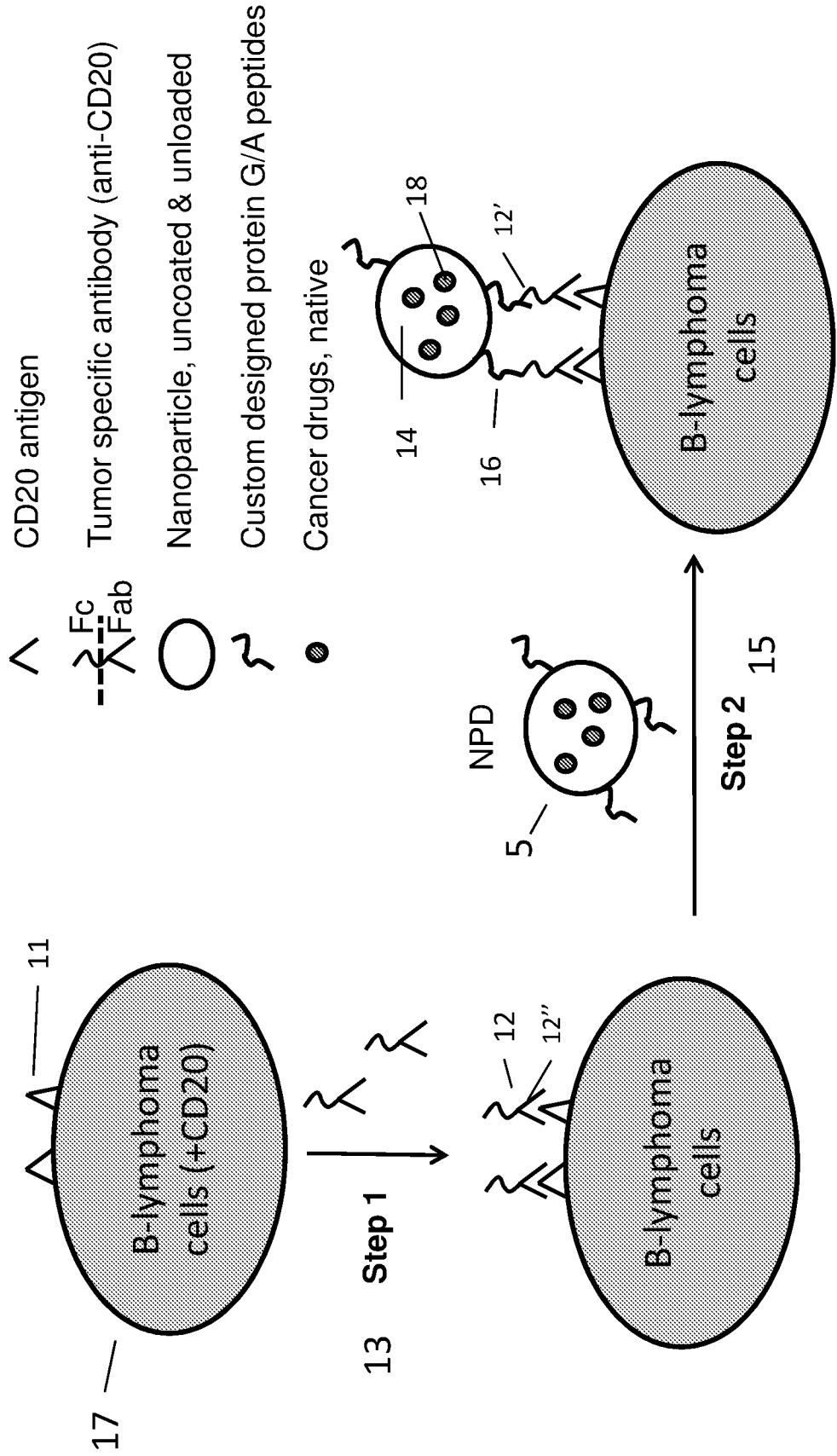


FIG. 3

