



(51) International Patent Classification:

A61K 39/12 (2006.01) A61K 39/38 (2006.01)
A61K 39/00 (2006.01)

(21) International Application Number:

PCT/US2014/013647

(22) International Filing Date:

29 January 2014 (29.01.2014)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/758,349 30 January 2013 (30.01.2013) US

(71) Applicant: UNIVERSITY OF MIAMI [US/US]; 1951
NW 7th Avenue, Suite 110, Miami, Florida 33136 (US).(72) Inventors: DAFTARIAN, Pirouz; 8208 SW 172 Terrace,
Palmetto Bay, Florida 33157 (US). DAUNERT, Sylvia;
148 Isla Dorada Blvd., Coral Gables, Florida 33143 (US).
FUKATA, Masayuki; 9972 Durant Drive, Beverly Hills,
California 90212 (US).(74) Agent: DOBBELAERE, Amy A.; Novak Druce Connolly
Bove + Quigg LLP, CityPlace Tower, 525 Okeechobee
Blvd., 15th Floor, West Palm Beach, Florida 33401 (US).

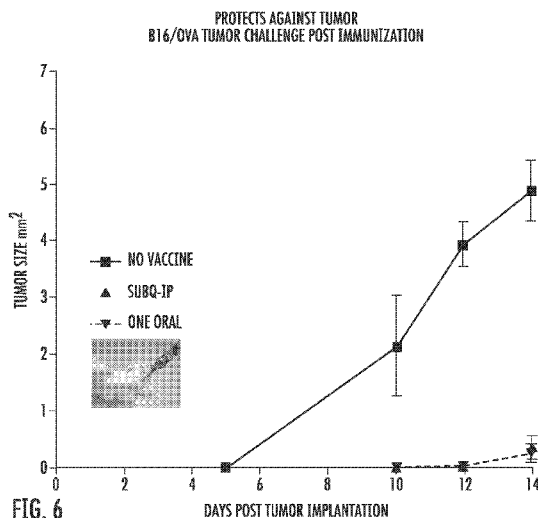
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, 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, 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))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: ENTERIC COATED NANOPARTICLES FOR ORAL VACCINE AND DRUG DELIVERY AND METHODS OF PRODUCTION AND USE THEREOF



(57) Abstract: Described herein are nanoparticle-based compositions, kits, methods and platforms for oral delivery of drugs and vaccines to professional APCs (PAPCs) in a subject's intestinal tract. The composition, kits, platforms and methods involve the combined use of MHC targeting peptides (e.g., PADRE, CLIP, etc.) with charged particles or charged polymers (e.g., positively-charged highly branched polymeric dendrimers) as vehicles for the gastrointestinal tract-targeted delivery of therapeutic agents such as drugs and vaccines.

ENTERIC COATED NANOPARTICLES FOR ORAL VACCINE AND DRUG DELIVERY AND METHODS OF PRODUCTION AND USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of Provisional Application Serial No. 61/758,349, filed January 30, 2013, which is hereby incorporated by reference in its entirety, for all purposes, herein.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on January 24, 2014, is named 59077-00349(7230-173)_SL.txt and is 13,058 bytes in size.

FIELD OF THE INVENTION

[0003] The invention relates generally to the fields of chemistry, immunology, and medicine. More particularly, the invention relates to compositions, kits, platforms and methods for oral delivery of vaccines and drugs using enteric-coated nanoparticles.

BACKGROUND

[0004] Drug and vaccine delivery via intravenous (i.v.) injections has a number of disadvantages. Untargeted drug delivery via i.v. injection is also associated with inefficient rates of drug absorption, as well as toxicity. For example, U.S. Centers for Disease Control and Prevention (CDC) reports that 150,000 patients have been impacted by unsafe injection practices since 2001. The unsafe injections led to at least 49 disease outbreaks.. As another example, implementing i.v. immunization of vaccines such as hepatitis B, which require booster immunizations, often face compliance difficulties. Formulating drugs for rapid rates of absorption and low toxicity, as well as formulating vaccines for lower doses and frequency of immunizations would address such difficulties.

SUMMARY

[0005] Polyamidoamine dendrimer-based oral vaccine and drug-delivery formulations disclosed herein involve a homing peptide-derivatized-dendrimer (PDD) encapsulated in a polymer that serves as an enteric coating. In these formulations, the PDD is targeted to antigen presenting cells (APCs) and the polymer is resistant to the pH of the stomach (e.g., human

stomach) while it degrades in a pH of > 7 (an intestinal pH) thus providing delivery of a drug or vaccine to APCs in a subject's intestinal tract. In one embodiment of a vaccine formulation, the PDD is entrapped in a polymer emulsion formulation of a) methyl-acrylate, methyl methacrylate, and methacrylic acid mixed with *Glycerol Monostearate*, Triethyl Citrate, and Polysorbate with b) PDD having conjugated thereto a nucleic acid (e.g., DNA, RNA) encoding an antigen of interest. Additionally, oral targeted drug delivery via the same formulation where the drug replaces the vaccine is also disclosed. PDD to which a drug is conjugated, or to which a drug-encapsulating particle (e.g., a liposome) is attached, are wrapped in a suitable enteric formulation (e.g., a polymer emulsion composed of either or combination of a) methyl-acrylate, methyl methacrylate, and methacrylic acid mixed with *Glycerol Monostearate*, Triethyl Citrate, and Polysorbate in generally known concentrations and ratios). The compositions, methods and kits described herein are unique because they involve enteric-coated PDD which is a targeted nanovehicle and a newly designed formulation to allow for intestinal release of a vaccine, drug, or other therapeutic agent.

[0006] Nanoparticles such as poly(amidoamine) (PAMAM) dendrimer-based platforms present an exciting opportunity for targeted drug delivery since they have a precise/controllable chemistry (e.g., size and number of cross-linkable surface amine branches), a nanometer scale size range that can pass biological barriers and penetrate most tissues, water solubility, an ability to prolong the circulation half-life of the payload (e.g., a vaccine, drug) when conjugated or complexed to vaccine or drugs, and fast renal/intestinal elimination (Shcharbin et al., *Colloids and Surfaces B, Biointerfaces*. 2007, 58(2):286-289; Sekowski et al., *Colloids and Surfaces B, Biointerfaces*. 2009, 69(1):95-98; Daftarian et al., *Cancer Res*. 2011, Oct 10.). PAMAM dendrimers may be decorated with homing ligands for a specific cell lineage to be able to selectively home in on the intended target organ and/or cells in a host (Daftarian et al., *Cancer Res*. 2011, Oct 10.). Various ligands may be used including peptides, aptamers, small molecules or even proteins. The resulting "ligand-dendrimer" can be conjugated to therapeutic(s) via covalent coupling or simply via electrostatic interactions.

[0007] Typical compositions and nanocarriers described herein include a charged (e.g., positively-charged) highly branched polymeric dendrimer conjugated to an MHC targeting peptide and at least one therapeutic agent (e.g., a vaccine, a drug). In some embodiments, particularly drug delivery embodiments, the MHC targeting peptide is a peptide that is neither

immunogenic nor has a known immunopotentiating effect, e.g., CLIP (class II-associated invariant-chain peptide) or a fragment thereof. For example, CLIP p88-99 is a CLIP fragment having the sequence of SKMRMATPLLMQ (SEQ ID NO:1) that can be used in the compositions described herein. In other embodiments, particularly vaccine or immunotherapeutic embodiments, the MHC targeting peptide has an immunopotentiating effect, such as a universal T helper peptide (e.g., an epitope such as the Pan-HLA-DR-binding epitope (PADRE) peptide). Other reported sequences that bind MHC class II with or without activation effects such as LRMKLPKPPKPVSKMR (SEQ ID NO:2) or all modified versions thereof (e.g. YRMKLPKPPKPVSKMR (SEQ ID NO:3)) may be used as an MHC targeting peptide. Thus, the platforms described herein for drug delivery and oral vaccination involve use of two APC-recognition peptides that share the ability to bind to MHC class II (which is expressed on APCs), one having an intrinsic immunopotentiating effect while the other one does not.

