(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 October 2016 (06.10.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/161129 A1

(51) International Patent Classification:
A61K 9/51 (2006.01) *A61P 35/00* (2006.01)
A61K 47/48 (2006.01)

(21) International Application Number:
PCT/US2016/025290

(22) International Filing Date:
31 March 2016 (31.03.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/140,696 31 March 2015 (31.03.2015) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

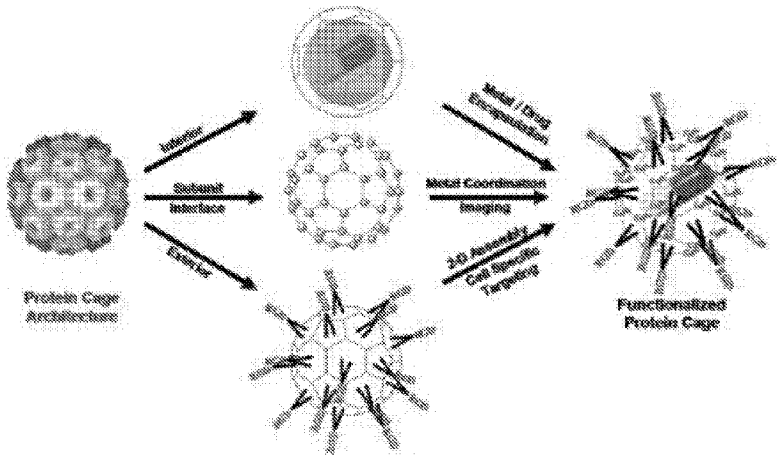
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

(54) **Title**: SELF ASSEMBLING MOLECULES FOR TARGETED DRUG DELIVERY

Figure 6



(57) **Abstract**: Described herein are self-assembling protein molecules for delivering a payload, for example, a toxic anti-cancer agent, a cancer immunotherapy, a toxic anti-cancer agent and a cancer immunotherapy, or an imaging agent, to specific tissues. Examples of self-assembled proteins include clathrin and derivatives of clathrin.

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Self Assembling Molecules for Targeted Drug Delivery

Related Applications

This application claims the benefit of priority to United States Provisional Patent Application serial number 62/140,696, filed March 31, 2015, the contents of which are hereby incorporated by reference.

Background

Many extremely useful chemotherapeutics lose their potential utility as effective cancer therapy due to their systemic toxicity. As a result, drug delivery systems have been a significant focus of research in the anti-cancer arena. For example, large particulate assemblies of biologically compatible materials, such as liposomes, have been used as carriers for administration of drugs and paramagnetic contrast agents. For example, liposome compositions containing an entrapped agent, such as a drug, are known; these compositions are engineered to control biodistribution and recirculatory half-life.

In order to provide a therapeutic effect, a sufficient concentration of an active agent must be delivered to a targeted site. So, there is a need for recirculation of the active agent in the body. Active agents and delivery systems that avoid rapid endocytosis by the reticuloendothelial (RE) system or rapid filtration by the kidney are desirable. Experience with magnetic resonance contrast agents has provided useful information regarding circulation lifetimes. Small molecules, such as gadolinium diethylenetriaminepentaacetic acid, tend to have limited circulation times due to rapid renal excretion while most liposomes, having diameters greater than 800 nm, are quickly cleared by the reticuloendothelial system. Attempts to solve these problems have involved use of macromolecular materials, such as gadolinium diethylenetriaminepentaacetic acid-derived polysaccharides, polypeptides, and proteins. These agents have not achieved the versatility in chemical modification to provide for both long recirculation times and active targeting. In addition, the use of targeted antibodies, immune-enhancing drugs, slow-release peptides, or polymers for targeted drug delivery results in extreme side-effects or low delivery efficiency (e.g, the delivery systems are not internalized by the cells).

Accordingly, there is a need for improved anti-cancer therapeutics and delivery systems.

Summary

In certain embodiments, the invention relates to a first composition comprising a protein, a first payload, and a first targeting agent, wherein the protein is in the form of a three-dimensional cage structure comprising an outer surface and an inner cavity; and the first targeting agent is conjugated to the outer surface of the three-dimensional cage structure.

In certain embodiments, the invention relates to any of the first compositions described herein, wherein the first payload is an anti-cancer agent.

In certain embodiments, the invention relates to any of the first compositions described herein, wherein the first payload is an imaging agent.

In certain embodiments, the invention relates to any of the first compositions described herein, wherein the first targeting agent selectively targets cancer cells as compared to healthy cells.

In certain embodiments, the invention relates to any of the first compositions described herein, wherein the first targeting agent specifically targets cancer cells.

In certain embodiments, the invention relates to any of the first compositions described herein, wherein the first targeting agent is an antibody.

In certain embodiments, the invention relates to any of the first compositions described herein, wherein the protein is clathrin or a clathrin derivative.

In certain embodiments, the invention relates to any of the first compositions described herein, wherein the first composition or the second composition is able to transfect cells in vivo.

In certain embodiments, the invention relates to a second composition comprising a protein, a second payload, and a second targeting agent, wherein the protein is in the form of a three-dimensional cage structure comprising an outer surface and an inner cavity; the second payload is an immunogen; and the second targeting agent conjugated to the outer surface of the three-dimensional cage structure.

In certain embodiments, the invention relates to any of the second compositions described herein, wherein the second targeting agent does not selectively target cancer cells as compared to healthy cells.

In certain embodiments, the invention relates to any of the second compositions described herein, wherein the second targeting agent is an antibody.

In certain embodiments, the invention relates to any of the second compositions described herein, wherein the second targeting agent is an anti-PD-1 antibody.

In certain embodiments, the invention relates to any of the second compositions described herein, wherein the protein is clathrin or a clathrin derivative.

In certain embodiments, the invention relates to any of the second compositions described herein, wherein the second composition is able to transfect cells in vivo.

In certain embodiments, the invention relates to a method of treating cancer in a subject in need thereof, comprising:

administering to the subject a therapeutically effective amount of any of the first compositions described herein wherein the first payload is an anti-cancer agent.

In certain embodiments, the invention relates to a method of treating cancer in a subject in need thereof, comprising:

administering to the subject a therapeutically effective amount of any of the first compositions described herein, wherein the first payload is an anti-cancer agent; and

administering to the subject a therapeutically effective amount of any of the second compositions described herein.

In certain embodiments, the invention relates to a method generating an image of a subject in need thereof, comprising:

administering to the subject a detectable amount of any of the first compositions described herein, wherein the first payload is an imaging agent; and

generating an image.

Brief Description of the Figures

Figure 1 depicts a schematic representation of an exemplary procedure for preparing a drug-loaded vehicle of the invention.

Figure 2 depicts a schematic representation of a mechanism by which the drug-loaded vehicles may be internalized.

Figure 3 depicts a schematic representation of the selectivity of the drug-loaded vehicles for cancer cells over normal healthy cells.

Figure 4 depicts the results of gel electrophoresis of the cloned clathrin heavy chain (M1: SDS-PAGE Protein Marker; Lane 1: PE1130119-1 protein; M2: Western-Blot Protein Marker; Lane 2: PE1130119-1 protein (using anti-6His antibody)).

Figure 5 depicts the results of gel electrophoresis of the cloned clathrin light chain (M1: SDS-PAGE Protein Marker; Lane 1: PE1130119-2 protein; M2: Western-Blot ProteinMarker; Lane 2: PE1130119-2 protein (using anti-6His antibody)).

Figure 6 depicts a schematic representation of protein cage functionalization. Protein cage architectures have three surfaces (interior, subunit interface, and exterior) amenable to both genetic and chemical modification.

Figure 7 depicts a schematic representation of the steps and components involved in cancer immunotherapy.

Detailed Description

Overview

In certain embodiments, this invention relates to the use of self-assembling protein molecules for delivering a payload, for example, a toxic anti-cancer agent, a cancer immunotherapy, or an imaging agent, to specific tissues. In certain embodiments, the protein is clathrin or a derivative of clathrin. In certain embodiments, the protein is endogenous. In certain embodiments, the protein is non-immunogenic. In certain embodiments, the protein is ferritin or a derivative of ferritin.

In some embodiments, the self-assembled protein cages or vehicles, made of heavy and light chains, mask the toxicity of the anti-cancer agent, thereby resulting in decreased serum and systemic toxicity.

In certain embodiments, the heavy chain and the light chain are fused (e.g., the protein may be a fusion protein).

In other embodiments, the self-assembled delivery vehicles are used to target specific tissues, such as cancer cells, using antigen biomarkers, antibodies, or peptides that are recognized by the cell membrane of the target cell. In certain embodiments, once delivered to the target tissues, the clathrin cages are internalized by the cell for in-cell deposition of drug.

In certain embodiments, the payload is an anti-cancer agent, for example, a chemotherapeutic, siRNA, miRNA, immunotherapeutics, or a radiotherapeutic. In certain embodiments, the payload is an imaging agent, such as a contrast medium or a fluorophore. In certain embodiments, the drug is a radiotherapeutic, such as a radionuclide.

In certain embodiments, the payload is conjugated to the protein, for example, to the light chain. “Conjugated” or “linked” as used herein means ionically or, preferably, covalently attached (e.g., via a crosslinking agent).

In certain embodiments, the invention relates to a method of treating a subject in need thereof comprising administering to the subject a therapeutically effective amount of any one of the drug-loaded vehicles described herein. In certain embodiments, the drug-loaded vehicle is administered to the subject intravenously or intraperitoneally.

This technology is expected to achieve synergistic results as compared to the protein alone, the payload alone, the targeting agent alone, or any combination of two of these components. The advantages include, but are not limited to: 1. The proteins self-assemble following their loading with known or newly developed therapeutic agents. 2. The proteins are easily internalized by cells. 3. The assembled, drug-loaded vehicles are stable in serum proteins and are non-toxic while transported in vivo via the blood and lymph system. 4. The proteins and vehicles are designed to specifically target diseased cells using specific antibodies or high-affinity fragments of antibodies. In some embodiments, the antibodies are designed to enhance the immune system by uncovering a cancer call not identified by the immune system. 5. Once targeted to diseased cells, the delivery vehicles are internalized and during this process they disassemble and release their therapeutic agent and specifically kill the diseased cell or allow the immune system to fight it. 6. This platform has the potential to provide mono-, bi- and multi-specific targeting. 7. Because of the ease of internalization, if the payload is an imaging agent or a radiotherapeutic, the vehicles may be used for tumor imaging or radiotherapy. 8. For therapeutic applications where longer half-life is desired, the vehicles may be modified by increasing the molecular weight of the proteins or adding polymeric extensions. 9. The combination of (i) endogenous, self-assembled, cell-internalized proteins with (ii) self-internalized antibodies and (iii) payloads can improve cancer imaging or treatment while lowering systemic toxicity.

Exemplary Proteins

In certain embodiments, the invention relates to a protein having a heavy chain, wherein the heavy chain has greater than 85% sequence homology to SEQ ID NO:3. In certain embodiments, the invention relates to any of the proteins described herein, wherein the heavy chain has greater than 90% sequence homology to SEQ ID NO:3. In certain embodiments, the invention relates to any of the proteins described herein, wherein the heavy chain has greater than 95% sequence homology to SEQ ID NO:3. In certain embodiments, the invention relates to any of the proteins described herein, wherein the heavy chain has greater than 98% sequence homology to SEQ ID NO:3. In certain

embodiments, the invention relates to any of the proteins described herein, wherein the heavy chain has greater than 99% sequence homology to SEQ ID NO:3. In certain embodiments, the invention relates to any of the proteins described herein, wherein the heavy chain has SEQ ID NO:3.

In certain embodiments, the invention relates to a protein having a light chain, wherein the light chain has greater than 85% sequence homology to SEQ ID NO:6. In certain embodiments, the invention relates to any of the proteins described herein, wherein the light chain has greater than 90% sequence homology to SEQ ID NO:6. In certain embodiments, the invention relates to any of the proteins described herein, wherein the light chain has greater than 95% sequence homology to SEQ ID NO:6. In certain embodiments, the invention relates to any of the proteins described herein, wherein the light chain has greater than 98% sequence homology to SEQ ID NO:6. In certain embodiments, the invention relates to any of the proteins described herein, wherein the light chain has greater than 99% sequence homology to SEQ ID NO:6. In certain embodiments, the invention relates to any of the proteins described herein, wherein the light chain has SEQ ID NO:6.

In certain embodiments, the invention relates to any of the proteins described herein, wherein the protein has a heavy chain and a light chain.

Exemplary Compositions

In certain embodiments, the invention relates to a first composition comprising, consisting essentially of, or consisting of a protein, a first payload, and a first targeting agent, wherein the protein is in the form of a three-dimensional cage structure comprising an outer surface and an inner cavity; and the first targeting agent is conjugated to the outer surface of the three-dimensional cage structure. In certain embodiments, the first targeting agent selectively targets cancer cells as compared to healthy cells. In certain embodiments, the first targeting agent specifically targets diseased cells, such as cancer cells.

In certain embodiments, the invention relates to a second composition comprising, consisting essentially of, or consisting of a protein, a second payload, and a second targeting agent, wherein the protein is in the form of a three-dimensional cage structure comprising an outer surface and an inner cavity; the second payload is an immunogen; and the second targeting agent conjugated to the outer surface of the three-dimensional cage structure. In certain embodiments, the second targeting agent does not selectively target cancer cells as compared to healthy cells.

In certain embodiments, the compositions (i.e., the first composition or the second composition) are able to identify or transfect cells in vivo.

Protein

In certain embodiments, the invention relates to any of the compositions described herein (e.g., the first composition or the second composition), wherein the protein is able to deliver a payload into a cell.

In certain embodiments, the invention relates to any of the compositions described herein (e.g., the first composition or the second composition), wherein the protein is clathrin or a clathrin derivative.

In certain embodiments, the invention relates to any of the compositions described herein (e.g., the first composition or the second composition), wherein the protein comprises a heavy chain or a light chain. In certain embodiments, the invention relates to any of the compositions described herein, wherein the protein comprises a heavy chain and a light chain. In some embodyments scaffolding of truncated clathrin and their repeated squances of these tranced peptides are used as payload carries of anticancer internalizing peptides.