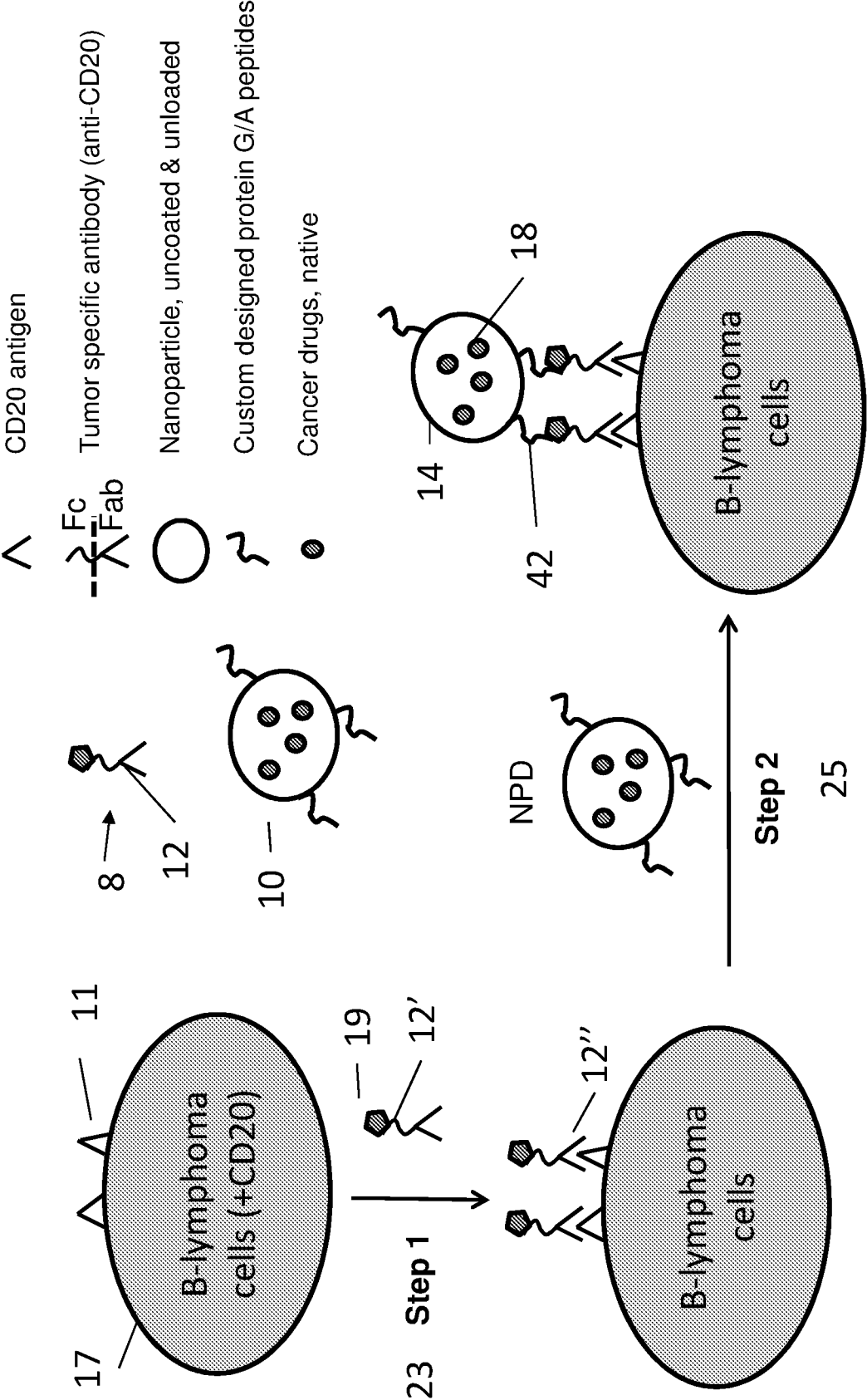


FIG. 4