[0008] The at least one MHC targeting peptide (e.g., universal T helper peptide, CLIP, etc.) and the at least one therapeutic agent are conjugated to the exterior surface of the at least one dendrimer, for example, a charged highly branched polymeric dendrimer such that the at least one MHC targeting peptide specifically binds to PAPCs, such a composition or nanocarrier capable of targeting PAPCs in the intestinal tract, surviving the pH of the stomach and degrading in the intestines, delivering a drug or vaccine to the intestinal tract, and in some embodiments (e.g., vaccine embodiments), inducing an immunoenhancing effect when administered to a subject. In some embodiments, a polymeric particle, e.g., a biodegradable polymeric particle is used in place of a dendrimer. The polymeric particle can be charged and the MHC targeting peptide (e.g., universal T helper peptide, CLIP, etc.) and the at least one therapeutic agent can be conjugated to the polymeric particle.

[0009] Accordingly, described herein is a composition including: a) at least one dendrimer (e.g., a charged highly branched polymeric dendrimer) having conjugated thereto (e.g., covalently attached) at least one MHC targeting peptide and at least one therapeutic agent, wherein the at least one MHC targeting peptide and the at least one therapeutic agent are conjugated to the exterior surface of or entrapped within the dendrimer (e.g., a charged highly branched polymeric dendrimer) such that the at least one MHC targeting peptide specifically binds to professional antigen presenting cells (APCs), and b) a polymer emulsion encapsulating the dendrimer, the MHC targeting peptide, and the at least one therapeutic agent, wherein the

polymer emulsion is resistant to a pH of about 7.0 or less and degrades in a pH of about 7.1 or higher. The at least one MHC targeting peptide specifically binds to APCs in a subject's gastrointestinal tract. In one embodiment, the therapeutic agent is a nucleic acid encoding an antigen, and the composition is a vaccine. In some embodiments, the therapeutic agent is a drug or a liposome encapsulating a drug. In some embodiments, the MHC targeting peptide is covalently attached to the dendrimer and the therapeutic agent is conjugated to the exterior surface of or entrapped within the dendrimer. The composition typically further includes a pharmaceutically acceptable carrier. The at least one MHC targeting peptide can be, for example, a Pan-DR T helper epitope (PADRE) or an epitope of influenza virus haemagglutinin molecule, and the dendrimer (e.g., the at least one charged highly branched polymeric dendrimer) can be, for example, a PAMAM dendrimer. In another embodiment, the at least one MHC targeting peptide is CLIP. The polymer emulsion is an enteric formulation that can include methyl-acrylate, methyl methacrylate, and methacrylic acid mixed with glycerol monostearate, triethyl citrate, and polysorbate. In a composition, the dendrimer, the MHC targeting peptide, and the nucleic acid encoding an antigen can be in amounts effective for inducing an immune response against the antigen when administered to a subject. In another composition, the dendrimer, the MHC targeting peptide, and the at least one therapeutic agent are in amounts effective for preventing or decreasing growth of a cancerous tumor when administered to a subject having a cancerous tumor or at risk of developing a cancerous tumor.

[00010] Also described herein is a method of delivering a therapeutic agent specifically to professional APCs in a subject. The method includes administering to the subject a therapeutically effective amount of a composition as described herein. Administering the composition to the subject results in internalization of the therapeutic agent by professional APCs in the subject's gastrointestinal tract. Generally, the composition is administered orally, and subsequently degrades in the subject's intestines. In one example of the method, the subject is a human, and the therapeutic agent is a nucleic acid encoding an antigen, and the composition is a vaccine. In such an embodiment, administering the composition to the subject results in an immune response against the antigen in the subject. In another example of the method, the subject is a human, and the therapeutic agent is a liposome or a liposome encapsulating a drug. For example, the subject has a cancerous tumor or is at risk of developing a cancerous tumor and administering the composition to the subject results in prevention of or decreased growth of the

cancerous tumor. In the method, the composition typically further includes a pharmaceutically acceptable carrier. In one embodiment, the at least one MHC targeting peptide is a PADRE or an epitope of influenza virus haemagglutinin molecule, and the at least one charged highly branched polymeric dendrimer is a PAMAM dendrimer. In another embodiment, the at least one MHC targeting peptide is CLIP and the at least one charged highly branched polymeric dendrimer is a PAMAM dendrimer. The polymer emulsion is an enteric formulation that can include methyl-acrylate, methyl methacrylate, and methacrylic acid mixed with glycerol monostearate, triethyl citrate, and polysorbate.

[00011] Further described herein is a kit for delivering a drug or vaccine to a subject. The kit includes a composition as described herein, a pharmaceutically acceptable carrier; instructions for use; and packaging.

[00012] Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[00013] As used herein, a "nucleic acid" or a "nucleic acid molecule" means a chain of two or more nucleotides such as RNA (ribonucleic acid) and DNA (deoxyribonucleic acid), and chemically-modified nucleotides. A "purified" nucleic acid molecule is one that is substantially separated from other nucleic acid sequences in a cell or organism in which the nucleic acid naturally occurs (e.g., 30, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, 100% free of contaminants). The terms include, e.g., a recombinant nucleic acid molecule incorporated into a vector, a plasmid, a virus, bacterial DNA, or a genome of a prokaryote or eukaryote. Examples of purified nucleic acids include cDNAs, fragments of genomic nucleic acids, nucleic acids produced polymerase chain reaction (PCR), nucleic acids formed by restriction enzyme treatment of genomic nucleic acids, recombinant nucleic acids, and chemically synthesized nucleic acid molecules. A "recombinant" nucleic acid molecule is one made by an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques.

[00014] When referring to an amino acid residue in a peptide, oligopeptide or protein, the terms "amino acid residue", "amino acid" and "residue" are used interchangeably and, as used herein, mean an amino acid or amino acid mimetic joined covalently to at least one other amino acid or amino acid mimetic through an amide bond or amide bond mimetic.

[00015] As used herein, "protein" and "polypeptide" are used synonymously to mean any peptide-linked chain of amino acids, regardless of length or post-translational modification, e.g., glycosylation or phosphorylation.

[00016] When referring to a nucleic acid molecule, polypeptide, or infectious pathogen, the term "native" refers to a naturally-occurring (e.g., a wild-type (WT)) nucleic acid, polypeptide, or infectious pathogen.

[00017] As used herein, the term "antigen" or "immunogen" means a molecule that is specifically recognized and bound by an antibody.

[00018] When referring to an epitope (e.g., T helper epitope), by biological activity is meant the ability to bind an appropriate MHC molecule and, in the case of peptides useful for stimulating CTL responses, induce a T helper response and a CTL response against a target antigen or antigen mimetic.

[00019] As used herein, "bind," "binds," or "interacts with" means that one molecule recognizes and adheres to a particular second molecule in a sample or organism, but does not substantially recognize or adhere to other structurally unrelated molecules in the sample.

[00020] The terms "specific binding" and "specifically binds" refer to that binding which occurs between such paired species as enzyme/substrate, receptor/agonist, antibody/antigen, etc., and which may be mediated by covalent or non-covalent interactions or a combination of covalent and non-covalent interactions. When the interaction of the two species produces a non-covalently bound complex, the binding which occurs is typically electrostatic, hydrogen-bonding, or the result of lipophilic interactions. Accordingly, "specific binding" occurs between a paired species where there is interaction between the two which produces a bound complex having the characteristics of an antibody/antigen or enzyme/substrate interaction. In particular, the specific binding is characterized by the binding of one member of a pair to a particular species and to no other species within the family of compounds to which the corresponding member of the binding member belongs.