In certain embodiments, the invention relates to any of the compositions described herein, wherein the heavy chain has a molecular weight from about 100 kDa to about 300 kDa. In certain embodiments, the invention relates to any of the compositions described herein, wherein the heavy chain has a molecular weight of about 100 kDa, about 110 kDa, about 120 kDa, about 130 kDa, about 140 kDa, about 150 kDa, about 160 kDa, about 170 kDa, about 180 kDa, about 190 kDa, about 200 kDa, about 210 kDa, about 220 Da, about 230 kDa, about 240 kDa, about 250 kDa, about 260 kDa, about 270 kDa, about 280 kDa, about 290 kDa, or about 300 kDa. In certain embodiments, the invention relates to any of the compositions described herein, wherein the heavy chain has a molecular weight of about 190 kDa.

In certain embodiments, the invention relates to any of the compositions described herein, wherein the heavy chain has greater than 85% sequence homology to SEQ ID NO:3. In certain embodiments, the invention relates to any of the compositions described herein, wherein the heavy chain has greater than 90% sequence homology to SEQ ID NO:3. In certain embodiments, the invention relates to any of the compositions described herein, wherein the heavy chain has greater than 95% sequence homology to SEQ ID NO:3. In certain embodiments, the invention relates to any of the compositions described

herein, wherein the heavy chain has greater than 98% sequence homology to SEQ ID NO:3. In certain embodiments, the invention relates to any of the compositions described herein, wherein the heavy chain has greater than 99% sequence homology to SEQ ID NO:3. In certain embodiments, the invention relates to any of the compositions described herein, wherein the heavy chain has SEQ ID NO:3.

In certain embodiments, the invention relates to any of the compositions described herein, wherein the light chain has a molecular weight from about 15 kDa to about 45 kDa. In certain embodiments, the invention relates to any of the compositions described herein, wherein the light chain has a molecular weight of about 15 kDa, about 16 kDa, about 17 kDa, about 18 kDa, about 19 kDa, about 20 kDa, about 21 kDa, about 22 kDa, about 23 kDa, about 24 kDa, about 25 kDa, about 26 kDa, about 27 kDa, about 28 kDa, about 29 kDa, about 30 kDa, about 31 kDa, about 32 kDa, about 33 kDa, about 34 kDa, about 35 kDa, about 36 kDa, about 37 kDa, about 38 kDa, about 39 kDa, about 40 kDa, about 41 kDa, about 42 kDa, about 43 kDa, about 44 kDa, or about 45 kDa. In certain embodiments, the invention relates to any of the compositions described herein, wherein the light chain has a molecular weight of about 28 kDa.

In certain embodiments, the invention relates to any of the compositions described herein, wherein the light chain has greater than 85% sequence homology to SEQ ID NO:6. In certain embodiments, the invention relates to any of the compositions described herein, wherein the light chain has greater than 90% sequence homology to SEQ ID NO:6. In certain embodiments, the invention relates to any of the compositions described herein, wherein the light chain has greater than 95% sequence homology to SEQ ID NO:6. In certain embodiments, the invention relates to any of the compositions described herein, wherein the light chain has greater than 98% sequence homology to SEQ ID NO:6. In certain embodiments, the invention relates to any of the compositions described herein, wherein the light chain has greater than 99% sequence homology to SEQ ID NO:6. In certain embodiments, the invention relates to any of the compositions described herein, wherein the light chain has SEQ ID NO:6.

In certain embodiments, the invention relates to any of the compositions described herein, wherein the three-dimensional cage structure has a diameter from about 10 nm to about 100 nm. In certain embodiments, the invention relates to any of the compositions described herein, wherein the three-dimensional cage structure has a diameter of about 10 nm, about 20 nm, about 30 nm, about 40 nm, about 50 nm, about 60 nm, about 70 nm,

about 80 nm, about 90 nm, or about 100 nm. In certain embodiments, the invention relates to any of the compositions described herein, wherein the three-dimensional cage structures have an average diameter from about 10 nm to about 100 nm. In certain embodiments, the invention relates to any of the compositions described herein, wherein the three-dimensional cage structures have an average diameter of about 10 nm, about 20 nm, about 30 nm, about 40 nm, about 50 nm, about 60 nm, about 70 nm, about 80 nm, about 90 nm, or about 100 nm. In certain embodiments, the diameter of the three-dimensional cage structures may be estimated or measured by techniques known in the art, such as dynamic light scattering or high-resolution NMR spectroscopy.

In certain embodiments, the invention relates to any of the compositions described herein, wherein the three-dimensional cage structure is substantially spherical.

In certain embodiments, the invention relates to any of the compositions described herein, wherein the three-dimensional cage structure is non-covalently assembled, for example, self-assembled.

In certain embodiments, the invention relates to any of the compositions described herein, wherein the three-dimensional cage structure is substantially stable at about 37 °C at about pH greater than or equal to 7.

In certain embodiments, the invention relates to any of the compositions described herein, wherein the three-dimensional cage structure is substantially stable at about 37 °C at about pH 7.

In certain embodiments, the invention relates to any of the compositions described herein, wherein the three-dimensional cage structure is substantially stable at about 37 °C at about pH 6.5 to about pH 8.5.

In certain embodiments, the invention relates to any of the compositions described herein, wherein the three-dimensional cage structure is substantially unstable at about 37 °C at about pH less than or equal to 5.5.

In certain embodiments, the invention relates to any of the compositions described herein, wherein the three-dimensional cage structure is substantially unstable at about 37 °C at about pH 5.5.

Cage-like proteins such as clathrin, ferritins, DNA-binding proteins (dps), and heat shock proteins have three distinct surfaces (inside, outside, interface) that can be exploited to generate nanomaterials with multiple functionality by design. Protein cages are biological in origin and each cage exhibits extremely homogeneous size distribution. This

homogeneity can be used to attain a high degree of homogeneity of the templated material and its associated property. A series of protein cages exhibiting diversity in size, functionality, and chemical and thermal stabilities can be utilized for materials synthesis under a variety of conditions. Since synthetic approaches to materials science often use harsh temperature and pH, in certain embodiments, it can be an advantage to utilize protein cages from extreme environments, such as acidic thermal hot springs.

Protein cage architectures, 10-100 nm in diameter, are self-assembled hollow spheres derived from viruses and other biological cages, including heat shock proteins (Hsp), DNA-binding proteins from starved cells (Dps), and ferritins. These architectures play critical biological roles. For example, heat shock proteins are thought to act as chaperones that prevent protein denaturation, and ferritins are known to store iron (which is both essential and toxic) as a nanoparticle of iron oxide. While each of these structures has evolved to perform a unique natural function, they are similar in that they are all essentially proteinaceous containers with three distinct surfaces (interior, exterior, and subunit interface) to which one can impart function by design. Protein cage architectures have demonstrated utility in nanotechnology with applications including inorganic nanoparticle synthesis and the development of targeted therapeutic and imaging delivery agents.

Protein cage architectures are naturally diverse; each has unique attributes (including size, structure, solvent accessibility, chemical and temperature stability, structural plasticity, assembly and disassembly parameters, and electrostatics) useful to particular applications. Importantly, one can capitalize on these features or alter them via genetic or chemical modification. Atomic level structural information identifies the precise location of amino acids within protein cage architectures and in turn allows for the rational inclusion, exclusion, and substitution of amino acid(s) (at the genetic level) resulting in protein cages with novel functional properties.

Protein cages isolated from thermophilic environments are desirable as building blocks for nanotechnology due to their potential stability in harsh reaction conditions including high temperature and pH extremes. Interestingly, one of the most stable protein cage architectures, ferritin, is commonly found in mesophilic organisms, including animals, plants, and microbes. For example, horse spleen ferritin exhibits broad pH (pH 2–8) and temperature stability (<70°C). Ferritins are involved in iron sequestration, which they accomplish through the oxidation of soluble Fe(II) using O_2. This oxidation results in the formation of a nanoparticle of Fe_2O_3 encapsulated (and rendered nontoxic) within the

protein cage. High charge density on the inner surface of the protein cage promotes this reaction, which is assisted by an enzymatic (ferroxidase) activity in some ferritin subunits. Ferritins are made up of 24 subunits, which form a spherical cage 12 nm in diameter. The ferritin family also includes the 24 subunit bacterioferritins and the Dps class of proteins, which assemble from 12 monomers.

A cavity forming protein cage is described in U.S. Pat. No. 7,393,924 (incorporated by reference). The cage is formed in vitro from a plurality of 3-legged triskelia, each triskelion having 6 protein subunits; 3 Clathrin heavy chain and 3 Clathrin light chain subunits. In certain embodiments, the 3-legged triskelia are not required (see, e.g., U.S. Patent Application Publication No. 2015/0307570, incorporated by reference). For example, the protein may be an isolated, synthetic or recombinant, protein comprising in whole or in part one or more types of clathrin proteins of one or more isoforms, including cloned isoforms.

Payload

In certain embodiments, the invention relates to any of the first compositions described herein, wherein the payload is any therapeutic agent, but preferably an anti-cancer agent, such as paclitaxel, gemcitabine, or an azonafide (e.g., a compound described in U.S. Patent No. 8,008,316, which is incorporated by reference).

As used herein, the terms "anti-cancer agent" and "therapeutic agent" are defined broadly as anything that cancer cells, including tumor cells, may be exposed to in a therapeutic protocol for the purpose of inhibiting their growth or kill the cells. In one embodiments, such agents can be used according to the compositions and methods described herein in conjunction with each other (*e.g.*, LY294002 plus gemcitabine, taxol plus U0126, taxol plus gemcitabine, etc.), or in any combination thereof. Such agents include, but are not limited to, chemotherapeutic agents, such as anti-metabolic agents, *e.g.*, Ara AC, 5-FU and methotrexate, antimitotic agents, *e.g.*, TAXOL, inblastine and vincristine, alkylating agents, *e.g.*, melphalan, BCNU and nitrogen mustard, topoisomerase II inhibitors, *e.g.*, VW-26, topotecan and Bleomycin, strand-breaking agents, *e.g.*, doxorubicin and DHAD, cross-linking agents, *e.g.*, cisplatin and CBDCA, radiation and ultraviolet light.

As used herein, the term "chemotherapeutic agent" is intended to include chemical reagents which inhibit the growth of proliferating cells or tissues wherein the growth of such cells or tissues is undesirable. Particular chemotherapeutic agents include, but are not

limited to (i) antimetabolites, such as cytarabine, fludarabine, 5-fluoro-2'-deoxyuiridine, gemcitabine, hydroxyurea or methotrexate; (ii) DNA-fragmenting agents, such as bleomycin, (iii) DNA-crosslinking agents, such as chlorambucil, cisplatin, cyclophosphamide or nitrogen mustard; (iv) intercalating agents such as adriamycin (doxorubicin) or mitoxantrone; (v) protein synthesis inhibitors, such as L-asparaginase, cycloheximide, puromycin or diphtheria toxin; (vi) topoisomerase I poisons, such as camptothecin or topotecan; (vii) topoisomerase II poisons, such as etoposide (VP-16) or teniposide; (viii) microtubule-directed agents, such as colcemid, colchicine, paclitaxel, vinblastine or vincristine; (ix) kinase inhibitors such as flavopiridol, staurosporin, STI571 (CPG 57148B) or UCN-01 (7-hydroxystaurosporine); (x) enhancers of the AMPK signaling pathway, (xi) inhibitors of the PI3K/AKT/mTORC1 signaling pathway, (xii) inhibitors of the MEK/ERK signaling pathway, (xiii) miscellaneous investigational agents such as thioplatin, PS-341, phenylbutyrate, ET-18-OCH3, or farnesyl transferase inhibitors (L-739749, L-744832); polyphenols such as quercetin, resveratrol, piceatannol, epigallocatechine gallate, theaflavins, flavanols, procyanidins, betulinic acid and derivatives thereof; (xiv) hormones such as glucocorticoids or fenretinide; and (xv) hormone antagonists, such as tamoxifen, finasteride or LHRH antagonists. In an embodiment, the chemotherapeutic compound is one or more of gemcitabine, cisplatin, doxorubicin, daunarubicin, paclitaxel, taxotere and mitomycin C. In a particular embodiment, the chemotherapeutic compound is one or more of gemcitabine, cisplatin and paclitaxel.

Chemotherapeutic agents are well known in the art (see *e.g.*, Gilman A.G., *et al.*, The Pharmacological Basis of Therapeutics, 8th Ed., Sec 12:1202-1263 (1990)), and are typically used to treat neoplastic diseases. The chemotherapeutic agents generally employed in chemotherapy treatments are listed below in Table 1.

Table 1

CLASS	TYPE OF AGENT	NONPROPRIETARY NAMES (OTHER NAMES)
Alkylating	Nitrogen Mustards	Mechlorethamine (HN_2) Cyclophosphamide Ifosfamide Melphalan (L-sarcolysin) Chlorambucil
	Ethylenimines And Methylmelamines	Hexamethylmelamine Thiotepa
	Alkyl Sulfonates	Busulfan

Alkylating	Nitrosoureas	Carmustine (BCNU) Lomustine (CCNU) Semustine (methyl-CCNU) Streptozocin (streptozotocin)
	Triazenes	Decarbazine (DTIC; imethyltriazenoimi-dazolecarboxamide)
	Alkylator	cis-diamminedichloroplatinum II (CDDP)
Antimetabolites	Folic Acid Analogs	Methotrexate (amethopterin)
	Pyrimidine Analogs	Fluorouracil ('5-fluorouracil; 5-FU) Floxuridine (fluorode-oxyuridine; FUdR) Cytarabine (cytosine arabinoside) gemcitabine (deoxycytidine analog)
	Purine Analogs and Related Inhibitors	Mercaptopuine (6-mercaptopurine; 6-MP) Thioguanine (6-thioguanine; TG) Pentostatin (2' - deoxycoformycin)
Natural Products	Vinca Alkaloids	Vinblastin (VLB) Vincristine
	Topoisomerase Inhibitors	Etoposide Teniposide Camptothecin Topotecan 9-amino-campotothecin CPT-11
	Antibiotics	Dactinomycin (actinomycin D) Adriamycin (Doxorubicin) Daunorubicin (daunomycin; rubindomycin) Doxorubicin Bleomycin Plicamycin (mithramycin) Mitomycin (mitomycin C) TAXOL (paclitaxel) Taxotere
	Enzymes	L-Asparaginase
	Biological Response Modifiers	Interfon alfa interleukin 2
Misc. Agents	Platinum Coordination Complexes	cis-diamminedichloroplatinum II (CDDP) Carboplatin Oxaliplatin Cisplatin
	Anthracendione	Mitoxantrone
	Substituted Urea	Hydroxyurea
	Methyl Hydraxzine Derivative	Procarbazine (N-methylhydrazine, (MIH)
	Adrenocortical Suppressant	Mitotane *(o,p'*-DDD) Aminoglutethimide
	Adrenocorticosteroids	Prednisone Dexamethasone

Hormones and Antagonists	Progestins	Hydroxyprogesterone Caproate Medroxyprogesterone Acetate Megestrol acetate
	Estrogens	Diethylstilbestrol Ethinyl estradiol
	Antiestrogen	Tamoxifen
	Androgens	Testosterone propionate Fluoxymesterone
	Antiandrogen	Flutamide
	Gonadotropin-releasing Hormone analog	Leuprolide

In certain embodiments, the chemotherapeutic agents used in the compositions and methods can be a single agent or a combination of agents. Preferred combinations will include agents that have different mechanisms of action, *e.g.*, the use of an anti-mitotic agent in combination with an alkylating agent.