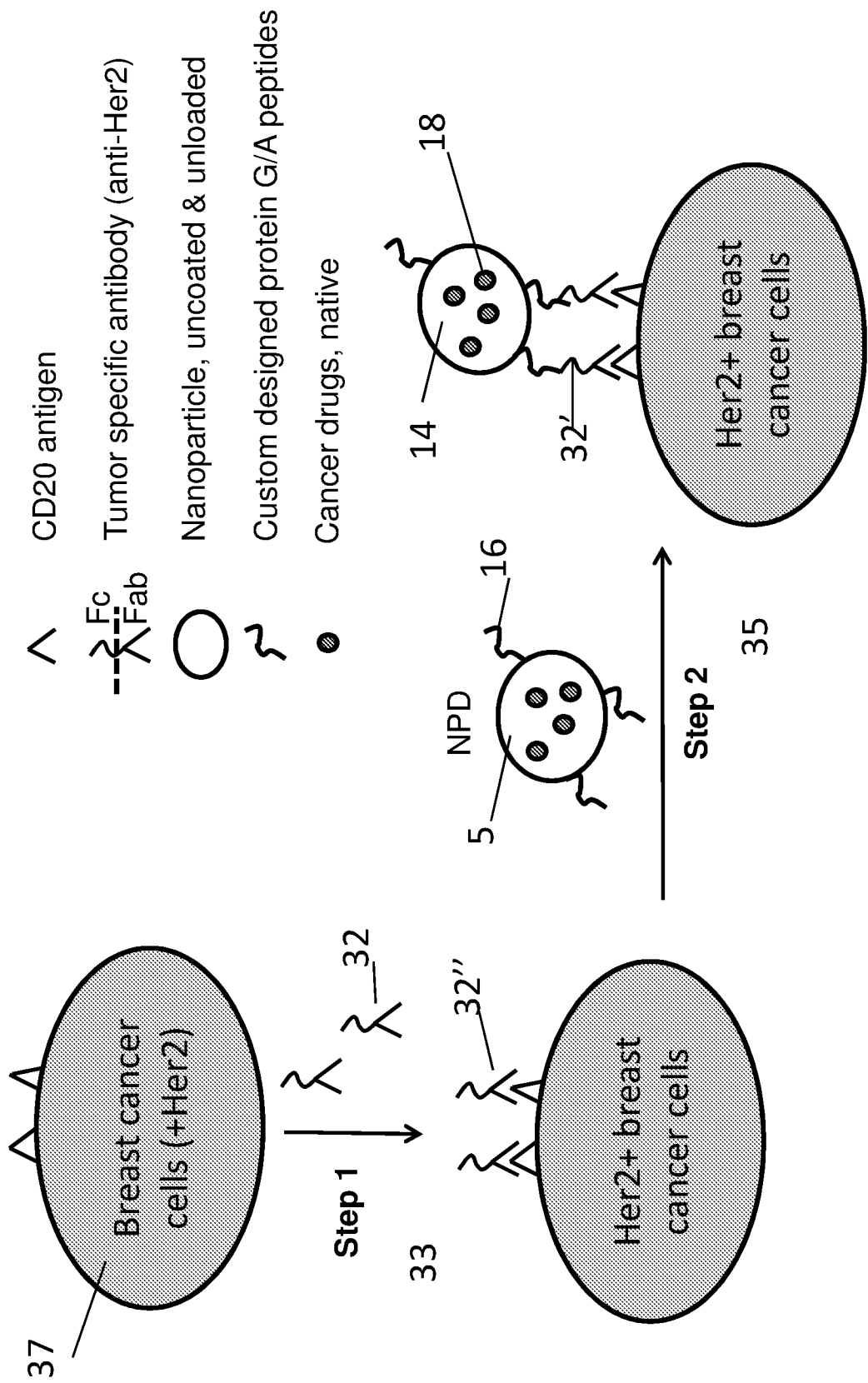


FIG. 5

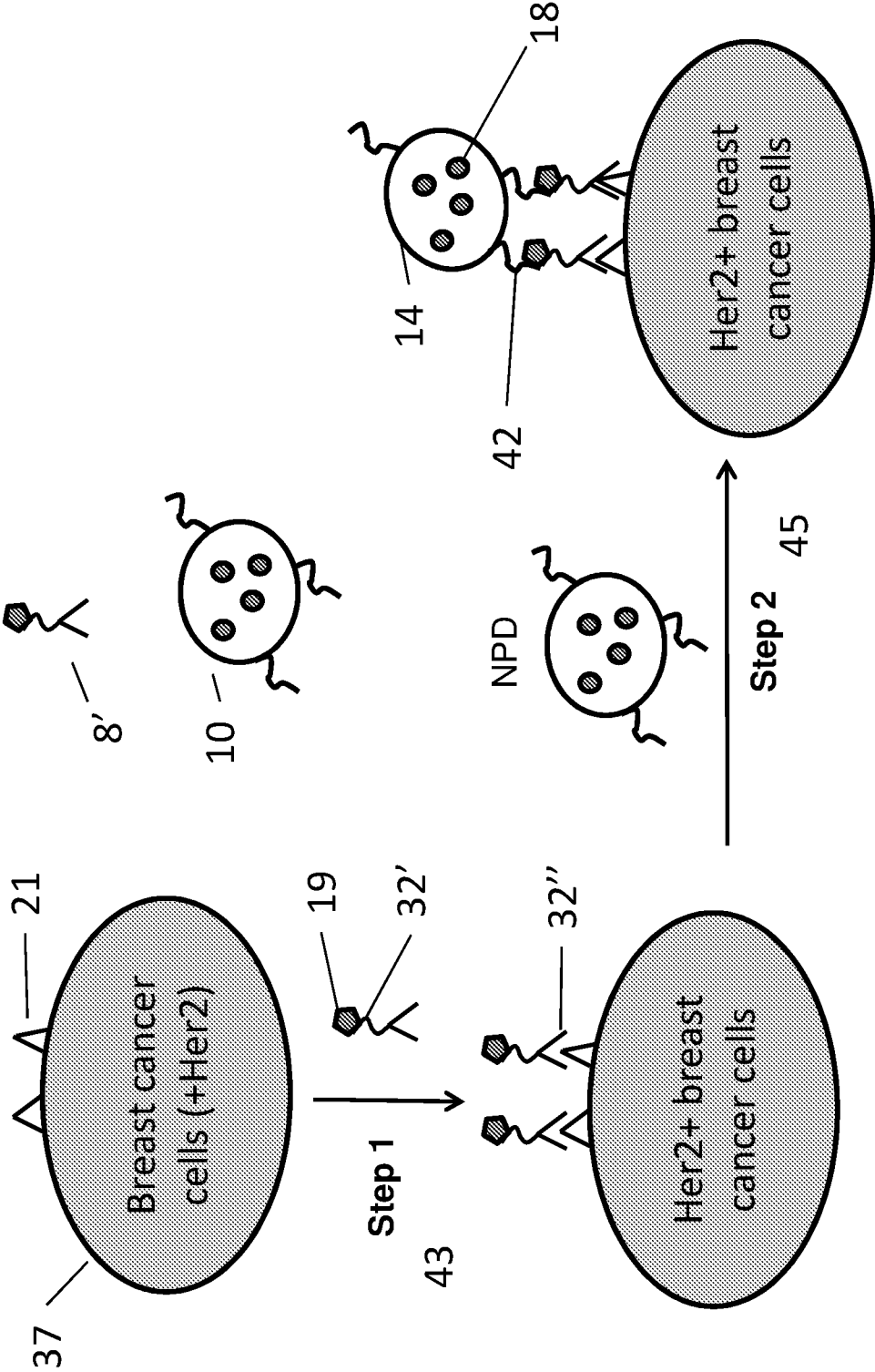