[00021] As used herein, the terms "Pan-DR epitope," "Pan DR T helper epitope," "Pan-HLA-DR-binding epitope," "PADRE" and "PADRE peptides" mean a peptide of between about 4 and about 20 residues that is capable of binding at least about 7 of the 12 most common DR alleles (DR1, 2w2b, 2w2a, 3, 4w4, 4w14, 5, 7, 52a, 52b, 52c, and 53) with high affinity. "High affinity" is defined herein as binding with an IC_{50} of less than 200 nM. For example, high affinity

binding includes binding with an IC_{50} of less than 3100 nM. For binding to Class II MHC, a binding affinity threshold of 1,000 nM is typical, and a binding affinity of less than 100nM is generally considered high affinity binding. Construction and use of PADRE peptides is described in detail in U.S. Patent No. 5,736,142 which is incorporated herein by reference.

[00022] A "T helper peptide" as used herein refers to a peptide recognized by the T cell receptor of T helper cells. For example, the PADRE peptides described herein are T helper peptides.

[00023] By the term "MHC targeting peptide" is meant any peptide that binds to an MHC class II molecule or domain thereof. For example, CLIP is an MHC class II peptide that binds MHC class II groove.

[00024] As used herein, the term "dendrimer" means a charged (e.g., positively-charged, negatively-charged), highly branched polymeric macromolecule with roughly spherical shape. An example of a positively-charged, highly branched polymeric dendrimer is a PAMAM dendrimer. By the terms "PAMAM dendrimer" and "poly-amidoamine dendrimer" is meant a type of dendrimer in which tertiary amines are located at branching points and connections between structural layers are made by amide functional groups. PAMAM dendrimers exhibit many positive charges on their surfaces.

[00025] By the term "derivatized dendrimer" is meant a dendrimer having one or more functional groups conjugated to its surface.

[00026] A "PADRE-derivatized dendrimer" or "PADRE-dendrimer" is a nanoconstruct (e.g., nanocarrier, nanovehicle) in which one or more PADRE peptides are covalently attached to the functional groups on the surface of a charged (e.g., positively-charged) highly branched polymeric dendrimer (e.g., a PAMAM dendrimer).

[00027] As used herein, "vaccine" includes all prophylactic and therapeutic vaccines.

[00028] As used herein, an "immune cell" refers to dendritic cells, macrophages, lymphocytes, mast cells, endothelial cells, lymphatic vessel cells and the like which can, when properly stimulated, serve as an antigen-presenting cell (APC) to initiate an immune response or as an effector cell of an immune response.

[00029] As used herein, the term "professional antigen presenting cell" means any MHC class II expressing cell including dendritic cells, macrophages, and B cells.

[00030] By the term “conjugated” is meant when one molecule or agent is physically or chemically coupled or adhered to another molecule or agent. Examples of conjugation include covalent linkage and electrostatic complexation. The terms “complexed,” “complexed with,” and “conjugated” are used interchangeably herein.

[00031] As used herein, the phrase “sequence identity” means the percentage of identical subunits at corresponding positions in two sequences (e.g., nucleic acid sequences, amino acid sequences) when the two sequences are aligned to maximize subunit matching, i.e., taking into account gaps and insertions. Sequence identity can be measured using sequence analysis software (e.g., Sequence Analysis Software Package from Accelrys CGC, San Diego, CA).

[00032] The phrases “isolated” or “biologically pure” refer to material which is substantially or essentially free from components which normally accompany it as found in its native state.

[00033] As used herein, the terms “nanoparticle,” “nanovehicle” and “nanocarrier” mean a microscopic particle whose size is measured in nanometers. For example, a nanoparticle, nanovehicle or nanocarrier is a PADRE-dendrimer conjugate (e.g., PDD) or a particle combining several PADRE-dendrimer conjugates with a total diameter in the range of approximately 2-500 nm. As another example, a nanoparticle, nanovehicle or nanocarrier is an HA-dendrimer conjugate (e.g., PDD2) or a particle combining several HA-dendrimer conjugates with a total diameter in the range of approximately 2-500 nm. As yet another example, a nanoparticle, nanovehicle or nanocarrier is a CLIP-dendrimer conjugate. Typically, an unloaded PDD (e.g., a PDD not formulated with a vaccine or drug) is sized such that at least 60% of the microparticles in a preparation are less than 10 nm, while in a typical preparation of vaccine or drug-loaded PDD, 60% or less of the microparticles are less than 600 nm.

[00034] As used herein, the term “therapeutic agent” is meant to encompass any molecule, chemical entity, composition, drug, vaccine, or biological agent capable of preventing, treating or mitigating a disease. An example of a therapeutic agent is a vaccine or a nucleic acid encoding a vaccine. Another example of a therapeutic agent is a drug (e.g., Amphotericin B), including a drug conjugated to or encapsulated by a carrier such as a liposome (e.g., AmBisome®). The term includes small molecule compounds, antisense reagents, nucleic acids (e.g., microRNA and siRNA reagents), antibodies, antimicrobial agents, enzymes, polypeptides, peptides, organic or inorganic molecules, natural or synthetic compounds and the like.

[00035] The term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies, humanized antibodies, anti-idiotypic (anti-Id) antibodies to antibodies that can be labeled in soluble or bound form, as well as fragments, regions or derivatives thereof, provided by any known technique, such as, but not limited to, enzymatic cleavage, peptide synthesis or recombinant techniques.

[00036] As used herein the term "adjuvant" means any material which modulates to enhance the humoral and/or cellular immune response.

[00037] As used herein, the terms "displayed" or "surface exposed" are considered to be synonyms, and refer to antigens or other molecules that are present (e.g., accessible to immune site recognition) at the external surface of a structure such as a nanoparticle or nanocarrier (e.g., PADRE-dendrimer).

[00038] The expression "biologically compatible form suitable for administration *in vivo*" as used herein means a form of the substance to be administered in which any toxic effects are outweighed by the therapeutic effects. The substances may be administered to any subject, e.g., humans.

[00039] By the phrase "immune response" is meant induction of phagocytic, cytokine, and cellular and/or humoral responses (cellular or humoral or both cellular and humoral) specific against an antigen, antigens, pathogen, pathogenic agent, cancer cells, etc. An immune response has many facets, some of which are exhibited by the cells of the immune system (e.g., B-lymphocytes, T-lymphocytes, macrophages, and plasma cells). In the innate phase of immune system, antigen-presenting cells interact with an antigen or pathogen or other cells of the immune system, and release cytokines to direct adaptive immunity. The adaptive immune responses are generally divided into two main categories--humoral and cell-mediated. The humoral component of the immune response includes production of antibodies specific for an antigen or pathogen. The cell-mediated component includes the generation of delayed-type hypersensitivity and cytotoxic effector cells against the antigen or pathogen. An immune response can include, for example, activation of a CD4 T helper response.

[00040] By the phrases "therapeutically effective amount" and "effective dosage" is meant an amount sufficient to produce a therapeutically (e.g., clinically) desirable result; the exact nature of the result will vary depending on the nature of the disorder being treated. For example, where the disorder to be treated is a pathogenic infection, the result can be elimination of the pathogen,

a reduction in growth of the pathogen, a reduction in size or elimination of a lesion associated with the pathogen, etc. As another example, where the disorder to be treated is cancer (e.g., a cancerous tumor), the result can be elimination of the cancer, a reduction in size or elimination of a cancerous tumor, a reduction in growth of cancerous cells, etc. The compositions and nanocarriers described herein can be administered from one or more times per day to one or more times per week. The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the compositions and nanocarriers described herein can include a single treatment or a series of treatments.

[00041] As used herein, the term "treatment" is defined as the application or administration of a therapeutic agent described herein, or identified by a method described herein, to a patient, or application or administration of the therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease, or the predisposition toward disease.