In some embodiments, the anti-cancer agent is an inhibitor of ERK signaling, such as an inhibitor of MEK. As used herein, the term "inhibitor of MEK" refers to a compound or agent, such as a small molecule, that inhibits, decreases, lowers, or reduces the activity of MEK. Examples of inhibitors of MEK include, but are not limited to, AZD6244 (6-(4-Bromo-2-chloro-phenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxy-ethoxy)-amide; selumetinib; Structure IV), and U0126 (1,4-diamino-2,3-dicyano-1,4-bis [2-aminophenylthio]butadiene; ARRY-142886; Structure V). Further non-limiting examples of MEK inhibitors include PD0325901, AZD2171, GDC-0973/XL-518, PD98059, PD184352, GSK1120212, RDEA436, RDEA119/BAY869766, AS703026, BIX 02188, BIX 02189, CI-1040 (PD184352), PD0325901, and PD98059. These and other inhibitors of MEK, as well as non-limiting examples of their methods of manufacture, are described in U.S. Pat. Nos. 5,525,625; 6,251,943; 7,820,664; 6,809,106; 7,759,518; 7,485,643; 7,576,072; 7,923,456; 7,732,616; 7,271,178; 7,429,667; 6,649,640; 6,495,582; 7,001,905; US Patent Publication No. US2010/0331334, US2009/0143389, US2008/0280957, US2007/0049591, US2011/0118298, International Patent Application Publication No. WO98/43960, WO99/01421, WO99/01426, WO00/41505, WO00/42002, WO00/42003, WO00/41994, WO00/42022, WO00/42029, WO00/68201, WO01/68619, WO02/06213 and WO03/077914, the contents of which are herein incorporated by reference in their entireties.

In another embodiment, the anti-cancer agent is an inhibitor of Epidermal Growth Factor Receptor (EGFR). EGFR is a member of the type 1 subgroup of receptor tyrosine kinase family of growth factor receptors which play critical roles in cellular growth, differentiation and survival. Activation of these receptors typically occurs via specific ligand binding which results in hetero- or homodimerization between receptor family members, with subsequent autophosphorylation of the tyrosine kinase domain. Specific ligands which bind to EGFR include epidermal growth factor (EGF), transforming growth factor alpha (TGF alpha), amphiregulin and some viral growth factors. Activation of EGFR triggers a cascade of intracellular signaling pathways involved in both cellular proliferation (the ras/raf/MAP kinase pathway) and survival (the PI3 kinase/Akt pathway). Members of this family, including EGFR and HER2, have been directly implicated in cellular transformation. A number of human malignancies are associated with aberrant or overexpression of EGFR and/or overexpression of its specific ligands. Aberrant or overexpression of EGFR has been associated with an adverse prognosis in a number of human cancers, including head and neck, breast, colon, prostate, lung (*e.g.*, NSCLC, adenocarcinoma and squamous lung cancer), ovarian, gastrointestinal cancers (gastric, colon, pancreatic), renal cell cancer, bladder cancer, glioma, gynecological carcinomas and prostate cancer. In some instances, overexpression of tumor EGFR has been correlated with both chemoresistance and a poor prognosis. Mutations in EGFR are associated with many types of cancer as well. For example, EGFR mutations are highly prevalent in non-mucinous BAC patients. Finberg, et al., J. Mol. Diagnostics. (2007) 9(3):320-26. In an embodiment the EGFR inhibitor is an antibody such as Erbitutux™ (cetuximab, Imclone Systems Inc.) and ABX-EGF (panitumumab, Abgenix, Inc.). In another embodiment the EGFR inhibitor is a small molecule that competes with ATP such as TarcevaTM (erlotinib, OSI Pharmaceuticals), Iressa™ (gefitinib, Astra-Zeneca), tyrphostins described by Dvir, et al., J Cell Biol., 113:857-865 (1991); tricyclic pyrimidine compounds disclosed in U.S. Pat. No. 5,679,683; compound 6-(2,6-dichlorophenyl)-2-(4-(2-diethylaininoethoxy)phenylamino)-- 8-methyl-8H-pyrido(2,3-d)pyrimidin-7-one (known as PD166285) disclosed in Panek, et al., Journal of Pharmacology and Experimental Therapeutics 283, 1433-1444 (1997).

In addition to the specific agents described above, it is further contemplated that a polypeptide, an antibody or antigen binding fragment thereof, a toxin, an RNA interfering molecule, an siRNA molecule, and shRNA molecule, an antisense oligonucleotide, a

peptide, a peptidomimetic, an aptamer, and the like, as well as combinations thereof, that appropriately enhance or inhibit the targets of pro-survival signaling pathways can also be used as a therapeutic agent according to the invention. In particular, the nucleic acid sequence, amino acid sequence, functional domain, structural domain, gene locus, and other identifying information for the signaling pathway targets described herein are well known in the art.

In certain embodiments, the payload is an siRNA moiety comprised of a sense strand and an antisense strand; the sense strand comprising a 3' end and a 5' end; and the antisense strand comprising a 3' end and a 5' end.

"Antisense" nucleic acids refer to nucleic acids that specifically hybridize (e.g., bind) with a complementary sense nucleic acid, e.g., cellular mRNA and/or genomic DNA, under cellular conditions so as to inhibit expression (e.g., by inhibiting transcription and/or translation). The binding may be by conventional base pair complementarity or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix.

The siRNA moiety may further include a guanosine at the 5'-end.

The sense and/or antisense strands of the siRNA moiety may equal to or less than 30, 25, 24, 23, 22, 21, 20, 19, 18 or 17 nucleotides in length. An siRNA moiety may include one or more overhangs. For example, the siRNA moiety may include one or two 3' overhangs of 2-3 nucleotides. In certain embodiments, the invention relates to any of the compositions described herein, wherein the siRNA moiety is composed of 21-nt sense and 21-nt antisense strands, paired in a manner to have a 19-nucleotide duplex region and a 2-nt 3' overhang at each 3' terminus. In certain embodiments, the invention relates to any of the compositions describe herein, wherein the 2-nt 3' overhang is either UU or dTdT. Symmetric 3'-overhangs ensure that the sequence-specific endonuclease complexes (siRNPs) are formed with approximately equal ratios of sense and antisense target RNA cleaving siRNPs. The 3'-overhang in the sense strand provides no contribution to recognition as it is believed the antisense siRNA strand guides target recognition. Therefore, the UU or dTdT 3'-overhang of the antisense sequences is complementary to the target mRNA but the symmetrical UU or dTdT 3'-overhang of the sense siRNA oligo does not need to correspond to the mRNA. The use of deoxythymidines in both 3'-overhangs may increase nuclease resistance, although siRNA duplexes with either UU or dTdT

overhangs work equally well. 2'-Deoxynucleotides in the 3' overhangs are as efficient as ribonucleotides, but are often cheaper to synthesize.

The targeted region in the mRNA, and hence the sequence in the siRNA duplex, are chosen using the following guidelines. The open reading frame (ORF) region from the cDNA sequence is recommended for targeting, preferably at least 50 to 100 nucleotides downstream of the start codon, most preferably at least 75-100. Both the 5' and 3' untranslated regions (UTRs) and regions near the start codon are not recommended for targeting as these may be richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNP endonuclease complex.

The sequence of the mRNA or cDNA is searched seeking the sequence AA(N19)TT. Sequences with approximately 50% G/C-content (30% to 70%) are used. If no suitable sequences are found, the search is extended to sequences AA(N21). The sequence of the sense siRNA corresponds to 5'-(N19)dTdT-3' or N21, respectively. In the latter case, the 3' end of the sense siRNA is converted to dTdT. The rationale for this sequence conversion is to generate a symmetric duplex with respect to the sequence composition of the sense and antisense 3' overhangs. It is believed that symmetric 3' overhangs help to ensure that the siRNPs are formed with approximately equal ratios of sense and antisense target RNA-cleaving siRNPs. The modification of the overhang of the sense sequence of the siRNA duplex is not expected to affect targeted mRNA recognition, as the antisense siRNA strand glides target recognition.

If the target mRNA does not contain a suitable AA(N21) sequence, it is recommended to search for NA(N21) The sequence of the sense and antisense strand may still be synthesized as 5' (N19)TT as the sequence of the 3' most nucleotide of the antisense siRNA does not appear to contribute to specificity.

It is further recommended to search the selected siRNA sequence against EST libraries in appropriate databases (e.g., NCBI BLAST database search) to ensure that only one gene is targeted.

The appropriately designed siRNAs are either obtained from commercial sources (such as Dharmacon Research, Lafayette, Colo.; Xergon, Huntsville, Ala.; Ambion, Austin, Tex.) or chemically synthesized used appropriately protected ribonucleoside phosphoramidites and a conventional DNA/RNA synthesizer according to standard protocols. The RNA oligonucleotides are 2'-deprotected, desalted and the two strands

annealed, according to manufacturer's specifications or conventional protocols, depending on how the siRNAs are obtained. All handling steps are conducted under strict sterile, RNase-free conditions.

In certain embodiments, linkers (also known as "linker molecules" or "cross-linkers" or "spacers") may be used to conjugate the payload to the protein. The majority of known cross-linkers react with amine, carboxyl, and sulfhydryl groups. Linker molecules may be responsible for different properties of the composition. The length of the linker should be considered in light of molecular flexibility during the conjugation step, and the availability of the conjugated molecule for its target. Longer linkers may thus improve the biological activity of the compositions of the invention, as well as the ease of preparation of them. The geometry of the linker may be used to orient a molecule for optimal reaction with a target. A linker with flexible geometry may allow the entire composition to conformationally adapt as it binds a target sequence. The nature of the linker may be altered for other various purposes. For example, the hydrophobicity of a polymeric linker may be controlled by the order of monomeric units along the polymer, e.g. a block polymer in which there is a block of hydrophobic monomers interspersed with a block of hydrophilic monomers.

The chemistry of preparing and utilizing a wide variety of molecular linkers is well-known in the art and many pre-made linkers for use in conjugating molecules are commercially available from vendors such as Pierce Chemical Co., Roche Molecular Biochemicals, United States Biological. Exemplary linker molecules for use in the compositions of the invention include, but are not limited to: aminocaproic acid (ACA); polyglycine, and any other amino acid polymer, polymers such as polyethylene glycol (PEG), polymethyl methacrylate (PMMA), polypropylene glycol (PPG); homobifunctional reagents such as APG, AEDP, BASED, BMB, BMDB, BMH, BMOE, BM[PEO]3, BM[PEO]4, BS3, BSOCOES, DFDNB, DMA, DMP, DMS, DPDPB, DSG, DSP (Lomant's Reagent), DSS, DST, DTBP, DTME, DTSSP, EGS, HBVS, Sulfo-BSOCOES, Sulfo-DST, Sulfo-EGS; heterobifunctional reagents such as ABH, AEDP, AMAS, ANB-NOS, APDP, ASBA, BMPA, BMPH, BMPS, EDC, EMCA, EMCH, EMCS, KMUA, KMUH, GMBS, LC-SMCC, LC-SPDP, MBS, MBuS, M2C2H, MPBH, MSA, NHS-ASA, PDPH, PMPI, SADP, SAED. SAND, SANPAH, SASD, SATP, SBAP, SFAD, SIA, SIAB, SMCC, SMPB, SMPH, SMPT, SPDP, Sulfo-EMCS, Sulfo-GMBS, Sulfo-HSAB, Sulfo-KMUS, Sulfo-LC-SPDP, Sulfo-MBS. Sulfo-NHS-LC-ASA, Sulfo-SADP, Sulfo-

SANPAH, Sulfo-SIAB, Sulfo-SMCC, Sulfo-SMPB, Sulfo-LC-SMPT, SVSB, TFCS; and trifunctional linkers such as Sulfo-SBED.

Branched linkers may be prepared or used so that multiple moieties per linker are able to react. Such multiply reactive linkers allow the creation of multimeric binding sites.

An appropriate linker may be a macromolecular polymer. Any of the above-mentioned polymers may comprise the macromolecular polymer. In certain embodiments, such macromolecular polymers may be comprised entirely of one type of polymeric molecule. In other embodiments, the macromolecular polymers may be comprised of more than one type of polymeric molecule. The macromolecular polymers may exist in many possible structures, for example, linear, comb-branched, dendrigraft, dendrimer, or a linear dendron architectural copolymer. For example, PEG and PPG may be used to create a variety of bi- and multivalent linkers. Methods of synthesizing, activating, and modifying branched PEG/PPG polymers and PEG/PPG block co-polymers are well-known in the art. PEG is hydrophilic, while PPG is hydrophobic. For instance, a linker could be synthesized with a PPG core and PEG branches.

In certain embodiments, the invention relates to any of the first compositions described herein, wherein the payload is an imaging agent or a diagnostic agent. For example, the imaging agent may be a fluorescent imaging agent, such as a fluorophore or a gadolinium chelator, or a magnetic imaging agent, such as a magnetite mineral, a paramagnetic metal ion, or a metal chelating peptide. The imaging agent may be bound to an endogenous site (e.g., a paramagnetic metal ion), bound to a chemically modified site (e.g., chemical modifications to covalently bind a fluorophore or a gadolinium chelator), or genetically incorporated (e.g., a metal chelating peptide).

Examples of imaging or diagnostic agents include fluorophores (e.g. Dy547), chromophores, chemoluminescing agents, radionuclides (e.g., In-111, Tc-99m, I-123, I-125 F-18, Ga-67, Ga-68) for Positron Emission Tomography (PET) and Single Photon Emission Tomography (SPECT), unpair spin atoms and free radicals (e.g., Fe, lanthanides, and Gd), and contrast agents (e.g., chelated (DTPA) manganese) for Magnetic Resonance Imaging (MRI).