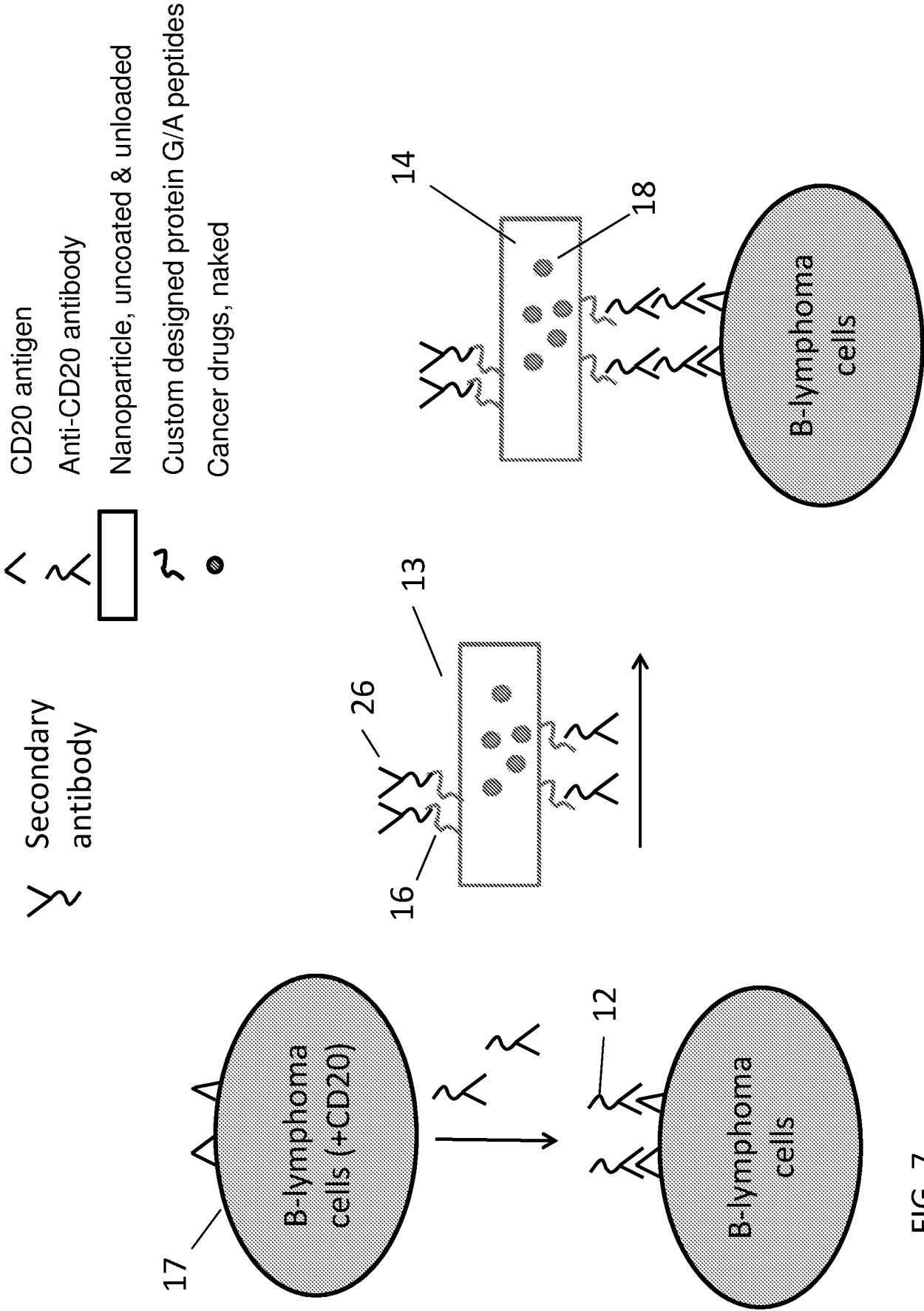