[00042] The terms "patient" "subject" and "individual" are used interchangeably herein, and mean an invertebrate or vertebrate animal to be treated, including humans. In some cases, the methods of the invention find use in experimental animals, in veterinary applications (e.g., equine, bovine, ovine, canine, feline, avian, etc.), and in the development of animal models for disease, including, but not limited to, rodents including mice, rats, and hamsters, as well as non-human primates.

[00043] Although compositions, kits, platforms and methods similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable compositions, kits, platforms and methods are described below. All publications, patent applications, and patents mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. The particular embodiments discussed below are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

[00044] FIG. 1 is transmission electron microscopy (TEM) of PDD/AmBisome complex. PDD was added to AmBisome at a 7.1 ratio. After a twenty-minute incubation at room-temperature the TEM image was obtained.

[00045] FIG. 2 is scanning electron microscopy (SEM) showing that PDD targets human APC. PPD was loaded with pcDNA3 and the complex was incubated for 40 minutes at room temperature (RT) with magnetically purified human B-cells. Cells were washed, fixed and imaged in the SEM. PDD/cargo is seen on 40 min post co-incubation.

[00046] FIG. 3 is a diagram and a photo illustrating oral vaccine delivery of PDD/vaccine that includes an enteric coating that resists stomach pH and degrades in a pH of >7 (e.g., in intestinal targeting, for human and rodent). A formulation as described herein wraps/protects the PDD from a low stomach pH and degrades in an intestinal pH resulting in shuttling the payload (i.e., the vaccine) into APCs in the gut.

[00047] FIG. 4 is a photo and a schematic illustration of formulating PDD for oral administration. An enteric coating and intestinal targeting was achieved by an emulsion formulation of PDD/vaccine into a mixture of EUDRAGIT FS 30 D (60%) plus PlasACRYL (40%).

[00048] FIG. 5 is a graph showing anti-OVA antibody titers from an experiment involving PDD/OVA plasmid. The results show that a sole oral administration elicits titers comparable to two biweekly intra-peritoneal (IP) immunizations. Mice in groups of 8 received either one oral gavage of the oral vaccine formulation or of the controls.

[00049] FIG. 6 is a graph showing results from an experiment demonstrating that a single oral dose of PDD/DNA is an effective prophylactic oral vaccine against B16/ova melanoma tumors. A sole PDD/ovaDNA oral formulation as described herein was comparable with two subcutaneous or two IP immunizations of PDD/ovaDNA.

[00050] FIG. 7 is a series of plots showing that orally administered PDD successfully delivers dsRNA to dendritic cells in Peyer's patches (PPs).

[00051] FIG. 8 is a graph showing results from an experiment in which orally administered PDD successfully delivered dsRNA to PPs.

[00052] FIG. 9 is a graph showing results from an experiment in which orally administered poly I:C-PDD did not induce a systemic pro-inflammatory response.

[00053] FIG. 10 is a graph showing results from an experiment involving a PP cell suspension infected with *Y. enterocolitica* 48 hrs post-administration.

[00054] FIG. 11 is a graph showing results from an experiment involving cytokine production in the MLN after stimulation with HKY (10 μ g/ml) 48 hrs post-administration.

[00055] FIG. 12 is a graph showing results from an experiment involving cytokine expression in the PPs 8 hrs post-administration.

[00056] FIG. 13 is a graph showing results from an experiment demonstrating the ability to kill *Salmonella* in a PP suspension (8 hrs post oral delivery).

DETAILED DESCRIPTION

[00057] Described herein are nanoparticle- or nanocarrier-based compositions, kits, platforms and methods for effective oral delivery of a therapeutic agent (e.g., a vaccine, a drug) to PAPCs or MHC class II positive cells, in the intestines of a subject in need thereof. A targeted delivery platform that offers targeted vaccine and drug delivery to phagocytes, lowered toxicity, lower doses, and enhanced efficacy of vaccines and drugs is described herein. In a typical composition, a charged (e.g., positively-charged), highly branched polymeric dendrimer is conjugated to 1) an MHC targeting peptide (e.g., an epitope such as the PADRE peptide, other universal T helper epitopes, or CLIP peptide, etc.) and 2) to at least one therapeutic agent (e.g., drug, vaccine, or liposome entrapped vaccines or drugs) for delivery of the therapeutic agent into PAPCs in the subject's intestinal tract. In another composition, the therapeutic agent is conjugated to the MHC targeting peptide rather than to the charged, highly branched polymeric dendrimer. The dendrimer makes a complex (conjugation) with a therapeutic agent based on the opposite charge of the dendrimer (positive) and that of the therapeutic agent (negative) or the conjugation may be a covalent chemical linkage.

[00058] The below described preferred embodiments illustrate adaptations of these compositions, kits, platforms and methods. Nonetheless, from the description of these embodiments, other aspects of the invention can be made and/or practiced based on the description provided below.

Biological Methods

[00059] Methods involving conventional molecular biology techniques are described herein. Such techniques are generally known in the art and are described in detail in methodology treatises such as *Molecular Cloning: A Laboratory Manual*, 3rd ed., vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001; and *Current Protocols in Molecular Biology*, ed. Ausubel et al., Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates). Immunology techniques are generally known in the art and are described in detail in methodology treatises such as *Advances in Immunology*, volume 93, ed. Frederick W. Alt, Academic Press, Burlington, MA, 2007; *Making and Using Antibodies: A Practical Handbook*, eds. Gary C. Howard and Matthew R. Kaser, CRC Press, Boca Raton, FL, 2006; *Medical Immunology*, 6th ed., edited by Gabriel Virella, Informa Healthcare Press, London, England, 2007; and Harlow and Lane *ANTIBODIES: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988. Construction and use of PAMAM dendrimers is also described, for example, in Arashkia et al., *Virus Genes* 40 (1): 44-52, 2010; Velders et al., *J Immunol.* 166:5366–5373, 2001; and S. Chauhan, N. K. Jain, P. V. Diwan. (2009) Pre-clinical and behavioural toxicity profile of PAMAM dendrimers in mice. *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences* (Online publication date: December 3, 2009).

Platform for Targeting Vaccines and Drugs to PAPCs In A Subject's Intestinal Tract

[00060] Dendrimers have demonstrated considerable promise in shuttling negatively charged payloads into cells (Shcharbin et al., *Colloids and surfaces B, Biointerfaces.* 2007;58(2):286-9; Sekowski et al., *Colloids and surfaces B, Biointerfaces.* 2009;69(1):95-8; Medina SH, and El-Sayed MEH, *Chemical reviews.* 2009;109(7):3141-57; Bracci et al., *The Journal of biological chemistry.* 2003;278(47):46590-5), and are an ideal vaccine and drug delivery vehicle as they provide structural control over size and shape (cargo-space), are biocompatible (non-toxic and nonimmunogenic), have precise scaffolding properties, have a well-defined surface-modifiable functionality for specific targeting moieties, have the ability for cellular adhesion and endocytosis and delivery into the cytoplasm or nucleus, have acceptable biodegradation (the ability to safely degrade within the body), and are associated with easy and consistently reproducible (clinical grade) synthesis. Dendrimers are highly branched macromolecules which

span from a central core and contain a series of structurally and synthetically distinct layers. Each layer added to the structure of a dendrimer is referred to as a “generation”, in a clear reference to the stepwise growth of the macromolecule. PAMAM dendrimers have been developed and are now commercially available. Described herein are novel dendrimer-based compositions and platforms that when complexed with a therapeutic agent, such as a drug or vaccine of interest, target the therapeutic agent (e.g., drug, vaccine) primarily to phagocytic cells in a subject’s intestinal tract via MHC class II molecules expressed on the surface of such cells and an enteric coating that is resistant to the pH of the stomach yet degrades in the pH of the intestines. These compositions and platforms involve the combined use of a cationic PAMAM dendrimer (e.g., peptide-derivatized dendrimer PAMAM generation 5 (G5) or any polymer), and one or more MHC class II targeting peptides (e.g., PADRE, or any universal T helper epitope or combination thereof, or CLIP epitope, or combination thereof). A drug or vaccine (therapeutic agent) may be complexed to the dendrimer-MHC targeting peptide by incubation in room temperature or be coupled via direct conjugation of the therapeutic agent(s) to the platform, e.g., dendrimer-PADRE, dendrimer-CLIP. Alternatively, the drug or vaccine may also be coupled to the MHC targeting peptide (e.g., PADRE, HA, or other universal T helper epitopes, CLIP, etc.).