Additional examples include radionuclides (e.g. F-18, I-124, I-123, I-125, I-131, Re-186, Re-188, Y-90, Bi-212, At-211, Sr-89, Ho-166, Sm-153, Cu-67, Cu-64, In-111, Tc-99m, Ga-67, and Ga-68).

In certain embodiments, the invention relates to any of the second compositions described herein, wherein the payload is an immunogen, for example, an immunogenic antigen. An immunogen is an antigen or any substance that may be specifically bound by components of the immune system (e.g., antibody, lymphocytes). An immunogen is capable of inducing humoral or cell-mediated immune response rather than immunological tolerance. For example, the immunogen may be selected from the group consisting of keyhole limpet hemocyanin (KLH), concholepas concholepas hemacyanin (CCH), bovine serum albumin (BSA), and ovalbumin (OVA). Further information may be found in Chen DS, et al. *Immunity*. 2013;39:1-10; and Chen DS, et al. *Clin Cancer Res*. 2012;18:6580-6587 (both incorporated by reference).

In certain embodiments, the invention relates to any of the second compositions described herein, wherein the payload is an adjuvant. In certain embodiments, the invention relates to any of the second compositions described herein, wherein the payload is an immunogen and an adjuvant. recruiting of professional antigen-presenting cells (APCs) to the site of antigen exposure; increasing the delivery of antigens by delayed/slow release (depot generation); immunomodulation by cytokine production (selection of Th1 or Th2 response); inducing T-cell response (prolonged exposure of peptide-MHC complexes [signal 1] and stimulation of expression of T-cell-activating co-stimulators [signal 2] on the APCs' surface) and targeting (e. g. carbohydrate adjuvants which target lectin receptors on APCs). Examples of adjuvants include, but are not limited to Freund's Complete Adjuvant, lipopolysaccharides, muramyldipeptide from TB, synthetic polynucleotides, aluminum hydroxide, aluminum phosphate, cytokines, and squalene.

Targeting Agent

In certain embodiments, the invention relates to any of the compositions described herein, wherein the composition is a cell-specific therapeutic and imaging-agent delivery system. Targeted therapeutic delivery systems can enhance the effective dose at the site, such as a tumor, while decreasing general exposure to the drug and its associated side effects.

Protein cage architectures have three surfaces (interior, subunit interface, and exterior) amenable to both genetic and chemical modification. Each surface can play a distinct role in the development of new targeted therapeutic and imaging agent delivery systems. *See* Figure 6. The cage interior can house therapeutics, the subunit interface

incorporates gadolinium (an MRI contrast agent) and the exterior presents cell-specific targeting ligands (such as peptides and antibodies).

Protein cages have many beneficial attributes that are useful in their development as targeted therapeutic and imaging agent delivery systems. Their size falls into the nanometer range shown to localize in tumors due to the enhanced permeability and retention effect. Their multivalent nature enables the incorporation of multiple functionalities (including targeting peptides and imaging agents) on a single protein cage. They are malleable to both chemical and genetic manipulation and can be produced in heterologous expression systems (including bacterial, yeast, and baculoviral systems). In addition, detailed atomic resolution structural information enables the rational design of genetic mutants with specific functions, including cell-specific targeting.

Another key component for the development of protein cage architectures as imaging and therapeutic agents is cell-specific targeting. In vivo application of the phage display library technique enabled the identification of peptides that bind specifically to the vasculature of particular organs as well as tumors. One of the most characterized of these targeting peptides is RGD-4C (CDCRGDCFC), which binds $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins that are more prevalently expressed within tumor vasculature. For example, RGD-4C and other targeting peptides may be incorporated on the exteriors of the proteins. Fluorescein labeling of cell-specific targeted cages enables their visualization by epifluorescence microscopy. In addition to genetic incorporation, cell-specific targeting ligands, including antibodies and peptides, have also been chemically coupled to protein cage platforms. For example, an anti-CD4 monoclonal antibody conjugated to a protein could enable targeting of $CD4^+$ lymphocytes within a population of splenocytes. The multivalent nature of protein cage architectures results in the presentation of multiple targeting ligands on their surfaces and may potentially aid in the interaction of these protein cages with many surfaces including receptors on a variety of cell types.

In certain embodiments, the invention relates to any of the compositions described herein, wherein the targeting agent is an anti-PD-1 antibody.

A targeting agent, or affinity reagent, is a molecule that binds to an antigen or receptor or other molecule. In some embodiments, a targeting agent is a molecule that specifically binds to an antigen or receptor or other molecule. In certain embodiments, some or all of a targeting agent is composed of amino acids (including natural, non-natural,

and modified amino acids), nucleic acids, or saccharides. In certain embodiments, a targeting agent is a small molecule.

Targeting agents in certain embodiments of the invention specifically bind to molecules or targets, such as a cell surface antigen, a cell surface receptor, or other cell surface molecule.

In some embodiments, the targeting agent is proteinaceous and may be present in a single peptide or polypeptide chain. In some embodiments, the polypeptide chain is a bispecific antibody.

Bispecific antibodies are well-established in the art as a Standard technique to create a single polypeptide that binds to two different determinants. Bispecific antibodies may be made in many different formats, including but not limited to quadroma, F(ab')2, tetravalent, heterodimeric scFv, bispecific scFv, tandem scFv, diabody and minibody formats, or scFvs appended to or recombinantly fused with whole antibodies.

Antibodies for use in the invention may be raised through any conventional method, such as through injection of immunogen into mice and subsequent fusions of lymphocytes to create hybridomas. Such hybridomas may then be used either (a) to produce antibody directly, which is purified and used for chemical conjugation to create a bispecific antibody, or (b) to clone cDNAs encoding antibody fragments for subsequent genetic manipulation. To illustrate one method employing the latter strategy, mRNA is isolated from the hybridoma cells, reverse-transcribed into cDNA using antisense oligo-dT or immunoglobulin gene-specific primers, and cloned into a plasmid vector. Clones are sequenced and characterized. They may then be engineered according to standard protocols to combine the heavy and light chains of each antibody, separated by a short peptide linker, into a bacterial or mammalian expression vector as previously described to produce a recombinant bispecific antibody, which are then expressed and purified according to well-established protocols in bacteria or mammalian cells. Antibodies, or other proteinaceous affinity molecules or targeting agents such as peptides, may also be created through display technologies that allow selection of interacting affinity reagents through the screening of very large libraries of, for example, immunoglobulin domains or peptides expressed by bacteriophage. Antibodies may also be humanized through grafting of human immunoglobulin domains, or made from transgenic mice or bacteriophage libraries that have human immunoglobulin genes/cDNAs.

In some embodiments, a targeting agent may comprise proteinaceous structures other than antibodies that are able to bind to protein targets specifically, including but not limited to avimers, ankyrin repeats and adnectins, and other such proteins with domains that can be evolved to generate specific affinity for antigens, collectively referred to as "antibody-like molecules." Modifications of proteinaceous affinity reagents through the incorporation of unnatural amino acids during synthesis may be used to improve their properties. Such modifications may have several benefits, including the addition of chemical groups that facilitate subsequent conjugation reactions.

In some embodiments, the targeting agent may be a peptide. In some embodiments, the peptide chain is a bispecific peptide. Peptides can readily be made and screened to create affinity reagents that recognize and bind to macromolecules such as proteins.

Bispecific affinity reagents may be constructed by separate synthesis and expression of the first and second affinity reagents. A polypeptide bispecific reagent can be expressed as two separately encoded chains that are linked by disulfide bonds during production in the same host cell, such as, for example, a bispecific scFv or diabody. Similarly, standard and widely used solid-phase peptide synthesis technology can be used to synthesize peptides, and chimeric bispecific peptides are well known in the art. A bispecific peptide strategy may be used to combine the first and second first and second affinity reagents in a single peptide chain. Alternatively, polypeptide chains or peptide chains can be expressed/synthesized separately, purified and then conjugated chemically to produce the bispecific affinity reagents useful in the compositions and methods described herein. Many different formats of antibodies may be used. Whole antibodies, $F(ab')_2$, F(ab'), scFv, as well as smaller Fab and single-domain antibody fragments may all be used to create the first and second affinity reagents. Following their expression and purification, the targeting agents can be chemically conjugated to the protein vehicle. Many conjugation chemistries may be used to effect this conjugation, including homofunctional or heterofunctional linkers that yield ester, amide, thioether, carbon-carbon, or disulfide linkages.

In some embodiments, the targeting agent is a peptide aptamer. A peptide aptamer is a peptide molecule that specifically binds to a target protein, and interferes with the functional ability of that target protein. Peptide aptamers consist of a variable peptide loop attached at both ends of a protein scaffold. Such peptide aptamers can often have a binding affinity comparable to that of an antibody (nanomolar range). Due to the highly selective

nature of peptide aptamers, they can be used not only to target a specific protein, but also to target specific functions of a given protein (e.g., a signaling function).

Peptide aptamers are usually prepared by selecting the aptamer for its binding affinity with the specific target from a random pool or library of peptides. Peptide aptamers can be isolated from random peptide libraries by yeast two-hybrid screens. They can also be isolated from phage libraries or chemically generated peptides/libraries.

In some embodiments, the targeting agent is a nucleic acid aptamer. Nucleic acid aptamers are nucleic acid oligomers that bind other macromolecules specifically; such aptamers that bind specifically to other macromolecules can be readily isolated from libraries of such oligomers by technologies such as SELEX.

In some embodiments, the targeting agent is an oligosaccharide. Certain oligosaccharides are known ligands for certain extracellular or cell surface receptors.

The targeting agent recognizes a cell surface antigen on the target cell. The targeting agent may be an antibody, antibody-like molecule, or a peptide, such as an integrin-binding RGD peptide, or a small molecule, such as vitamins, e.g., folate, sugars such as lactose and galactose, or other small molecules. The cell surface antigen may be any cell surface molecule that undergoes internalization, such as a protein, sugar, lipid head group or other antigen on the cell surface. Examples of cell surface antigens useful in the context of the invention include but are not limited to the transferrin receptor type 1 and 2, the EGF receptor, HER2/Neu, VEGF receptors, integrins, CD33, CD19, CD20, CD22 and the asialoglycoprotein receptor.

Following their expression/synthesis and purification, the targeting agents are associated with the protein (for example, the heavy chain or the light chain of clathrin) through a covalent coupling, either through recombinant fusion, or chemical conjugation or association.

In certain embodiments, the targeting agent is an HER-2-targeting antibody, for example, trastuzumab or pertuzumab.

In certain embodiments, the targeting agent is an EGFR-targeting antibody, such as IMC-225.

In certain embodiments, the targeting agent is a VEGFR-2-targeting antibody.

In certain embodiments, the targeting agent is a CD-20-targeting antibody.

In certain embodiments, the targeting agent is a CD-22-targeting antibody.

In certain embodiments, the targeting agent is a CD-4-targeting antibody.

Exemplary Methods of Therapy or Diagnostic Imaging

One aspect of the invention relates to a method of treating cancer in a subject in need thereof, comprising:

administering to the subject a therapeutically effective amount of any one of the first compositions described herein wherein the first payload is an anti-cancer agent.

One aspect of the invention relates to a method of treating cancer in a subject in need thereof, comprising:

administering to the subject a therapeutically effective amount of any one of the first compositions described herein wherein the first payload is an anti-cancer agent; and

administering to the subject a therapeutically effective amount of any one of the second compositions described herein.

The language "effective amount" of a targeted therapeutic agent refers to that amount necessary or sufficient to eliminate, reduce, or maintain (e.g., prevent the spread of) a tumor, or other target. The effective amount can vary depending on such factors as the disease or condition being treated, the particular targeted constructs being administered, the size of the subject, or the severity of the disease or condition. One of ordinary skill in the art can empirically determine the effective amount of a particular composition without undue experimentation.

In certain embodiments, the invention relates to any of the methods described herein, wherein the cancer is lung cancer. In certain embodiments, the invention relates to any of the methods described herein, wherein the cancer is non-small cell lung cancer (NSCLC).

In certain embodiments, the invention relates to any of the methods described herein, wherein the cancer is pancreatic cancer.

In certain embodiments, the invention relates to any of the methods described herein, wherein the first composition and the second composition are co-administered, i.e., wherein the first composition and the second composition are administered sequentially, simultaneously, or separately.

In certain embodiments, the invention relates to any of the methods described herein, wherein the first composition and the second composition are administered simultaneously, for example, in one pharmaceutical formulation.

Another aspect of the invention relates to a method generating an image of a subject in need thereof, comprising:

administering to the subject a detectable amount of any of the first compositions described herein wherein the first payload is an imaging agent or a diagnostic agent; and

generating an image.

The language "effective amount" of a targeted imaging agent refers to that amount necessary or sufficient to visualize a tumor, or other target. The effective amount can vary depending on such factors as the cells or tissue being imaged, the particular targeted constructs being administered, the size of the subject, or the severity of the disease or condition. One of ordinary skill in the art can empirically determine the effective amount of a particular composition without undue experimentation.

In certain embodiments, the invention relates to any one of the methods described herein, wherein the subject is a mammal; preferably, the subject is a human.

Exemplary Pharmaceutical Compositions

In another aspect, the invention provides pharmaceutically acceptable compositions which comprise a therapeutically-effective amount of one or more of the compositions described above, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. As described in detail below, the pharmaceutical compositions of the invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; (3) topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; (5) sublingually; (6) ocularly; (7) transdermally; or (8) nasally.

As set out above, certain embodiments of the compositions may contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable acids. The term "pharmaceutically-acceptable salts" in this respect, refers to the relatively non-toxic, inorganic and organic acid addition salts of components of the compositions of the invention. These salts can be prepared *in situ* in the administration vehicle or the dosage form manufacturing process, or by separately reacting a purified compound in its free base

form with a suitable organic or inorganic acid, and isolating the salt thus formed during subsequent purification. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, for example, Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19)

The pharmaceutically acceptable salts of the subject components include the conventional nontoxic salts or quaternary ammonium salts of the compounds, e.g., from non-toxic organic or inorganic acids. For example, such conventional nontoxic salts include those derived from inorganic acids such as hydrochloride, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmitic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicyclic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isothionic, and the like.