FIG. 6



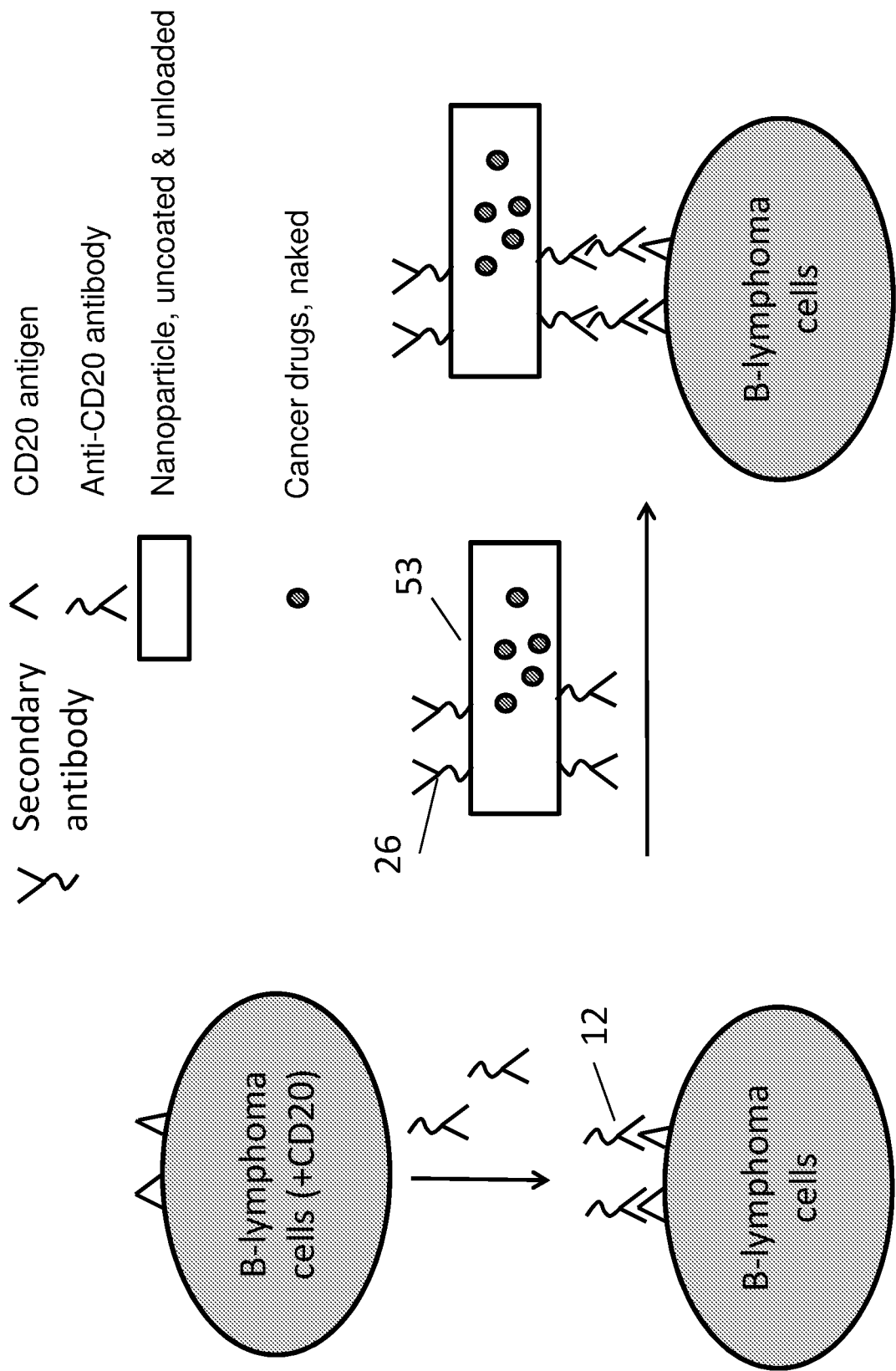


FIG. 8

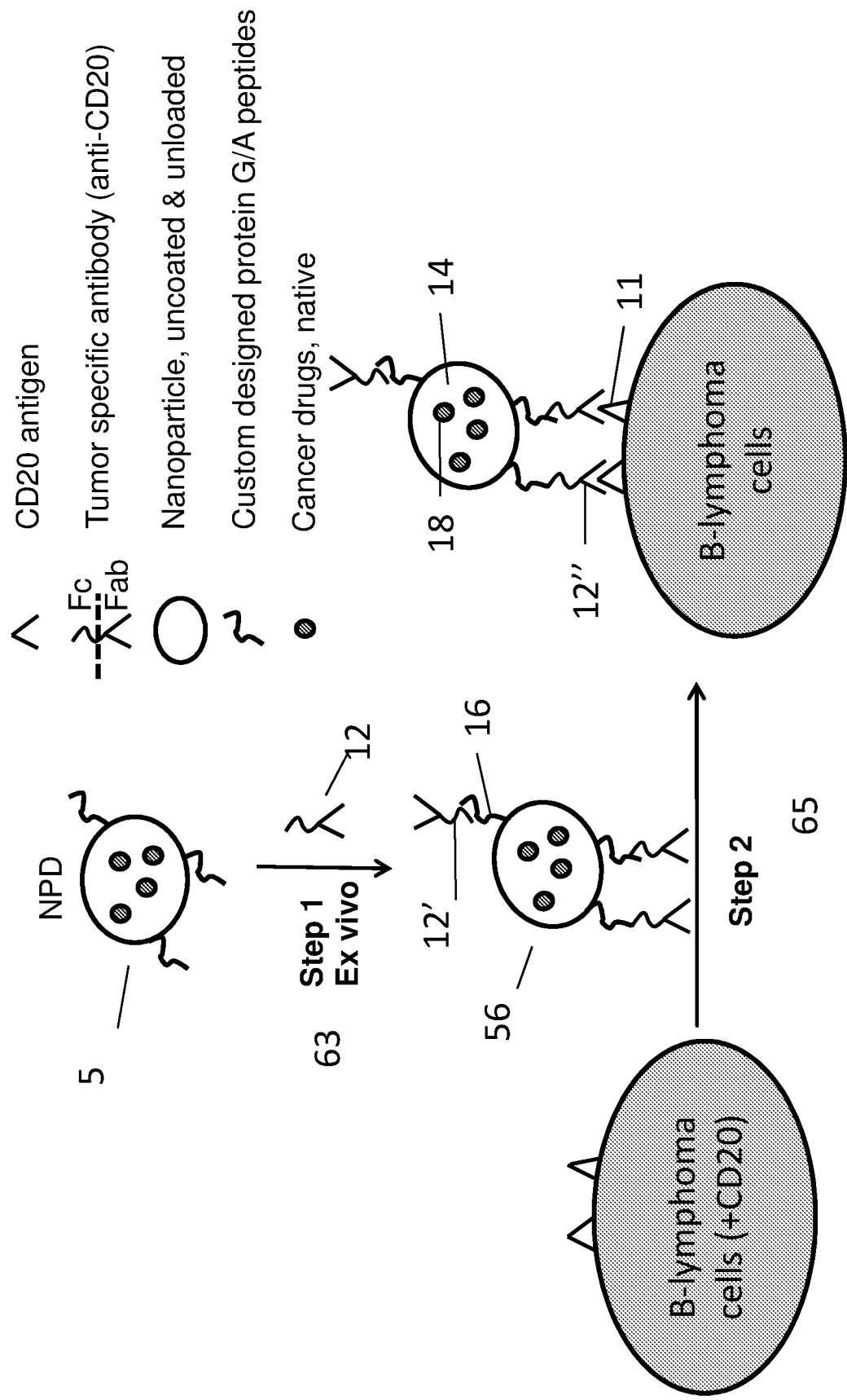