[00061] A typical composition for oral drug or vaccine delivery as described herein includes at least one charged highly branched polymeric dendrimer having conjugated thereto at least one MHC targeting peptide (e.g., a universal T helper peptide, CLIP) and at least one therapeutic agent, wherein the at least one MHC targeting peptide and the at least one therapeutic agent are conjugated to the exterior surface of the at least one charged highly branched polymeric dendrimer such that the at least one MHC targeting peptide specifically binds to PAPCs, and a polymer emulsion encapsulating the dendrimer, the MHC targeting peptide, and the at least one therapeutic agent. In this composition, the polymer emulsion is resistant to a pH of about 7.0 or less and degrades in a pH of about 7.1 or higher. The at least one MHC targeting peptide specifically binds to APCs in a subject’s gastrointestinal tract. Any suitable enteric coating can be used. In one example, the enteric coating is a polymer emulsion including methyl-acrylate, methyl methacrylate, and methacrylic acid mixed with glycerol monostearate, triethyl citrate, and polysorbate. In some vaccine embodiments, the therapeutic agent is a DNA or RNA-encoded antigen, and the dendrimer, the MHC targeting peptide, and the DNA or RNA-encoded antigen are in an amount effective for inducing an immune response against the antigen when

administered to a subject. In some embodiments, the dendrimer, the MHC targeting peptide, and the at least one therapeutic agent are in an amount effective for preventing or decreasing growth of a cancerous tumor when administered to a subject having a cancerous tumor (e.g., a melanoma) or at risk of developing a cancerous tumor.

[00062] Examples of therapeutic agents include but are not limited to drugs, toxins, iRNA, siRNA, microRNA, antibodies, polypeptides, peptides, nucleic acids, ligands, cytokines, enzymes, hormones, clotting factors, immunotherapeutics including vaccines and vaccine adjuvants, (humanized monoclonal antibodies) or any combination of such agents. Examples of drugs include AmBisome®, Amphotericin B, Taxol® (paclitaxel) (Bristol-Myers Squibb), melphalan, prednisone, thalidomide (MPT), Velcade® (bortezomib) (Millenium Pharmaceuticals), Ampligen (Hemispherx Biopharma), chloroquine, hydroxychloroquine, quinacrine, lenalidomide, dexamethasone, drugs that target intracellular pathogens including Clofazimine, Amoxicillin plus Clavulonic acid, Streptomycin, or Capreomycin, nevirapine, zidovudine, lamivudine, ritonavir and lopinavir. A drug or vaccine (e.g., a nucleic acid encoding an antigen of interest) may be complexed with a liposome or other type of vesicle. The compositions described herein may be used with adjuvants such as (but not limited to) Poly I:C which is negatively charged and makes a complex with a nanoparticle platform as described herein.

[00063] In one embodiment of delivering a drug to PAPCs, an approved marketed drug wrapped in liposomes is used. For example, liposomal amphotericin B (for example, AmBisome®) is Amphotericin B wrapped in a 60-70 nm diameter DSPG monolayer liposome, and shows a higher LD50 and better safety profile than conventional amphotericin B. AmB contains Hydrogenated soy phosphatidylcholine, vitamin E, cholesterol, and Distearoylphosphatidylglycerol. The net negative charge of the external surface of AmB increases affinity for fungal and mammalian cells. The liposome is useful for preventing the release of drug and limiting the off-target interaction of amphotericin B. Cholesterol in the platform increases the stability of the drug. Most drugs may be encapsulated in liposome and be complexed with PDD; the complex will then be enteric coated by this enteric coated platform. Alternatively, any drug can be covalently coupled to PDD using a pH sensitive cleavable or an enzyme sensitive cleavable linker. Examples are cathepsin B-cleavable tetrapeptide spacer sequence (GFLG) (SEQ ID NO:4), MaAhxFKFLAhxK10 (SEQ ID NO:5), which is susceptible

to both endopeptidase and exopeptidase activity, and MaAhxFKFLAhx(d)K10 (SEQ ID NO:6), which is susceptible only to endopeptidase activity. In addition, pH-sensitive linkers such as, N-ethoxybenzylimidazoles, or Bis (acryloyl) cystamine can be used as linkers to be assessed and compared with the “enzyme-sensitive” linkers. A list of drugs that can be used either wrapped in negatively charged liposomes or via cleavable cross-linkers are:

2nd/3 rd line TB Drugs	PK liabilities			Significance of delivery improvement
	Significant non-target tissue deposition	No/poor intracellular penetration and/or activity	No oral bioavailability	
Clofazimine	X			Little/no drug resistance, less skin pigmentation
Amoxacillin + Clavulonic acid		X		Highly bactericidal
Streptomycin		X	X	Oral; Less toxicity
Capreomycin			X	Oral; possible reduction in Tx duration

[00064] Methods of preparing and using liposomes and other nanocarriers are well known in the art, and are described in, for example, Multifunctional Nanoparticles for Drug Delivery Applications: Imaging, Targeting, and Delivery (Nanostructure Science and Technology), Sonke Svenson & Robert K. Prud'homme, editors, Springer, 2012 edition; Beija et al., Trends Biotechnol. Jun 4, 2012; Parveen et al., Nanomedicine. Feb;8(2):147-66, 2012; and Puri et al., Crit Rev Ther Drug Carrier Syst. 26(6):523-80, 2009.

[00065] Macrophage/dendritic cells are major PAPCs that are central in mounting protective immune responses against cancer and the many parasites, bacteria, and viruses. They also dictate the tolerogenic immutherapies for autoimmune disorders, including diabetes. Examples of pathogens that can be treated and/or vaccinated for using the compositions, methods, kits and platforms described herein include but are not limited to, pathogenic parasitic, bacterial, fungal, and viral organisms. Examples include:

Leishmania species (e.g., *L. major*, *L. tropica*, *L. aethiopica*, *L. mexicana*, *L. donovani*, *L. infantum* syn. *L. chagas*),

<i>Streptococcus</i> species,
<i>Candida</i> species,
<i>Brucella</i> species,
<i>Salmonella</i> species,
<i>Shigella</i> species,
<i>Yersinia</i> species
<i>Pseudomonas</i> species,
<i>Bordetella</i> species,
<i>Clostridium</i> species,
Norwalk virus,
<i>Bacillus anthracis</i> ,
<i>Mycobacterium tuberculosis</i> ,
HIV,
<i>Chlamydia</i> species,
human Papillomaviruses,
Influenza virus,
Paramyxovirus species,
Herpes virus,
Cytomegalovirus,
Varicella-Zoster virus,
Epstein-Barr virus,
Hepatitis viruses,
<i>Plasmodium</i> species (e.g., <i>Plasmodium(p) falciparum</i> , <i>P. malariae</i> , <i>P. ovale</i> , <i>P. vivax</i> and <i>P. knowlesi</i>),
<i>Trichomonas</i> species,
sexually transmitted disease agents,
arthropod-borne virus including West Nile virus and dengue
viral encephalitis agents,
protozoan disease agents,
fungal disease agents,

bacterial disease agents,
or mixtures thereof.

[00066] In embodiments for treating or preventing cancer in a subject by orally delivering a drug or vaccine, any type of cancer may be treated or prevented. Examples of cancers that can be prevented or treated using the methods, platforms and kits described herein include melanoma, HPV-induced cervical cancers, prostate cancer, lung cancer, breast cancer, leukemia, etc.