In other cases, components of the compositions of the invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable bases. The term "pharmaceutically-acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of components of the compositions of the invention. These salts can likewise be prepared *in situ* in the administration vehicle or the dosage form manufacturing process, or by separately reacting the purified component in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation, with ammonia, or with a pharmaceutically-acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations of the invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 0.1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

In certain embodiments, a formulation of the invention comprises an excipient selected from the group consisting of cyclodextrins, celluloses, liposomes, micelle forming agents, e.g., bile acids, and polymeric carriers, e.g., polyesters and polyanhydrides; and a compound of the invention. In certain embodiments, an aforementioned formulation renders orally bioavailable a composition of the invention.

Methods of preparing these formulations or compositions include the step of bringing into association a composition of the invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a composition of the invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and

acacia) and/or as mouth washes and the like, each containing a predetermined amount of a composition of the invention as an active ingredient. A composition of the invention may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules, trouches and the like), the composition is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds and surfactants, such as poloxamer and sodium lauryl sulfate; (7) wetting agents, such as, for example, cetyl alcohol, glycerol monostearate, and non-ionic surfactants; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, zinc stearate, sodium stearate, stearic acid, and mixtures thereof; (10) coloring agents; and (11) controlled release agents such as crospovidone or ethyl cellulose. In the case of capsules, tablets and pills, the pharmaceutical formulation may also comprise buffering agents. Solid formulations of a similar type may also be employed as fillers in soft and hard-shelled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered composition moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical formulations of the invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow

or controlled release of the composition or the payload therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be formulated for rapid release, e.g., freeze-dried. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These formulations may also optionally contain opacifying agents and may be formulated so that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the compositions of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral formulations can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the compositions, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Dosage forms for the topical or transdermal administration of a composition of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The composition may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

Pharmaceutical formulations of this invention suitable for parenteral administration comprise one or more compositions of the invention in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain sugars, alcohols, antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical formulations of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These formulations may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms upon the subject compounds may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

When the compositions of the invention are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical formulation containing, for example, 0.1 to 99% (more preferably, 10 to 30%) of composition in combination with a pharmaceutically acceptable carrier.

The formulations of the invention may be given orally, parenterally, topically, or rectally. They are of course given in forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories.

These formulations may be administered to humans and other animals for therapy by any suitable route of administration, including orally, nasally, as by, for example, a

spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

Regardless of the route of administration selected, the compositions of the invention, which may be used in a suitable hydrated form, and/or the pharmaceutical formulations of the invention, are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical formulations of this invention may be varied so as to obtain an amount of the payload which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular composition of the invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion or metabolism of the particular composition being employed, the rate and extent of absorption, the duration of the treatment, other drugs, compositions and/or materials used in combination with the particular composition employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical formulation required. For example, the physician or veterinarian could start doses of the compositions of the invention employed in the pharmaceutical formulation at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In general, a suitable daily dose of a composition of the invention will be that amount of the composition that is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above.

If desired, the effective daily dose of the composition may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. Preferred dosing is one administration per day.

While it is possible for a composition of the invention to be administered alone, it is preferable to administer the composition as a pharmaceutical formulation.

The composition according to the invention may be formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other pharmaceuticals.

In another aspect, the invention provides pharmaceutically acceptable formulations that comprise a therapeutically-effective amount of one or more of the subject compositions, as described above, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. As described in detail below, the pharmaceutical formulations of the invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension; (3) topical application, for example, as a cream, ointment or spray applied to the skin, lungs, or mucous membranes; or (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; (5) sublingually or buccally; (6) ocularly; (7) transdermally; or (8) nasally.

The patient receiving this treatment is any animal in need, including primates, in particular humans, and other mammals such as equines, cattle, swine and sheep; and poultry and pets in general.

Conjunctive or combination therapy, thus includes sequential, simultaneous and separate administration of the compositions in a way that the therapeutical effects of the first administered one is not entirely disappeared when the subsequent is administered.

Exemplary Kits

In certain embodiments, the invention relates to a kit for treating or imaging cancer. For example, a kit may comprise one or more compositions as described above and optionally instructions for their use; preferably the kit comprises a first composition and a second composition. In still other embodiments, the invention provides kits comprising one or more pharmaceutical or diagnostic formulations and/or one or more devices for accomplishing administration. For example, a subject kit may comprise a pharmaceutical or diagnostic formulation and catheter for accomplishing direct injection.

EXEMPLIFICATION

The invention now being generally described, it will be more readily understood by reference to the following examples which are included merely for purposes of illustration

of certain aspects and embodiments of the invention, and are not intended to limit the invention.

Example 1 – Expression of clathrin heavy chain

Clathrin human isoform 2 heavy chain was optimized for an *E. coli* expression system as follows:

(SEQ ID NO:1):

MAQILPIRFQEHLQLQNLGINPANIGFSTLTMESDKFICIREKVGEQAQVVIIDMNDPS
NPIRRPISADSAIMNPASKVIALKAGKTLQIFNIEMKSKMKAHTMTDDVTFWKWISLNT
VALVTDNAVYHWSMEGESQPVKMFDRHSSLAGCQIINYRTDAKQKWLLLTGISAQQNRV
VGAMQLYSVDRKVSQPIEGHAASFAQFKMEGNAEESTLFCFAVRGQAGGKLHIIEVGTP
PTGNQPFPKKAVDVFFPPEAQNDFPVAMQISEKHDVVFLITKYGYIHLYDLETGTCIYM
NRISGETIFVTAPHEATAGIIGVNRKGQVLSVCVEEENIIPYITNVLQNPDLALRMAVR
NNLAGAEELFARKFNALFAQGNYSEAAKVAANAPKGILRTPDTIRRFQSVPAQPGQTSP
LLQYFGILLDQGQLNKYESLELCRPVLQQGRKQLLEKWLKEDKLECSEELGDLVKSV
DPTLALSVYLRANVPNKVIQCFAETGQVQKIVLYAKKVGYTPDWIFLLRNVMRISPDQG
QQFAQMLVQDEEPLADITQIVDVFMEYNLIQQCTAFLLDALKNNRPSEGPLQTRLLEMN
LMHAPQVADAILGNQMFTHYDRAHIAQLCEKAGLLQRALEHFTDLYDIKRAVVHTHLLN
PEWLVNYFGSLSVEDSLECLRAMLSANIRQNLQICVQVASKYHEQLSTQSLIELFESFK
SFEGLFYFLGSIVNFSQDPDVHFKYIQAACKTGQIKEVERICRESNCYDPERVKNFLKE
AKLTDQLPLIIVCDRFDFVHDLVLYLYRNNLQKYIEIYVQKVNPSRLPVVIGGLLDVDC
SEDVIKNLILVVRGQFSTDELVAEVEKRNRLKLLLPWLEARIHEGCEEPATHNALAKIY
IDSNNNPERFLRENPYYDSRVVGKYCEKRDPHLACVAYERGQCDLELINVCNENSLF
KSLSRYLVRRKDPELWGSVLLESNPYRRPLIDQVVQTALSETQDPEEVSVTVKAFMTAD
LPNELIELLEKIVLDNSVFSEHRNLQNLLILTAIKADRTRVMEYINRLDNYDAPDIANI
AISNELFEEAFAIFRKFDVNTSAVQVLIEHIGNLDRAYEFAERCNEPAVWSQLAKAQLQ
KGMVKEAIDSYIKADDPSSYMEVVQAANTSGNWEELVKYLQMARKKARESYVETELIFA
LAKTNRLAELEEFINGPNNAHIQQVGDRCYDEKMYDAAKLLYNNVSNFGRLASTLVHLG
EYQAAVDGARKANSTRTWKEVCFACVDGKEFRLAQMCGLHIVVHADELEELINYYQDRG
YFEELITMLEAALGLERAHMGMFTELAILYSKFKPQKMREHLELFWSRVNIPKVLRAAE
QAHLWAELVFLYDKYEEYDNAIITMMNHPTDAWKEGQFKDIITKVANVELYYRAIQF
YLEFKPLLLNDLLMVLSPRLDHTRAVNYFSKVKQLPLVKPYLRSVQNHNNKSVNESLNL
FITEEDYQALRTSIDAYDNFDNISLAQRLEKHELIEFRRIAAYLFKGNNRWKQSVELCK
KDSLYKDAMQYASESKDTELAEELLQWFLQEEKRECFGACLFTCYDLLRPDVVLETAWR
HNIMDFAMPYFIQVMKEYLTKVDKLDASESLRKEEEQATETQPIVYGNLSL

(SEQ ID NO:2):

ATGGCGCAGATCCTGCCGATTCGCTTCCAGGAACACCTGCAaCTGCAaAACCTGGGCAT
CAACCCGGCAAACATCGGTTTCTCTACCCTGACtATGGAGTCTGATAAGTTTATCTGTA
TCCGTGAGAAAGTGGGTGAGCAGGCTCAGGTGGTGATTATTGACATGAACGACCCGTCT
AACCCGATCCGTCGCCCGATCTCCGCAGATTCCGCAATCATGAACCCGGCGTCCAAGGT
TATCGCGCTGAAAGCTGGTAAGACCCTGCAaATCTTTAACATTGAGATGAAGTCCAAAA
TGAAGGCGCATACCATGACCGACGACGTTACCTTCTGGAAGTGGATCTCTCTGAACACC
GTTGCACTGGTTACTGACAACGCGGTGTACCACTGGTCTATGGAAGGTGAATCCCAGCC
GGTTAAAATGTTCGACCGTCATTCTTCTCTGGCGGGTTGCCAGATTATCAACTACCGTA
CCGACGCGAAACAGAAATGGCTGCTGCTGACTGGCATTTCCGCACAGCAGAACCGCGTG
GTTGGTGCAATGCAGCTGTACTCTGTGGACCGTAAGGTGTCTCAGCCGATCGAAGGTCA

CGCTGCGTCCTTTGCGCAGTTCAAGATGGAGGGTAACGCGGAAGAATCCACCCTGTTTT
GCTTTGCGGTGCGTGGCCAGGCGGGTGGTAAACTGCATATTATCGAGGTTGGCACTCCG
CCGACCGGCAACCAGCCGTTCCCGAAAAAAGCGGTTGACGTTTTCTTTCCGCCGGAAGC
TCAGAACGACTTCCCGGTTGCGATGCAGATTAGCGAGAAACACGACGTGGTTTTCCTGA
TTACCAAGTACGGCTACATCCACCTGTACGACCTGGAGACTGGcACCTGCATCTATATG
AACCGTATCTCTGGTGAAACCATCTTCGTTACTGCTCCGCATGAGGCGACCGCtGGTAT
CATCGGTGTTAACCGTAAAGGTCAGGTGCTGTCTGTTTGTGTTGAGGAAGAGAACATCA

TCCCGTACATCACTAACGTTCTGCAaAACCCGGACCTGGCGCTGCGCATGGCGGTTCGC
AACAACCTGGCAGGCGCTGAGGAGCTGTTCGCGCGTAAATTCAACGCGCTGTTTGCTCA
GGGCAACTATTCTGAAGCGGCGAAAGTTGCTGCAAACGCGCCGAAAGGCATCCTGCGTA
CTCCGGACACCATCCGCCGTTTCCAGTCCGTGCCGGCGCAGCCGGGTCAGACCTCCCCG
CTGCTGCAaTATTTTGGTATCCTGCTGGACCAGGGTCAGCTGAACAAGTATGAAAGCCT
GGAACTGTGCCGTCCGGTGCTGCAaCAGGGCCGTAAACAGCTGCTGGAGAAGTGGCTGA
AGGAAGACAAACTGGAATGCTCCGAAGAGCTGGGTGACCTGGTTAAATCCGTGGACCCG
ACTCTGGCACTGAGCGTGTATCTGCGTGCGAACGTGCCGAACAAAGTTATCCAGTGCTT
CGCGGAAACCGGCCAGGTGCAGAAGATTGTTCTGTACGCAAAAAAAGTTGGCTATACCC
CGGATTGGATCTTTCTGCTGCGTAACGTGATGCGTATCAGCCCGGATCAGGGCCAGCAG
TTTGCACAGATGCTGGTTCAGGACGAGGAGCCGCTGGCGGACATTACCCAGATCGTTGA
TGTTTTTATGGAATATAACCTGATTCAGCAGTGTACTGCGTTCCTGCTGGATGCTCTGA
AAAACAACCGTCCGTCTGAGGGTCCGCTGCAaACTCGTCTGCTGGAAATGAACCTGATG
CACGCGCCGCAGGTGGCAGATGCAATTCTGGGCAACCAGATGTTCACTCACTATGACCG
CGCTCATATCGCGCAGCTGTGCGAAAAAGCGGGTCTGCTGCAaCGTGCGCTGGAGCATT
TCACCGACCTGTACGACATTAAGCGTGCTGTGGTGCATACTCATCTGCTGAACCCGGAA
TGGCTGGTTAACTATTTCGGTTCTCTGAGCGTGGAAGACTCCCTGGAGTGCCTGCGCGC
GATGCTGTCCGCAAACATCCGTCAGAACCTGCAaATTTGTGTTCAGGTGGCTTCTAAAT
ACCATGAACAGCTGAGCACCCAGTCTCTGATTGAGCTGTTTGAATCTTTCAAGTCCTTC
GAGGGCCTGTTCTACTTCCTGGGTTCTATCGTGAACTTCTCTCAGGAcCCGGACGTTCA
TTTCAAATACATTCAGGCTGCGTGCAAAACtGGTCAGATCAAAGAAGTGGAACGTATCT
GCCGCGAATCTAACTGCTACGACCCGGAGCGCGTGAAGAACTTTCTGAAAGAAGCGAAG
CTGACCGACCAGCTGCCGCTGATCATCGTTTGTGACCGTTTCGACTTCGTTCATGATCT
GGTGCTGTACCTGTATCGTAACAACCTGCAaAAGTACATTGAGATtTACGTTCAGAAGG
TGAACCCGTCTCGTCTGCCGGTGGTTATTGGTGGCCTGCTGGATGTGGACTGCTCTGAA
GACGTTATCAAAAACCTGATCCTGGTTGTTCGTGGCCAGTTCTCCACCGATGAACTGGT
GGCTGAGGTTGAAAAGCGTAACCGTCTGAAACTGCTGCTGCCGTGGCTGGAAGCGCGTA
TCCACGAAGGTTGTGAGGAACCGGCGACCCATAACGCGCTGGCGAAAATCTATATCGAC
TCTAACAACAACCCGGAACGCTTCCTGCGTGAAAACCCGTATTACGACTCTCGTGTTGT
GGGTAAATACTGTGAGAAACGTGATCCGCACCTGGCGTGTGTTGCGTACGAACGTGGTC
AGTGCGACCTGGAACTGATCAACGTTTGTAACGAAAACTCTCTGTTCAAATCTCTGTCT
CGTTACCTGGTGCGTCGCAAAGATCCGGAGCTGTGGGGTAGCGTTCTGCTGGAATCCAA
CCCGTACCGTCGTCCGCTGATTGACCAGGTGGTTCAGACTGCGCTGAGCGAGACTCAGG
ACCCGGAGGAAGTTAGCGTTACCGTTAAAGCATTCATGACTGCgGACCTGCCGAACGA
GCTGATCGAGCTGCTGGAGAAAATTGTTCTGGACAACTCCGTTTTTAGCGAACACCGCA
ACCTGCAaAACCTGCTGATTCTGACTGCGATCAAGGCGGATCGTACCCGCGTGATGGAA
TATATCAACCGCCTGGATAACTATGATGCGCCGGACATCGCGAACATCGCTATCTCTAA
CGAACTGTTCGAAGAAGCgTTTGCGATTTTCCGTAAATTCGACGTTAACACCTCTGCGG
TGCAGGTGCTGATCGAACATATCGGTAACCTGGACCGTGCGTATGAGTTCGCAGAGCGC
TGCAACGAGCCGGCAGTTTGGTCCCAGCTGGCAAAGGCTCAGCTGCAaAAGGGTATGGT
TAAAGAAGCAATCGACTCTTACATCAAAGCGGATGATCCGTCTAGCTATATGGAAGTTG
TGCAGGCAGCGAACACCTCCGGTAACTGGGAGGAGCTGGTGAAGTACCTGCAaATGG
CGCGCAAAAAGGCGCGTGAATCTTATGTGGAGACCGAGCTGATTTTCGCGCTGGCGAAA