FIG. 9

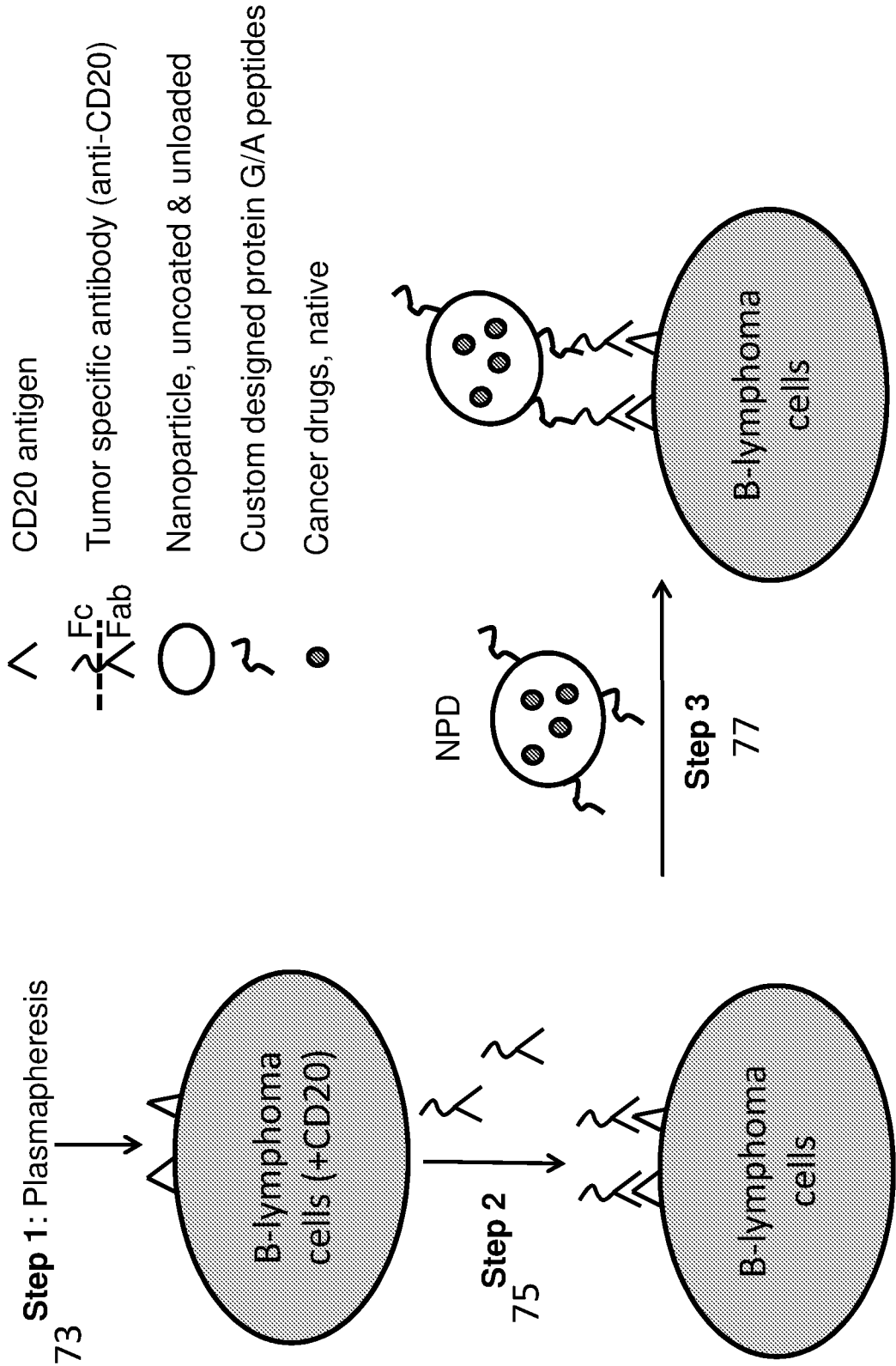
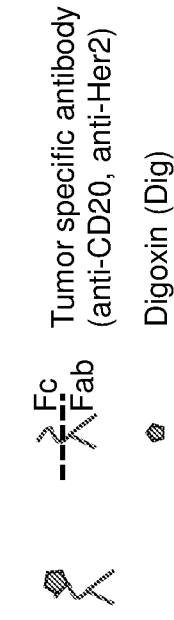