[00067] In one embodiment, the at least one MHC targeting peptide is a T helper epitope such as a Pan-DR epitope, e.g., PADRE. PADRE is an artificially designed peptide that binds to the majority of murine and human MHC Class II molecules, and conjugating PADRE peptides to dendrimers (e.g., a PADRE-derivatized dendrimer) makes the resultant complex or conjugate a ligand for PAPCs that express high levels of MHC class II. PADRE is a synthetic, non-natural T helper epitope [AKchxAVAAWTLKAAA (SEQ ID NO:7) (chxA = cyclohexylalanine)]. When fused to the surface of the dendrimer, PADRE will bind and activate primarily cells that have MHC class II including all PAPCs. Several PADRE epitopes (e.g., 2, 3, 4, 5, etc.) can be attached to each dendrimer. The attachment is done with or without suitable spacers to preserve the binding properties of the peptide that give rise to its targeting and immunoenhancing properties. A linker or spacer molecule may be used in conjugating a therapeutic agent to the dendrimer conjugates described herein. Spacers may be any combination of amino acids including AAA, KK, GS, GSGGGGS (SEQ ID NO:8), RS, or AAY. As used herein, the terms "linker" or "spacer" mean the chemical groups that are interposed between the dendrimer and the surface exposed molecule(s) such as the MHC class II ligand, CD4+ T helper epitope, and therapeutic agent (e.g., drug, vaccine) that is conjugated or bound to the dendrimer (e.g., PADRE-dendrimer) and the surface exposed molecule(s). Preferably, linkers are conjugated to the surface molecule at one end and at their other end to the nanoparticle (e.g., PADRE-dendrimer). Linking may be performed with either homo- or heterobifunctional agents, i.e., SPDP, DSS, SIAB. Methods for linking are disclosed in PCT/DK00/00531 (WO 01/22995) to deJongh, et al., which is hereby incorporated by reference in its entirety. Cleavable linkers may also be used in the compositions and methods described herein. For example, drug molecules may be coupled to targeted dendrimer (PDD) using a pH sensitive cleavable spacer (e.g., GFLG

(SEQ ID NO: 4)) that releases the drug inside the cells. GFLG (SEQ ID NO: 4) is a tetrapeptide spacer, composed of glycylphenylalanylleucylglycine (SEQ ID NO: 4).

[00068] In another embodiment, the MHC targeting peptide is a T helper epitope such as influenza HA. The T helper epitope, however, can be any epitope that activates or contributes to activation of CD4+ T helper cells. T helper epitope activation of CD4 + T helper cells is required for the expansion and stimulation of CD8 T cells as well as for antibody production by B cells, both of which are essential for induction of protective immune responses against infectious agents and cancerous cells, for example.

[00069] In yet another embodiment, the MHC targeting peptide is one that is not immunopotentiating or lacks known intrinsic immunoenhancing effects, such as CLIP. A CLIP peptide is a “self” peptide that masks the MHC class II for protection (from autoimmune responses) purposes. CLIP is the part of the invariant chain (Ii) that binds MHC class II groove and remains there until the MHC receptor is fully assembled. The purpose of CLIP is to prevent the binding of self-peptide fragments prior to MHC II localization within the endo/lysosome. It is particularly useful in some drug delivery embodiments as described herein for its ability to tag MHC class II as a targeting means to home the nanocarriers described herein to APC (unlike PADRE, CLIP has no T helper activity). This is important for immunoregulation, as activation of the immune system is not desired for many conditions (Crohn's disease, diabetes, etc.). CLIP enables delivery of a drug to APC to silence an immune response, for example. The CLIP peptide may form any part of the entire CLIP sequence, (LPKPPKPVSKMRMATPLLMQALPM (SEQ ID NO:9), including fragments and derivatives thereof. For example, the sequence KMRMATPLLMQALPM (SEQ ID NO:10) of CLIP that was shown to bind multiple human MHC class II including HLA-DRA*01:01/DRB1*01:01, HLA-DR4Dw4, or HLA-DR1, can be used. Another example is SKMRMATPLLMQ (SEQ ID NO:1) that binds to the antigen binding groove of MHC class II of humans (DR1, DR2, DR3(17) and DR7).

[00070] The compositions, methods, platforms and kits described herein have both prophylactic and treatment applications, i.e., can be used as a prophylactic to prevent onset of a disease or condition in a subject, as well as to treat a subject having a disease or condition. A composition or targeted delivery platform as described herein can be used to reduce the growth

of or eliminate any infectious pathogen or cancer cells, mount an immune response against any infectious pathogen or cancer cells, and deliver a drug to cells.

[00071] As an oral drug/vaccine delivery platform, the dendrimer-based platform described herein provides several advantages. The platform targets intestinal PAPCs via its MHC class II ligand, binds and penetrates the cell membrane by its highly positively-charged outer membrane, is resistant to the pH of the stomach yet degrades in the intestines for intestinal release of the drug or vaccine, is safe and easy to scale up for high-volume production, and in some embodiments (e.g., vaccine embodiments, anti-cancer embodiments), acts as a strong adjuvant due to the nature of modifications on the molecule. In a typical embodiment, the at least one dendrimer is a G5 PAMAM dendrimer that is a highly branched polymeric macromolecule and an ideal excipient for its enhanced solubility. In embodiments in which an immune enhancing effect is desired, inclusion of a universal T helper agonist, e.g., PADRE, which binds to the flank of the MHC class II molecules, results in an opsinizing dendrimer complex for PAPCs as well as helper T cells. This alteration changes an inert and weak dendrimer to a robust immune modulator.

[00072] Vaccine formulations and vaccine adjuvants including dendrimers are also described herein. A vaccine formulation for preventing or treating a disease or condition in a subject includes a pharmaceutically acceptable excipient and a composition as described herein. The at least one therapeutic agent is in an amount sufficient to induce an immune response in a subject. In an embodiment in which the subject has a viral infection, typically, administration of the composition induces anti-viral factors that directly reduce viral replication in an infected (e.g., chronically infected) subject. Typically, the vaccine formulation is administered to vaccinate a subject against cancer or infection.

Synthesis of Dendrimers Conjugated to Therapeutic Agents

[00073] Described herein are dendrimers (PDD) having conjugated thereto at least one MHC targeting peptide (e.g., a universal T helper peptide such as the PADRE peptide or Influenza HA, a CLIP peptide, etc.) and a therapeutic agent (e.g., drug, vaccine), wherein the at least one MHC targeting peptide and the therapeutic agent are conjugated to the exterior surface of the dendrimer such that the at least one MHC targeting peptide specifically binds to PAPCs. The PDD are delivered specifically to PAPCs in a subject's intestinal tract due to an enteric coating

which is resistant to the pH in the stomach but that is degraded by the pH in the intestines. Dendrimers can be prepared and conjugated to an MHC targeting peptide (e.g., an epitope such as the PADRE peptide or Influenza HA, CLIP peptide, etc.) and bound to or complexed with a therapeutic agent using any suitable method. Methods of producing and using dendrimers are well known in the art and are described, for example, in Zhang J-T et. al. *Macromol. Biosci.* 2004, 4, 575–578, and U.S. Patent Nos. 4,216,171 and 5,795,582, both incorporated herein by reference. See also: D.A. Tomalia, A.M. Naylor, and W.A. Goddard III, "Starburst Dendrimers: Molecular-Level Control of Size, Shape, Surface Chemistry, Topology, and Flexibility from Atoms to Macroscopic Matter", *Angew. Chem. Int. Ed. Engl.* 29 (1990), 138-175. In the experiments described herein, PAMAM dendrimers were used. However, any suitable positively charged, highly branched polymeric dendrimer can be used. Examples of additional positively charged, highly branched polymeric dendrimers include poly(propylene imine) (PPI) dendrimers or, more generally, any other dendrimers with primary amine groups on their surfaces. Depending on the type of therapeutic agent, more than one (e.g., a plurality) positively charged, highly branched polymeric dendrimers may be conjugated to a single therapeutic agent. For example, multiple PDD may bind to one AmBisome®, as AmBisome® is 100 nm and a PDD as described herein is approximately 8 nm.