ACCAACCGCCTGGCGGAACTGGAGGAGTTTATCAACGGTCCGAACAACGCTCATATCCA
GCAGGTTGGCGATCGTTGCTACGACGAAAAAATGTACGACGCGGCGAAGCTGCTGTACA
ACAACGTTTCTAACTTCGGCCGTCTGGCTTCTACTCTGGTGCATCTGGGCGAGTATCAG
GCTGCGGTGGACGGTGCGCGTAAAGCGAACTCTACCCGCACTTGGAAAGAAGTTTGCTT

CGCGTGTGTTGACGGCAAAGAATTTCGTCTGGCGCAGATGTGCGGTCTGCACATTGTGG
TGCACGCTGACGAGCTGGAAGAGCTGATCAACTACTATCAGGATCGTGGTTACTTTGAA
GAACTGATCACCATGCTGGAGGCGGCACTGGGTCTGGAACGTGCTCACATGGGTATG
TTCACCGAACTGGCAATCCTGTACTCTAAATTCAAGCCGCAGAAAATGCGCGAGCACCT
GGAACTGTTTTGGAGCCGCGTTAACATCCCGAAGGTTCTGCGTGCGGCGGAGCAGGCGC
ATCTGTGGGCTGAACTGGTGTTTCTGTATGATAAGTATGAGGAATATGACAACGCGATT
ATCACTATGATGAACCATCCGACCGACGCGTGGAAGGAAGGTCAGTTTAAGGACATCAT
CACTAAAGTGGCGAACGTGGAGCTGTACTACCGTGCGATCCAGTTTTACCTGGAGTTCA
AACCGCTGCTGCTGAACGATCTGCTGATGGTGCTGTCTCCGCGTCTGGACCACACCCGT
GCTGTGAACTACTTCTCTAAGGTTAAACAGCTGCCGCTGGTTAAGCCGTATCTGCGTAG
CGTTCAGAACCATAACAACAAGAGCGTGAACGAATCCCTGAACAACCTGTTCATTACCG
AAGAAGACTACCAGGCACTGCGTACCTCTATCGATGCTTACGACAACTTTGATAACATC
TCTCTGGCACAGCGCCTGGAAAAACATGAACTGATTGAGTTCCGTCGCATCGCGGCTTA
TCTGTTCAAGGGCAACAACCGTTGGAAACAGTCTGTTGAGCTGTGCAAAAAAGATTCTC
TGTATAAAGATGCAATGCAGTACGCGTCCGAATCTAAAGACACTGAGCTGGCTGAGGAA
CTGCTGCAaTGGTTCCTGCAaGAGGAGAAGCGCGAGTGCTTCGGTGCTTGCCTGTTTAC
TTGCTATGACCTGCTGCGTCCGGATGTTGTTCTGGAAACTGCTTGGCGTCATAACATTA
TGGACTTTGCGATGCCGTACTTTATCCAGGTTATGAAAGAATATCTGACCAAAGTGGAC
AAGCTGGACGCGAGCGAAAGCCTGCGCAAGGAGGAAGAACAGGCTACCGAAACCCAGCC
GATCGTGTACGGTAACCTGTCTCTG

The preparation yielded a protein with the following characteristics:

Protein Description:	1) 22.4 mg, >85%, soluble protein with 6His tag from *E. coli*; 2) QC by SDS-PAGE and Western-Blot.
Protein Concentration:	0.8 mg/mL, as determined by Bradford protein assay with BSA as a standard.
Final Prep:	Fusion protein: 22.4 mg; 1.0 mL/vial; 28 vials.
Purity:	>85 % as estimated by a Coomassie blue-stained SDS-PAGE gel
Storage Buffer:	20 mM Tris.HCl, pH 7.5, 20% Glycerol
Storage:	Immediate Storage at -20 °C upon receiving; At first use, aliquot and store at -20 °C to avoid multiple freeze-thaws.
Intended Use:	This product is intended for research use only. It is not for any human or animal diagnostic and therapeutic use.
Isoelectric Point	5.67
Molecular Weight	188,955 Da
Quality Assurance (*see* Figure 4)	M1: SDS-PAGE Protein Marker Lane 1: PE1130119-1 protein M2: Western-Blot Protein Marker Lane 2: PE1130119-1 protein (using anti-6His antibody)

Sequence	(*see* below)

(SEQ ID NO:3):

1 MAQILPIRFQ EHLQLQNLGI NPANIGFSTL TMESDKFICI REKVGEQAQV
VIIDMNDPSN PIRRPISADS AIMNPASKVI FNIEMKSKMK AHTMTDDVTF
5 WKWISLNTVA LVTDNAVYHW SMEGESQPVK MFDRHSSLAG CQIINYRTDA
81ALKAGKTLQI 161

KQKWLLLTGI SAQQNRVVGA MQLYSVDRKV SQPIEGHAAS FAQFKMEGNA
EESTLFCFAV RGQAGGKLHI IEVGTPPTGN 241

QPFPKKAVDV FFPPEAQNDF PVAMQISEKH DVVFLITKYG YIHLYDLETG
10 TCIYMNRISG ETIFVTAPHE ATAGIIGVNR 321

KGQVLSVCVE EENIIPYITN VLQNPDLALR MAVRNNLAGA EELFARKFNA
LFAQGNYSEA AKVAANAPKG ILRTPDTIRR 401

FQSVPAQPGQ TSPLLQYFGI LLDQGQLNKY ESLELCRPVL QQGRKQLLEK
WLKEDKLECS EELGDLVKSV DPTLALSVYL 481

15 RANVPNKVIQ CFAETGQVQK IVLYAKKVGY TPDWIFLLRN VMRISPDQGQ
QFAQMLVQDE EPLADITQIV DVFMEYNLIQ 561

QCTAFLLDAL KNNRPSEGPL QTRLLEMNLM HAPQVADAIL GNQMFTHYDR
AHIAQLCEKA GLLQRALEHF TDLYDIKRAV 641

VHTHLLNPEW LVNYFGSLSV EDSLECLRAM LSANIRQNLQ ICVQVASKYH
20 EQLSTQSLIE LFESFKSFEG LFYFLGSIVN 721

FSQDPDVHFK YIQAACKTGQ IKEVERICRE SNCYDPERVK NFLKEAKLTD
QLPLIIVCDR FDFVHDLVLY LYRNNLQKYI 801

EIYVQKVNPS RLPVVIGGLL DVDCSEDVIK NLILVVRGQF STDELVAEVE
KRNRLKLLLP WLEARIHEGC EEPATHNALA 881

25 KIYIDSNNNP ERFLRENPYY DSRVVGKYCE KRDPHLACVA YERGQCDLEL
INVCNENSLF KSLSRYLVRR KDPELWGSVL 961

LESNPYRRPL IDQVVQTALS ETQDPEEVSV TVKAFMTADL PNELIELLEK
IVLDNSVFSE HRNLQNLLIL TAIKADRTRV 1041

MEYINRLDNY DAPDIANIAI SNELFEEAFA IFRKFDVNTS AVQVLIEHIG
30 NLDRAYEFAE RCNEPAVWSQ LAKAQLQKGM 1121

VKEAIDSYIK ADDPSSYMEV VQAANTSGNW EELVKYLQMA RKKARESYVE
TELIFALAKT NRLAELEEFI NGPNNAHIQQ 1201

VGDRCYDEKM YDAAKLLYNN VSNFGRLAST LVHLGEYQAA VDGARKANST
RTWKEVCFAC VDGKEFRLAQ MCGLHIVVHA 1281

DELEELINYY QDRGYFEELI TMLEAALGLE RAHMGMFTEL AILYSKFKPQ KMREHLELFW SRVNIPKVLR AAEQAHLWAE 1361

LVFLYDKYEE YDNAIITMMN HPTDAWKEGQ FKDIITKVAN VELYYRAIQF YLEFKPLLLN DLLMVLSPRL DHTRAVNYFS 1441

KVKQLPLVKP YLRSVQNHNN KSVNESLNNL FITEEDYQAL RTSIDAYDNF DNISLAQRLE KHELIEFRRI AAYLFKGNNR 1521

WKQSVELCKK DSLYKDAMQY ASESKDTELA EELLQWFLQE EKRECFGACL FTCYDLLRPD VVLETAWRHN IMDFAMPYFI 1601

QVMKEYLTKV DKLDASESLR KEEEQATETQ PIVYGNLSLL EHHHHHH

Example 2 – Expression of clathrin light chain

Clathrin light chain (below) was expressed in *E. coli*:

(SEQ ID NO:4):

MAELDPFGAPAGAPGGPALGNGVAGAGEEDPAAAFLAQQESEIAGIENDEAFAILDGGA
PGPQPHGEPPGGPDAVDGVMNGEYYQESNGPTDSYAAISQVDRLQSEPESIRKWREEQM

ERLEALDANSRKQEAEWKEKAIKELEEWYARQDEQLQKTKANNRVADEAFYKQPFADVI
GYVTNINHPCYSLEQAAEEAFVNDIDESSPGTEWERVARLCDFNPKSSKQAKDVSRMRS
VLISLKQAPLVH

(SEQ ID NO:5):

ATGGCGGAACTGGACCCGTTCGGCGCTCCGGCAGGCGCACCGGGCGGTCCGGCGCTGGG
TAACGGCGTTGCGGGTGCTGGTGAAGAAGACCCGGCAGCAGCGTTCCTGGCGCAGCAGG
AATCTGAAATCGCAGGTATCGAAAACGATGAAGCGTTCGCGATCCTGGACGGTGGTGCT
CCGGGTCCGCAGCCGCACGGTGAACCGCCGGGTGGTCCGGATGCGGTTGACGGTGTTAT
GAACGGCGAGTACTACCAGGAGTCTAACGGTCCGACCGATTCTTACGCGGCAATTAGCC
AGGTTGATCGTCTGCAaTCCGAACCGGAATCTATCCGTAAATGGCGTGAGGAGCAGATG
GAACGCCTGGAAGCTCTGGACGCGAACTCTCGCAAACAGGAGGCGGAATGGAAAGAAAA
AGCGATCAAAGAGCTGGAAGAATGGTATGCGCGTCAGGACGAACAGCTGCAaAAAACCA
AAGCGAACAACCGTGTGGCGGACGAAGCATTCTACAAACAGCCGTTTGCGGACGTTATC
GGTTACGTTACCAACATCAACCATCCGTGCTACTCTCTGGAGCAGGCAGCGGAAGAAGC
gTTCGTGAACGACATCGACGAATCTAGCCCaGGcACCGAATGGGAACGTGTTGCGCGCC
TGTGCGACTTCAACCCGAAATCTTCTAAACAGGCTAAAGACGTTTCTCGTATGCGTTCT
GTTCTGATCTCTCTGAAGCAGGCTCCGCTGGTTCAC

The preparation yielded a protein with the following characteristics:

Protein Description:

12.96 mg, >85%, soluble protein with 6His tag from *E. coli*;

Protein Concentration:

0.60 mg/mL, as determined by Bradford protein assay with BSA as a standard.

Final Prep:

1.8 mL/tube, 12 tubes

Purity:

>85% as estimated by a Coomassie blue-stained SDS-PAGE gel

Storage Buffer:

50 mM Tris, 150 mM NaCl, 10% Glycerol, pH 8.0

Storage:

Immediate Storage at -20 °C upon receiving

At first use, aliquot and store at -20 °C to avoid multiple freeze-thaws.

Intended Use:

This product is intended for research use only. It is not for any human or animal diagnostic and therapeutic use.

Protein Sequence (SEQ ID NO:6):

```
1 MAELDPFGAP AGAPGGPALG NGVAGAGEED PAAAFLAQQE SEIAGIENDE AFAILDGGAP
61 GPQPHGEPPG  GPDAVDGVMN GEYYQESNGP TDSYAAISQV DRLQSEPESI RKWREEQMER
121 LEALDANSRK QEAEWKEKAI KELEEWYARQ DEQLQKTKAN NRVADEAFYK QPFADVIGYV
181 TNINHPCYSL EQAAEEAFVN DIDESSPGTE WERVARLCDF NPKSSKQAKD VSRMRSVLIS
241 LKQAPLVHLE HHHHHH
```

Protein Length
256

MW
28136.9

Predicted pI
4.37

Quality Assurance (*see* Figure 5):

M1: SDS-PAGE Protein Marker

Lane 1: PE1130119-2 protein

M2: Western-Blot ProteinMarker

Lane 2: PE1130119-2 protein (using Anti-6His antibody)

Example 3 – Loading of self-assembled protein

The self-assembled protein was loaded with a fluorescent compound to assess its ability to self-assembling following loading.