FIG. 10

Figure 11
New therapeutic
bi- and tri-specific
antibodies

Product C is a digoxin-conjugated anti-CD20 (or anti-HER2) antibody



Product A-B is a bi-specific immunoglobulin

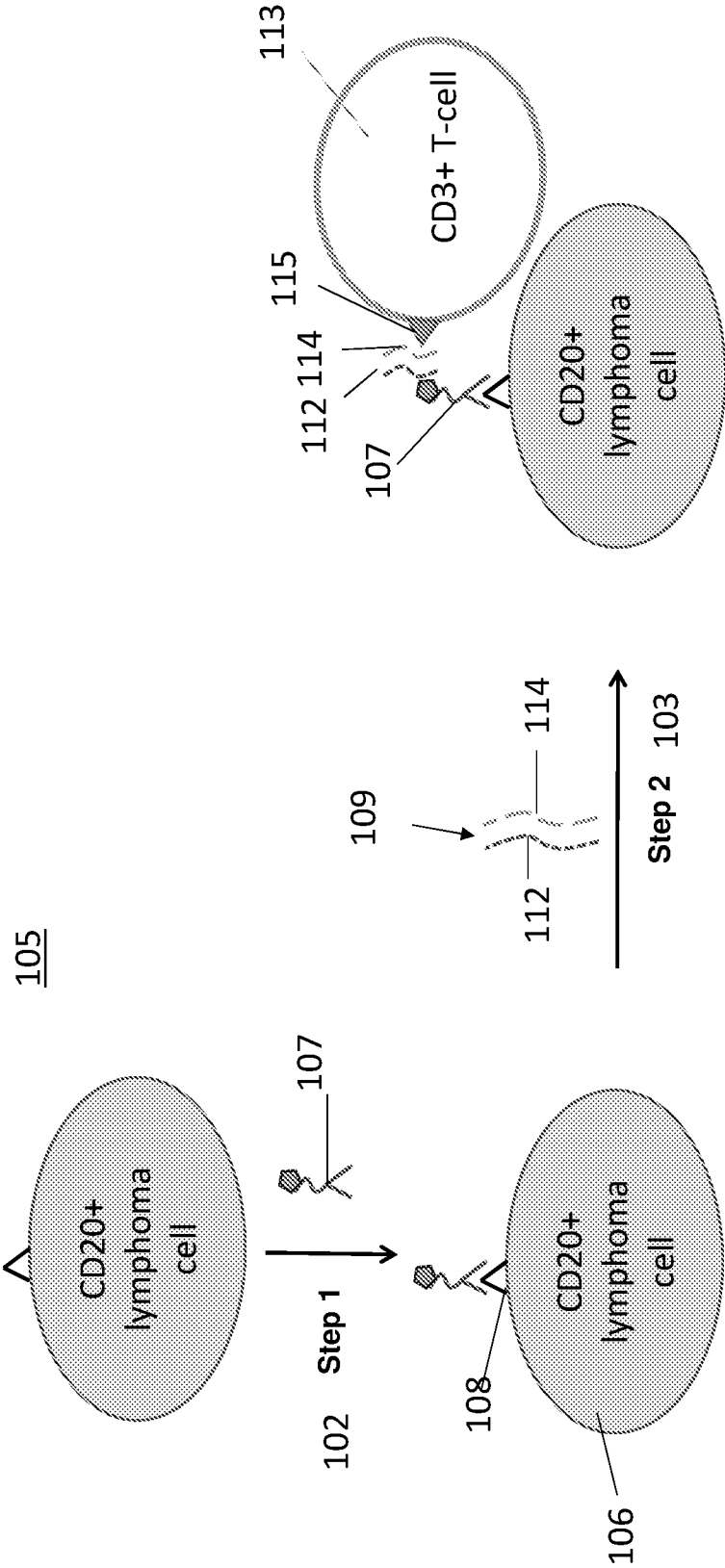
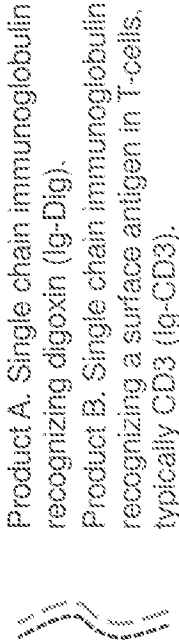
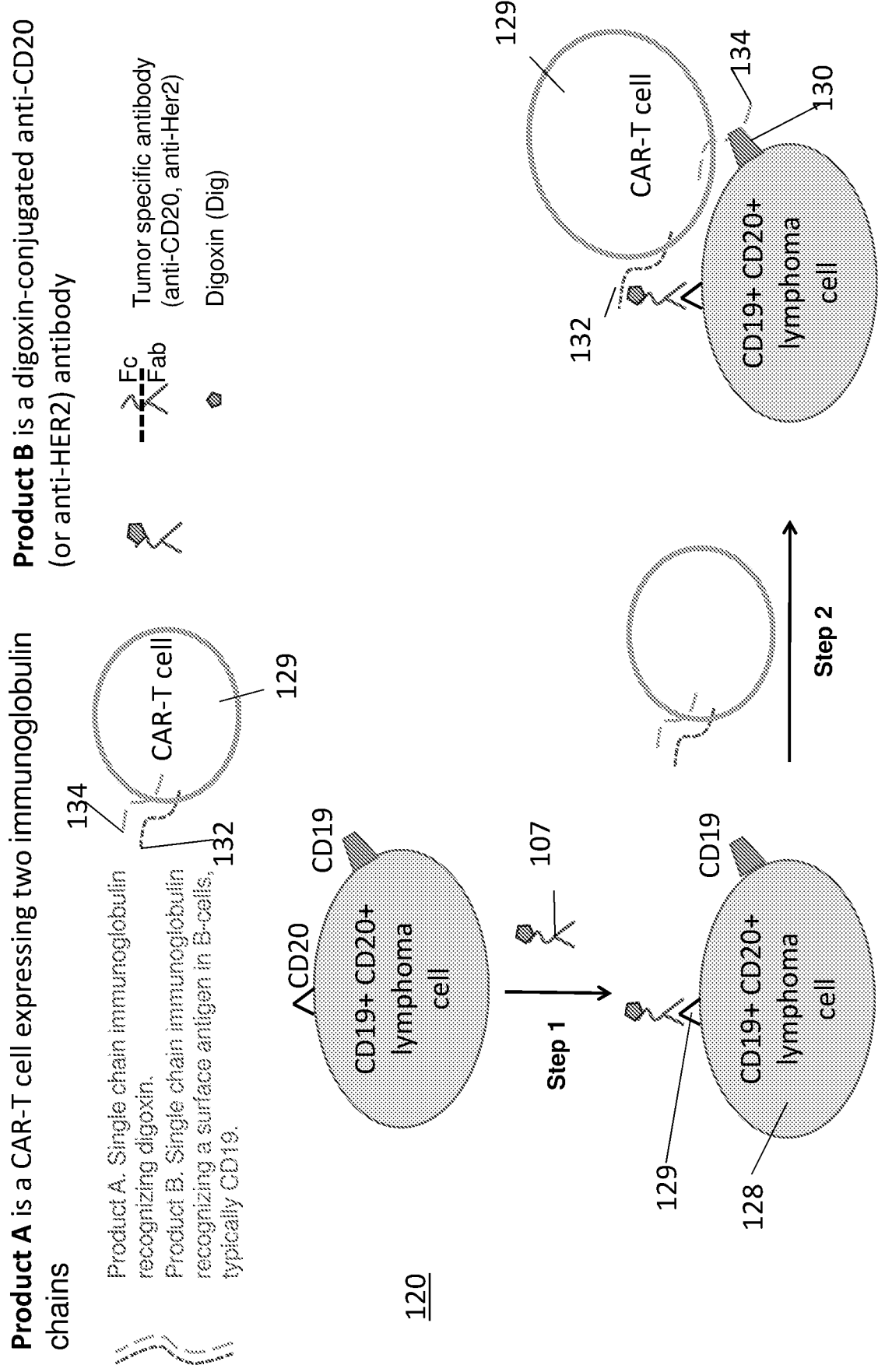