[00074] In one embodiment, dendrimers are conjugated to at least one PADRE peptide (e.g., 2, 3, 4, 5, etc.) and a drug or vaccine. The PADRE-dendrimers (PADRE-derivatized dendrimers) described herein can be prepared by any suitable method. Methods of making and using PADRE are known in the art. See, for example, U.S. Patent No. 5,736,142. PADRE peptides can be prepared according to the methods described in U.S. Patent No. 5,736,142, for example, or they can be purchased (e.g., from Anaspec, Inc., Fremont, CA). Because of their relatively short size, the PADRE peptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. Alternatively, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes a T helper epitope is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression. These procedures are generally known in the art, as described generally in Sambrook et al., (*supra*), which is incorporated herein by reference. PADRE peptides as described herein may include modifications to the N- and C-terminal residues. As

will be well understood by the artisan, the N- and C-termini may be modified to alter physical or chemical properties of the peptide, such as, for example, to affect binding, stability, bioavailability, ease of linking, and the like. The PADRE peptides described herein may be modified in any number of ways to provide desired attributes, e.g., improved pharmacological characteristics, while retaining substantially all of the biological activity of the unmodified peptide.

[00075] In the experiments described herein, the PADRE-dendrimer conjugate was made by simple amide coupling between the –COOH terminus of the PADRE peptide and one of the dendrimer amine groups. The PADRE peptide (Ac-D-Ala-Lys-Cha-Val-Ala-Ala-Trp-Thr-Leu-Lys-Ala-Ala-Ala-D-Ala-Ahx-Cys-OH) (SEQ ID NO:11) (Ac= acetylated; D-Ala = D-alanine; Cha = cyclohexylalanine; Ahx = aminohexanoic acid) was purchased from Twentyfirst Century Biochemicals, Inc. (Marlboro, MA) in its acetylated form in order to protect the amine terminus and prevent its reaction. The purchased peptide had a minimum purity of 95%. The amide coupling reaction was carried out under standard conditions in DMF solution or in MBS. There are variants of PADRE, and all such variants are encompassed by the compositions and methods described herein. For oral delivery, PDD-drug platforms are formulated in a biocompatible pH-responsive anionic polymer, EUDRAGIT FS 30 D (Evonic, Germany) which is composed of methyl-acrylate, methyl methacrylate, and methacrylic acid with determined percentages of *Glycerol Monostearate*, and Polysorbate 80. EUDRAGIT FS 30 D is the aqueous dispersion of an anionic copolymer based on methyl acrylate, methyl methacrylate and methacrylic acid (Kucera et al., Drug Dev Ind Pharm. 2012;38(3):341-50). It is insoluble in acidic media, but dissolves by salt formation above pH 7.0. Apart from its enteric properties, its dissolution at a higher pH value allows targeted intestinal delivery. The best results were obtained with formulations made by two luer lock glass syringes connected by a three-way. By changing the ratio of EUDRAGIT FS 30 D and PlasAcryl, two formulations were developed i) a syrup-like formulation for Oral gavage and ii) a soft solid formulation that is chewable.

[00076] In order to control the number of PADRE epitopes attached to the surface of each dendrimer, a 2:1 peptide/dendrimer challenge ratio was used in the reaction, seeking attachment of just a few peptides per dendrimer in order to keep most of the amine groups free to develop large positive charges on the dendrimer. In a typical embodiment, a plurality of PADRE-dendrimer conjugates as described herein will be a distribution of dendrimers containing 0, 1, 2,

3, etc., PADREs (or other peptide) attached thereto. Relative populations are expected to follow the Poisson distribution. The PADRE peptide variants including aKXVAAWTLKAAa (SEQ ID NO:12) bind with high or intermediate affinity ($IC_{50} < 1,000$ nM) to 15 out of 16 of the most prevalent HLA-DR molecules ((Kawashima et al., Human Immunology 59:1-14 (1998); Alexander et al., Immunity 1:751-761 (1994)). However, other peptides which also can bind MHC class II and activate CD4 T helper cells in most humans may also be used to tag the dendrimer.

[00077] Examples of APC targeting peptides include but are not limited to: tetanus toxoid (TT) peptide 830-843; the "universal" epitope described in Panina-Bordignon et al., (Eur. J. Immunology 19:2237-2242 (1989)); and the following peptides that react with MHC class II of most human HLA, and many of mice: aKFVAAWTLKAAa (SEQ ID NO:13), aKYVAAWTLKAAa (SEQ ID NO:14), aKFVAAAYTLKAAa (SEQ ID NO:15), aKXVAAAYTLKAAa (SEQ ID NO:16), aKYVAAAYTLKAAa (SEQ ID NO:17), aKFVAAHTLKAAa (SEQ ID NO:18), aKXVAAHTLKAAa (SEQ ID NO:19), aKYVAAHTLKAAa (SEQ ID NO:20), aKFVAAANTLKAAa (SEQ ID NO:21), aKXVAAANTLKAAa (SEQ ID NO:22), aKYVAAANTLKAAa (SEQ ID NO:23), AKXVAAWTLKAAA (SEQ ID NO:38), AKFVAAWTLKAAA (SEQ ID NO:24), AKYVAAWTLKAAA (SEQ ID NO:25), AKFVAAAYTLKAAA (SEQ ID NO:26), AKXVAAAYTLKAAA (SEQ ID NO:27), AKYVAAAYTLKAAA (SEQ ID NO:28), AKFVAAHTLKAAA (SEQ ID NO:29), AKXVAAHTLKAAA (SEQ ID NO:30), AKYVAAHTLKAAA (SEQ ID NO:31), AKFVAAANTLKAAA (SEQ ID NO:32), AKXVAAANTLKAAA (SEQ ID NO:33), AKYVAAANTLKAAA (SEQ ID NO:34), FNNFTVSFWLRVPKVSASHLE (SEQ ID NO:35), SSVFNVVNSSIGLIM (SEQ ID NO:36), SKMRMATPLLMQ (SEQ ID NO:1), and QYIKANSKFIGITEL (SEQ ID NO:37), (a = D-alanine, X = cyclohexylalanine). Such peptides bind to MHC class II molecules present on T cells of more than 95% of all humans.

[00078] The product was purified by dialysis against pure water for at least 24 h and then dried under vacuum. The collected product, a clear oil, was characterized by 1H NMR, UV-Vis and MALDI-TOF mass spectroscopy. The NMR spectra of the PADRE-dendrimer conjugate shows large peaks corresponding to the dendrimer protons and a small set of peaks for the peptide protons. The MALDI-TOF mass spectrum of the PADRE-dendrimer conjugate shows a

peak at a m/z ratio ca. 3,000 units higher than the peak observed for the dendrimer on its own. The excess mass corresponds to approximately 2 peptide epitopes. The UV-Vis spectrum of the conjugate shows a clear absorption in the wavelength range where tryptophan absorbs.

[00079] Dendrimers that are conjugated to T helper epitopes other than PADRE are typically prepared by a method similar to that described above for PADRE-derivatized dendrimers. For example, the acid terminus of the peptide can be covalently attached to one of the amine groups on the dendrimer surface by a number of well-known synthetic methods, such as amidation using carbodiimides as activating reagents. As another example, attachment of these peptides to amino-terminated dendrimers is performed using two synthetic routes. The amino terminus of the peptide epitope is protected by acetylation. The first route uses the carboxylic acid of the terminal cysteine residue to achieve attachment via standard amidation chemistry. The second route takes advantage of the cysteine's thiol (if present on the peptide, otherwise may be added) to react it with the alkene groups added to the dendrimer surface by previous treatment with maleimide. Both routes allow the functionalization of dendrimers with epitopes. Up to several peptide epitopes (e.g., 2, 3, 4, 5, 6, etc.) per dendrimer will enhance the targeting property of the drug and vaccine delivery agents. However, it is important to leave a large number of unreacted amine groups so that the dendrimer will acquire a large positive charge via protonation at physiological pH values. Dendrimers as described herein can be conjugated to any T helper epitope (e.g., Influenza HA and Pan DRT helper epitope).