Recombinant clathrin heavy chain (HC) and light chain (LC) were diluted at 300 μg/mL and 800 μL/mL, respectively in 10 mM Tris-HCl (pH 7.9). A fluoresceinated test compound (FTC) was diluted at 500 μg/mL in the same buffer. Assembly of 100 μL in a 96-well assay plate was initiated by adding 4 μL of 1 M 2-(N-morpholino)ethanesulfonic acid (MES) buffer, pH 6.5 supplemented with 10 mM ethylene glycol tetraacetic acid (EGTA) and 75 mM $CaCl_2$. A control was used with pH 7 MES buffer. OD320 nm readings were measured using the SpectraMax M3 (molecular devices) and the results were plotted by the software provided by the equipment.

Example 4 – Loading of self-assembled protein (prophetic)

A variety of ratios of HC, LC, and FTC, as well as low pH, are being tested in order to investigate assembling efficiency.

Other experiments to study drug loading are being tested.

1. Load or attach the drug to the light chain assembly cage and then load the loaded light chain to the heavy chain in self-assembling conditions (indirect loading to the main cage). The light chain may increase the stability of the main heavy chain cage.
2. Use direct mixing of drug and cages to change drug loading under different open and self-assembling conditions.
3. Use different size drugs, such as paclitaxel or gemcitabine.

Example 5 – Animal studies (prophetic)

Compare efficacy of loaded vehicles to efficacy of drugs alone in animal models.

Perform acute and chronic toxicity studies in two animal species with the lead drug.

Example 6 – Co-administration of a first composition and a second composition to enhance immunogenic response (prophetic)

Co-administration of a first composition (comprising a first self-assembled clathrin vehicle, an anti-cancer agent, and a targeting agent) with a second composition (comprising a second self-assembled clathrin vehicle and an anti-PD-1 antibody) is expected to provide enhanced therapeutic effect as compared to the first composition alone, the second composition alone, and the additive effect of the first composition and the second composition. The second composition may further comprise an immunogen payload.

References

All publications and patents mentioned herein, including those items listed below, are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

Equivalents

While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification.

We claim:

1. A first composition comprising a protein, a first payload, and a first targeting agent, wherein the protein is in the form of a three-dimensional cage structure comprising an outer surface and an inner cavity; and the first targeting agent is conjugated to the outer surface of the three-dimensional cage structure.

2. The first composition of claim 1, wherein the first payload is an anti-cancer agent.

3. The first composition of claim 2, wherein anti-cancer agent is paclitaxel, gemcitabine, or an azonafide.

4. The first composition of claim 1, wherein the first payload is an imaging agent.

5. The first composition of claim 4, wherein the imaging agent is a fluorophore, a chromophore, a chemoluminescing agent, a radionuclide, or a contrast agent.

6. The first composition of any of claims 1-5, wherein the first targeting agent selectively targets cancer cells as compared to healthy cells.

7. The first composition of any of claims 1-5, wherein the first targeting agent specifically targets cancer cells.

8. The first composition of any of claims 1-7, wherein the first targeting agent is an antibody.

9. The first composition of any of claims 1-8, wherein the protein is clathrin or a clathrin derivative.

10. The first composition of any of claims 1-9, wherein the protein comprises a heavy chain or a light chain.

11. The first composition of any of claims 1-9, wherein the protein comprises a heavy chain and a light chain.

12. The first composition of claim 11, wherein the heavy chain has a molecular weight from about 100 kDa to about 300 kDa.

13. The first composition or the second composition of claim 11, wherein the heavy chain has a molecular weight of about 190 kDa.

14. The first composition of any of claims 11-13, wherein the heavy chain has greater than 85% sequence homology to SEQ ID NO:3.

15. The first composition of any of claims 11-13, wherein the heavy chain has SEQ ID NO:3.

16. The first composition of any of claims 11-15, wherein the light chain has a molecular weight from about 15 kDa to about 45 kDa.

17. The first composition of any of claims 11-15, wherein the light chain has a molecular weight of about 28 kDa.

18. The first composition of any of claims 11-19, wherein the light chain has greater than 85% sequence homology to SEQ ID NO:6.

19. The first composition of any of claims 11-19, wherein the light chain has SEQ ID NO:6.

20. The first composition of any of claims 1-19, wherein the three-dimensional cage structure has a diameter from about 10 nm to about 100 nm.

21. The first composition of any of claims 1-20, wherein the three-dimensional cage structure is substantially spherical.

22. The first composition of any of claims 1-21, wherein the three-dimensional cage structure is non-covalently assembled.

23. The first composition of any of claims 1-22, wherein the three-dimensional cage structure is substantially stable at about 37 °C at about pH 7.

24. The first composition of any of claims 1-23, wherein the three-dimensional cage structure is substantially unstable at about 37 °C at about pH 5.5.

25. The first composition of any of claims 1-24, wherein the first composition is able to transfect cells in vivo.

26. A second composition comprising a protein, a second payload, and a second targeting agent, wherein the protein is in the form of a three-dimensional cage structure comprising an outer surface and an inner cavity; the second payload is an immunogen; and the second targeting agent conjugated to the outer surface of the three-dimensional cage structure.

27. The second composition of claim 26, wherein the second targeting agent does not selectively target cancer cells as compared to healthy cells.

28. The second composition of claim 26 or claim 27, wherein the second targeting agent is an antibody.

29. The second composition of claim 26 or claim 27, wherein the second targeting agent is an anti-PD-1 antibody.

30. The second composition of any of claims 26-29, wherein the protein is clathrin or a clathrin derivative.

31. The second composition of any of claims 26-29, wherein the protein comprises a heavy chain or a light chain.

32. The second composition of any of claims 26-29, wherein the protein comprises a heavy chain and a light chain.

33. The second composition of claim 32, wherein the heavy chain has a molecular weight from about 100 kDa to about 300 kDa.

34. The second composition of claim 32, wherein the heavy chain has a molecular weight of about 190 kDa.

35. The second composition of any of claims 32-34, wherein the heavy chain has greater than 85% sequence homology to SEQ ID NO:3.

36. The second composition of any of claims 32-34, wherein the heavy chain has SEQ ID NO:3.

37. The second composition of any of claims 32-36, wherein the light chain has a molecular weight from about 15 kDa to about 45 kDa.

38. The second composition of any of claims 32-36, wherein the light chain has a molecular weight of about 28 kDa.

39. The second composition of any of claims 32-38, wherein the light chain has greater than 85% sequence homology to SEQ ID NO:6.

40. The second composition of any of claims 32-38, wherein the light chain has SEQ ID NO:6.

41. The second composition of any of claims 26-40, wherein the three-dimensional cage structure has a diameter from about 10 nm to about 100 nm.

42. The second composition of any of claims 26-41, wherein the three-dimensional cage structure is substantially spherical.

43. The second composition of any of claims 26-42, wherein the three-dimensional cage structure is non-covalently assembled.

44. The second composition of any of claims 26-43, wherein the three-dimensional cage structure is substantially stable at about 37 °C at about pH 7.

45. The second composition of any of claims 26-44, wherein the three-dimensional cage structure is substantially unstable at about 37 °C at about pH 5.5.

46. The second composition of any of claims 26-45, wherein the second composition is able to transfect cells in vivo.

47. A method of treating cancer in a subject in need thereof, comprising:

administering to the subject a therapeutically effective amount of a first composition of any of claims 1-3 or 6-25 wherein the first payload is an anti-cancer agent.

48. A method of treating cancer in a subject in need thereof, comprising:

administering to the subject a therapeutically effective amount of a first composition of any of claims 1-3 or 6-25 wherein the first payload is an anti-cancer agent; and

administering to the subject a therapeutically effective amount of a second composition of any of claims 26-46.

49. The method of claim 47 or claim 48, wherein the cancer is lung cancer.

50. The method of claim 47 or claim 48, wherein the cancer is non-small cell lung cancer (NSCLC).

51. The method of claim 47 or claim 48, wherein the cancer is pancreatic cancer.

52. A method generating an image of a subject in need thereof, comprising:

administering to the subject a detectable amount of a first composition of any of claims 1 or 4-25, wherein the first payload is an imaging agent; and

generating an image.

53. The method of any of claims 47-52, wherein the subject is a mammal.

54. The method of any of claims 47-52, wherein the subject is a human.

55. A pharmaceutical formulation comprising a first composition of any of claims 1-25 and a pharmaceutically acceptable carriers or diluent.

56. The pharmaceutical formulation of claim 55, wherein the pharmaceutical formulation further comprises a second composition of any of claims 26-46.

57. A pharmaceutical formulation comprising a second composition of any of claims 26-46 and a pharmaceutically acceptable carriers or diluent.