Figure 12
New CAR-T



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/51319

A. CLASSIFICATION OF SUBJECT MATTER
IPC - A61K 47/42; A61K 9/48; A61P 35/00 (2019.01)
CPC - A61K 47/62; A61K 47/6925; A61K 49/225

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)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2011/0200530 A1 (Allemann et al.) 18 August 2011 (18.08.2011) para [0013]-[0014], [0022]-[0024], [0075], [0239]-[0240]	1-3, 10-11, 35-37 ----- 50-53
Y	Zaroff. CAR T-Cell Therapies with a Bispecific Twist. Genetic Engineering & Biotechnology News, July 1, 2018 (Vol.38, No. 13), title, pg2, ln1-8 from bottom	50-53
Y	Huston. Protein engineering of antibody binding sites: Recovery of specific activity in an anti-digoxin single-chain Fv analogue produced in Escherichia coli. Proc. Natl. Acad. Sci. USA, Biochemistry, Vol. 85, pp. 5879-5883, August 1988, abstract, pg5883, col1, ln32-38	53
Y	US 2006/0083781 A1 (Shastri et al.) 20 April 2006 (20.04.2006) abstract	1
Y	WO 2015/053434 A1 (THE JOHNS HOPKINS UNIVERSITY) 22 March 2018 (22 03.2018) abstract	1
Y	US 2012/0207754 A1 (Vaxiion Therapeutics, Inc.) 16 August 2012 (16.08.2012)	1

☐ 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

"D" document cited by the applicant in the international application

"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 or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel 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

"&" document member of the same patent family

Date of the actual completion of the international search

06 November 2019

Date of mailing of the international search report

27 JAN 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/51319

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-9, 12-14, 19-23, 30-34, 38-44, 48, 54-55, 60-62
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

---please see supplemental box---

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1-3, 10-11, 35-37, 50-53

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US 19/51319

Box III: lack of unity

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-3, 10-11, 35-37, 50-53, directed to a method of treating cancer in a subject in need comprising administering an amount of a primary targeting molecule that targets cancer cells and co-administering an amount of agent loaded delivery vehicles, wherein the agent-loaded delivery vehicles are coated with a secondary targeting molecule that targets the primary targeting molecule.

Group II: Claims 15-18, 24-29, directed to a nano-peptide drug complex (NPD) comprising an agent-loaded delivery vehicle loaded with a cancer therapeutic agent and the method of making the same.

Group III: Claims 45-47, 49, 56-59, 63 directed to a system and kit comprising a primary targeting molecule that targets a cancer cell and a secondary targeting molecule that comprises a first portion that binds to the primary targeting molecule and a second portion that binds an antigen associated with an immune cell in the subject

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I requires a method of treating cancer in a subject in need comprising administering an amount of a primary targeting molecule that targets cancer cells and co-administering an amount of agent loaded delivery vehicles, wherein the agent-loaded delivery vehicles are coated with a secondary targeting molecule that targets the primary targeting molecule, not required by groups II-III.

Group II requires a nano-peptide drug complex (NPD) comprising an agent-loaded delivery vehicle loaded with a cancer therapeutic agent and the method of making the same, not required by groups I and III

Group III requires a system and kit comprising a primary targeting molecule that targets a cancer cell and a secondary targeting molecule that comprises a first portion that binds to the primary targeting molecule and a second portion that binds an antigen associated with an immune cell in the subject, not required by group II.

Common Technical Features:

Groups I and III share the technical feature of a primary targeting molecule that targets cancer cells and co-administering an amount of agent loaded delivery vehicles, wherein the agent-loaded delivery vehicles wherein the agent-loaded delivery vehicles are coated with a secondary targeting molecule that targets the primary targeting molecule.

However, these shared technical features do not represent a contribution over prior art, because the shared technical feature is being obvious over US 2011/0200530 A1 to Allemann et al. (hereinafter 'Allemann'). Allemann teaches a method of treating cancer (para [0240], tumours) in a subject in need comprising administering (para [0240], injected) an amount of a primary targeting molecule (para [0014] a targeting construct) that targets cancer cells (para [0239], tumour targeting) and co-administering an amount of agent-loaded delivery vehicles (para [0240], liposomes loaded with doxorubicin), wherein the agent-loaded (para [0240], liposomes loaded with doxorubicin) delivery vehicles comprise a secondary targeting molecule (para [0013], a plurality of polymer) that targets the primary targeting molecule (para [0014], antibody capable of selective binding to said polymer). Although Allemann doesn't explicitly teach the delivery vehicles are coated with a secondary targeting molecule, it does teach the delivery vehicles (liposome or said gas-filled microvesicle) having a stabilizing envelope comprising a plurality of said polymer (para [0023]). It would have been obvious to one ordinary skilled in the art to coat the delivery vehicles with a secondary targeting molecule since coating is an easy and common way to attach the secondary targeting molecule to the delivery vehicle.

As the shared technical features were known in the art at the time of the invention, they cannot be considered common technical features that would otherwise unify the groups. Therefore, Groups I-III lack unity under PCT Rule 13.

Note:

Claims 4-9, 12-14, 19-23, 30-34, 38-44, 48, 54-55 and 60-62 determined unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).