Figure 1

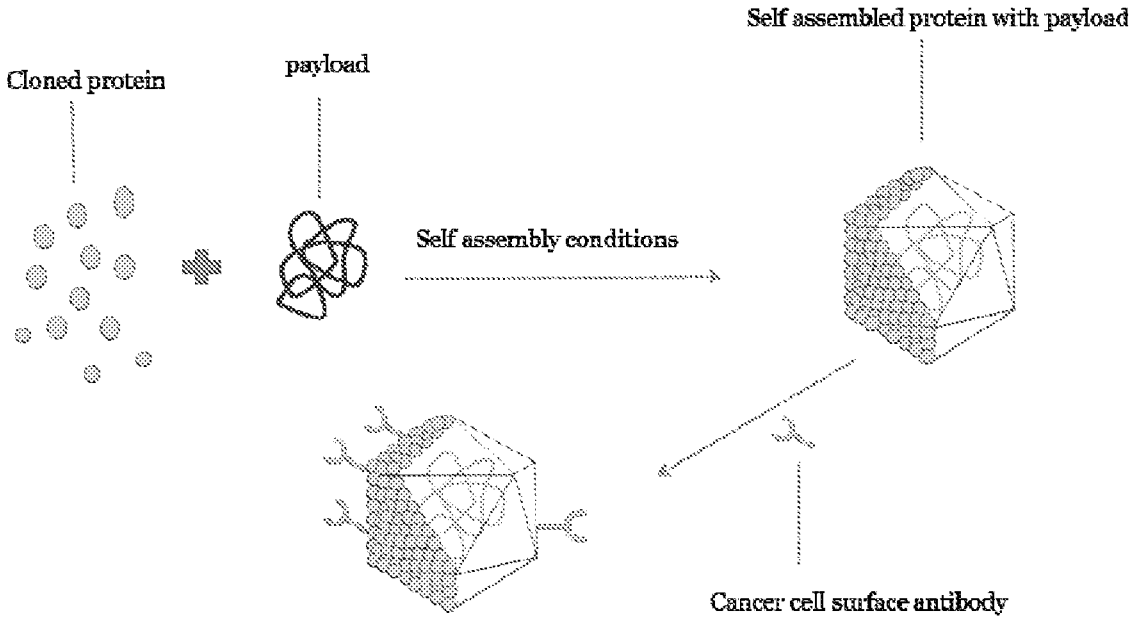


Figure 2

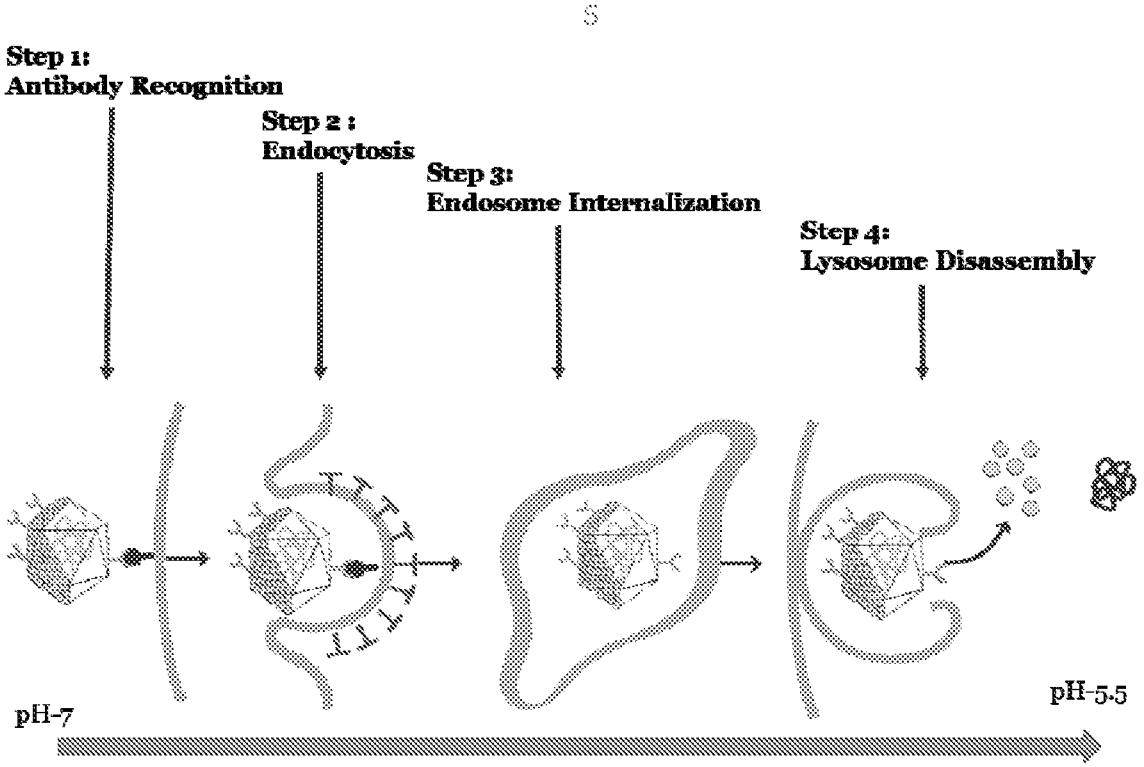


Figure 3

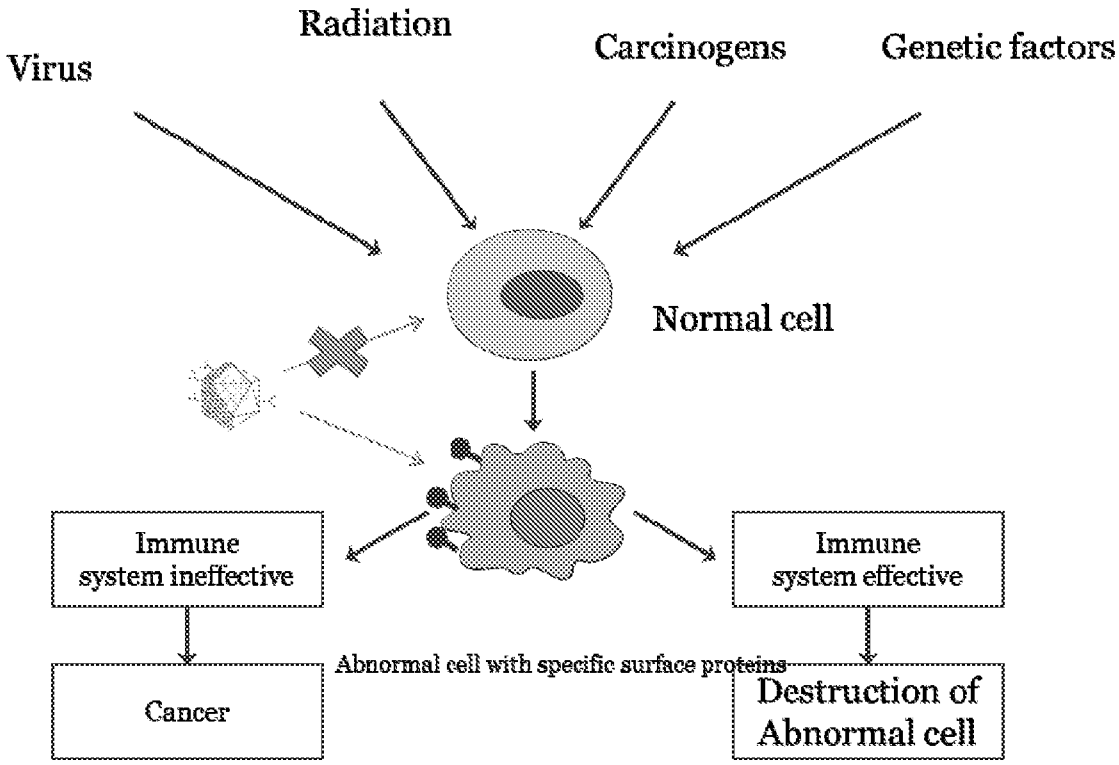


Figure 4

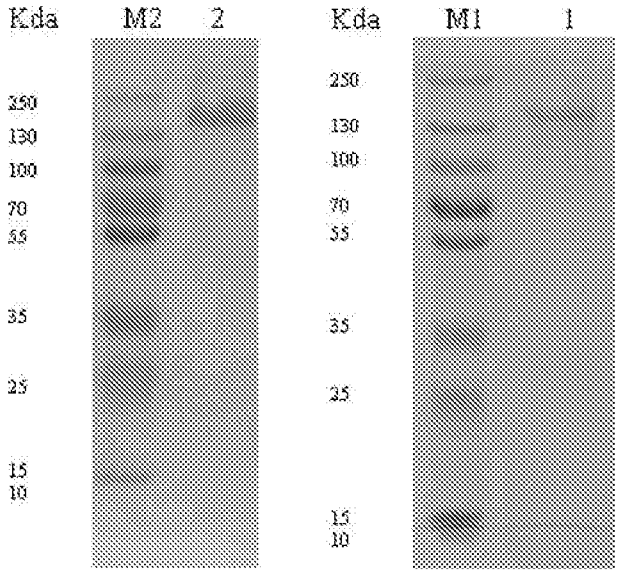


Figure 5

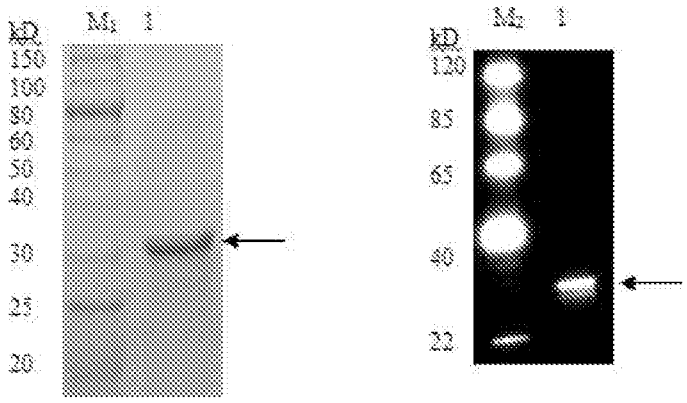


Figure 6

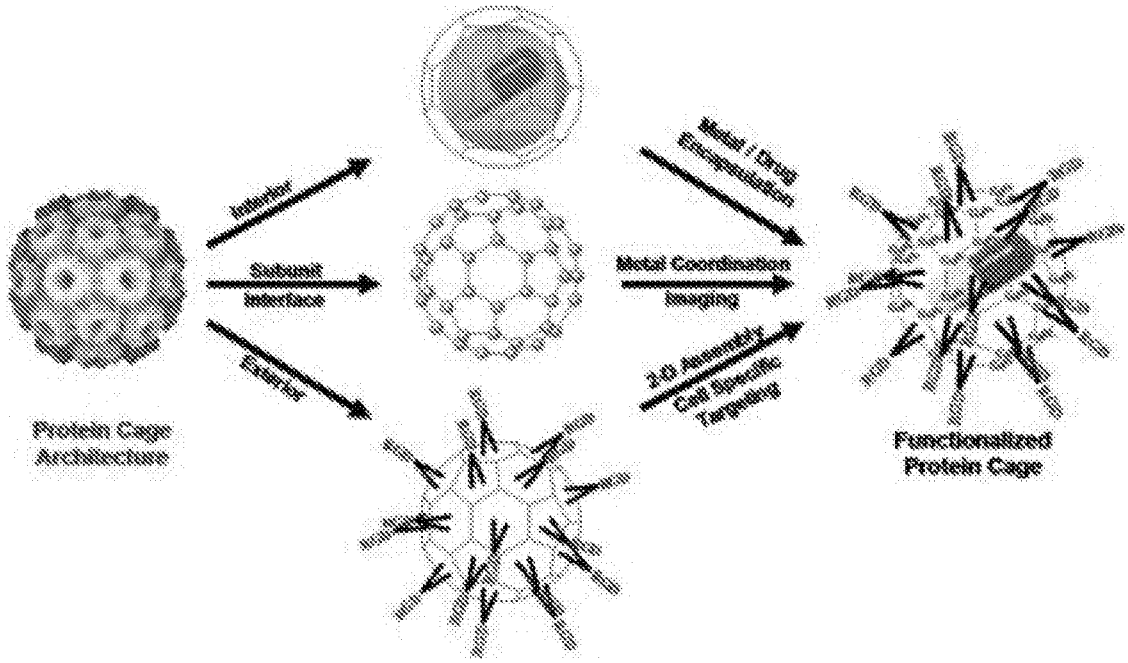
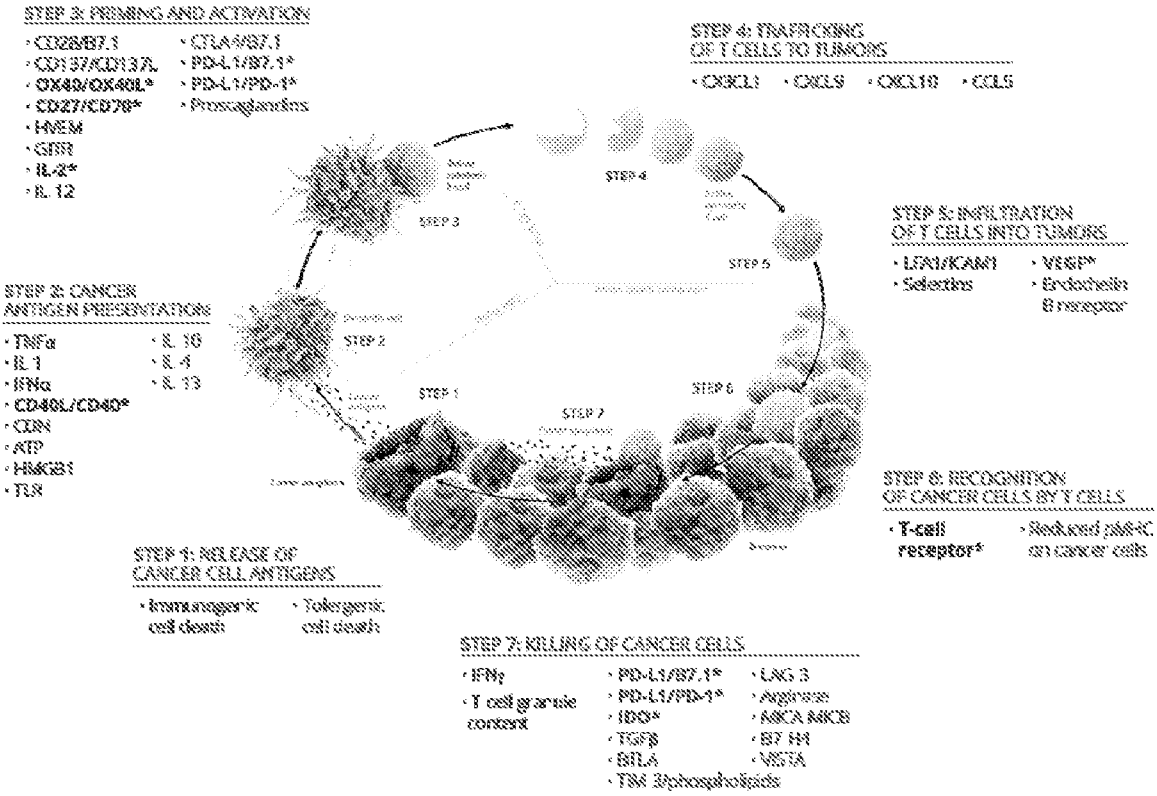


Figure 7


STEP 3: PRIMING AND ACTIVATION
• CD28/B7.1
• CD137/CD137L
• OX40/OX40L*
• CD27/CD70*
• HVEM
• GITR
• IL-2*
• IL-12
• CTLA4/B7.1
• PD-L1/B7.1*
• PD-L1/PD-1*
• Prostaglandins
STEP 4: TRAFFICKING OF T CELLS TO TUMORS
• CXCL1 • CXCL9 • CXCL10 • CCL5
STEP 5: INFILTRATION OF T CELLS INTO TUMORS
• LFA1/ICAM1
• Selectins
• VEGF*
• Endothelin B receptor
STEP 2: CANCER ANTIGEN PRESENTATION
• TNFα
• IL-1
• IFNα
• CD40L/CD40*
• CDN
• ATP
• HMGB1
• TLR
• IL-10
• IL-4
• IL-13
STEP 6: RECOGNITION OF CANCER CELLS BY T CELLS
• T-cell receptor*
• Reduced pMHC on cancer cells
STEP 1: RELEASE OF CANCER CELL ANTIGENS
• Immunogenic cell death
• Tolerogenic cell death
STEP 7: KILLING OF CANCER CELLS
• IFNγ
• T cell granule content
• PD-L1/B7.1*
• PD-L1/PD-1*
• IDO*
• TGFβ
• BTLA
• TIM 3/phospholipids
• LAG 3
• Arginase
• MICA MICB
• B7 H4
• VISTA

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/025290

A. CLASSIFICATION OF SUBJECT MATTER
IPC (2016.01) A61K 9/51, A61K 47/48, A61P 35/00

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)
IPC (2016.01) A61K, A61P

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010101694 A1 VITALIANO FRANCO?[US]; VITALIANO GORDANA?[US] 10 Sep 2010 (2010/09/10) whole document, especially: paras. 90203, 90172, 90368, 90249, Figure 1	1-57
X	WO 2008103920 A3 SPECIGEN INC?[US]; CAMPION BRIAN K?[US]; SIEGEL MARVIN I?[US] 06 Nov 2008 (2008/11/06) whole document, especially: paras. 0025, 0076. 0078, 0086, Examples 2 and 4,	1-57
X	Falvo, Elisabetta, et al. "Antibody–drug conjugates: targeting melanoma with cisplatin encapsulated in protein-cage nanoparticles based on human ferritin." Nanoscale 5.24 (2013): 12278-12285. Retrieved from the internet on: 05.07.2016; URL: http://s3.amazonaws.com/academia.edu.documents/33469291/2013_Nanoscale_Falvo_final.pdf?AWSAccessKeyId=AKIAJ56TQJRTWSMTNPEA&Expires=1467706618&Signature=DAe2bleyCfLSkpXrTGmGTLjrP3o%3D&response-content-disposition=inline%3B%20filename%3DAntibody_drug_conjugates_targeting_melan.pdf 21 Dec 2013 (2013/12/21) abstract, Fig. 7	1-57

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle 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	Date of mailing of the international search report
11 Jul 2016	11 Jul 2016

Name and mailing address of the ISA:	Authorized officer
Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616	HERMAN Karin Telephone No. 972-2-5651749

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2016/025290

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
WO 2010101694 A1	10 Sep 2010	WO 2010101694 A1	10 Sep 2010
		EP 2408821 A1	25 Jan 2012
		EP 2408821 A4	07 Mar 2012
		US 2010226856 A1	09 Sep 2010
		US 2013156697 A1	20 Jun 2013
		US 2015307570 A1	29 Oct 2015
		US 2016060309 A1	03 Mar 2016
		US 2016143992 A1	26 May 2016
		US 2016158313 A1	09 Jun 2016
		US 2016158366 A1	09 Jun 2016
		US 2016158367 A1	09 Jun 2016
		US 2016178652 A1	23 Jun 2016
WO 2008103920 A3	06 Nov 2008	WO 2008103920 A2	28 Aug 2008
		WO 2008103920 A3	06 Nov 2008
		US 2009035389 A1	05 Feb 2009

B. FIELDS SEARCHED:

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

Databases consulted: BLAST, PATENTSCOPE, THOMSON INNOVATION, Esp@cenet, Google Patents, CAPLUS, BIOSIS, REGISTRY, PubMed, Google Scholar, PatBase

Search terms used: Protein cage architectures, drug-loaded vehicle for cancer cells, self-assembling protein, molecules for delivering a payload, Cage-like proteins, clathrin, ferritins, DNA-binding proteins (dps), Heat-Shock proteins, an anti cancer agent, targeting agent, anti-PD-1 antibody, immunogen payload, nanotransporters

摘要

本文描述了用于向特定组织递送有效载荷例如毒性抗癌剂、癌症免疫疗法、毒性抗癌剂和癌症免疫疗法或者显像剂的自组装蛋白分子。自组装蛋白的实例包括网格蛋白和网格蛋白衍生物。