[00080] Generally, generation-5 (G5) dendrimers are used in the compositions, kits, platforms and methods described herein. However, other generation dendrimers (see Table 1) can be used.

Table 1 PAMAM Dendrimers

Generation	Molecular Weight	Diameter (nm)	Surface Groups
0	517	1.5	4
1	1,430	2.2	8
2	3,256	2.9	16
3	6,909	3.6	32
4	14,215	4.5	64

5	28,826	5.4	128
6	58,0548	6.7	256

Methods of Delivering a Therapeutic Agent to PAPCs

[00081] Described herein are methods of orally delivering a therapeutic agent for treating or preventing a disease, disorder, infection, etc., specifically to PAPCs in a subject's (e.g., human) intestinal tract, and in some embodiments, inducing an immune response (e.g., activation of CD4 T helper cells, production of monoclonal antibodies) against an antigen, pathogen, or cancer cells. A typical method of delivering a therapeutic agent specifically to professional APCs in a subject's intestinal tract includes administering to the subject a therapeutically effective amount of a composition for orally delivering a drug or vaccine as described herein. Generally, the at least one dendrimer is a G5 dendrimer (a PAMAM dendrimer). In some embodiments, the at least one MHC targeting peptide is a universal T helper peptide such as PADRE or a T helper epitope of influenza HA molecule. In the method, the composition is administered orally, and subsequently degrades in the subject's intestines. Generally, administering the composition to the subject results in internalization of the therapeutic agent by professional APCs by phagocytosis in the subject's gastrointestinal tract. In one embodiment of the method, administering the composition to the subject results in an immune response against the antigen in the subject. In another embodiment, the subject has a cancerous tumor or is at risk of developing a cancerous tumor and administering the composition to the subject results in prevention of or decreased growth of the cancerous tumor.

[00082] Administration of the composition generally results in no local adverse reactions in the subject. The compositions, platforms, kits and methods described herein can be utilized with any suitable subject, including invertebrate and vertebrate subjects. In a typical embodiment, a subject to be treated is an animal such as a mammal (e.g., human beings, rodents, dogs, cats, goats, sheep, cows, horses, etc.). A human patient suffering from or at risk of contracting an infectious disease, having or developing cancer, or suffering from another disease or disorder requiring treatment is a typical subject. For example, the subject is a human in need of a vaccine against an infectious pathogen or cancer.

[00083] In some embodiments, prior to or after administration of a composition as described herein to a subject, the subject's immune response is analyzed or measured. Any suitable biological sample can be tested for analyzing or measuring a subject's immune response. Examples of biological samples include blood, serum, plasma, urine, saliva and tissue. The sample may be tested using any suitable protocol or assay. Examples of suitable assays include enzyme-linked immunosorbent assays (ELISAs), Western blots, flow cytometry assays, immunofluorescence assays, qPCR, microarray analysis, etc.

Kits for Orally Delivering Drugs and Vaccines to APCs

[00084] A kit for delivering a drug or vaccine to a subject typically includes a composition for oral delivery of a drug or vaccine as described herein, a pharmaceutically acceptable carrier; instructions for use; and packaging. Lyophilized PDD or any other feasible form of PDD (universal T helper epitope such as PADRE conjugated to dendrimer, CLIP conjugated to dendrimer, etc.) may be included in a kit. In one example of a kit, the kit includes the therapeutic agent (e.g., a drug, vaccine) and a buffer composed of physiological saline, phosphate buffer saline, or OptiMem, that has a physiological pH and is buffered.

[00085] In a kit, the instructions generally include one or more of: a description of the composition; dosage schedule and administration for prevention or treatment (e.g., vaccination) of cancer or infectious disease; precautions; warnings; indications; counter-indications; overdose information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container. Generally, a kit as described herein also includes packaging. In some embodiments, the kit includes a sterile container which contains a therapeutic or prophylactic composition; such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding cells or medicaments.

Administration of Compositions

[00086] The compositions described herein may be administered to invertebrates, animals, and mammals (e.g., dog, cat, pig, horse, rodent, non-human primate, human) in any suitable formulation. For example, a composition including a PADRE-dendrimer conjugated to a therapeutic agent may be formulated in pharmaceutically acceptable carriers or diluents such as physiological saline or a buffered salt solution. Suitable carriers and diluents can be selected on the basis of mode and route of administration and standard pharmaceutical practice, those for oral delivery are generally preferred. A description of exemplary pharmaceutically acceptable carriers and diluents, as well as pharmaceutical formulations, can be found in Remington's Pharmaceutical Sciences, a standard text in this field, and in USP/NF. Other substances may be added to the compositions to stabilize and/or preserve the compositions.

[00087] The compositions described herein may be administered to a subject (e.g., mammals) by any conventional technique. Typically, such administration will be oral. In therapeutic applications, the compositions described herein are administered to an individual already suffering from a disease or disorder or infection, for example, an individual infected with a pathogen of interest or having cancer cells (e.g., a cancerous tumor). In prophylactic applications, the compositions described herein are administered to an individual at risk of developing (e.g., genetically predisposed to, or environmentally exposed to) or contracting an infectious disease, cancer, or other disease or disorder. The compositions and vaccines may be formulated according to conventional pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy (20th ed.), ed. A. R. Gennaro, Lippincott Williams & Wilkins, (2000) and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, Marcel Dekker, New York (1988-1999)).

Effective Doses

[00088] The compositions described herein are preferably administered to a subject (e.g., invertebrates, animals, mammals (e.g., dog, cat, pig, horse, rodent, non-human primate, human)) in an effective amount, that is, an amount capable of producing a desirable result in a treated subject (e.g., protection against cancer, infectious disease(s), or other disease or disorder). Such a therapeutically effective amount can be determined as described below.

[00089] Toxicity and therapeutic efficacy of the compositions described herein can be determined by standard pharmaceutical procedures, using either cells in culture or experimental animals to determine the LD₅₀ (the dose lethal to 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Those compositions that exhibit large therapeutic indices are preferred. While those that exhibit toxic side effects may be used, care should be taken to design a delivery system that minimizes the potential damage of such side effects. The dosage of preferred compositions lies preferably within a range that includes an ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. Generally, the range of concentrations for the MHC targeting peptide/dendrimer is 0.001mg/Kg to 200 mg/Kg per day for at least one day. Generally, the range of concentrations for the therapeutic agent is 0.001mg/Kg to 200 mg/Kg per day for at least one day. In an embodiment in which the complex of MHC targeting peptide/dendrimer is conjugated to AmBisome®, the concentration of AmBisome® per dose is in the range of about 0.1 mg/kg to about 50 mg/kg. As is well known in the medical and veterinary arts, dosage for any one subject depends on many factors, including the subject's size, body surface area, age, the particular composition to be administered, time and route of administration, general health, and other drugs being administered concurrently.

EXAMPLES

[00090] The present invention is further illustrated by the following specific examples. The examples are provided for illustration only and should not be construed as limiting the scope of the invention in any way.

Example 1 – PDDs complex with drug-encapsulating liposomes

[00091] A drug/vaccine delivery platform as described herein uses PAN-HLA-DR-binding epitope (PADRE) as a macrophage (APC) homing moiety, a promiscuous CD4+ Th determinant that binds to most murine and human MHC class II molecules (Daftarian et al., Cancer Res. 2011 Oct 10; Daftarian et al., Vaccine. 2005 May 16;23(26):3453-68). The platform is positively charged and thus makes a complex with liposomes that have a negative net charge (Figure 1). Figure 1 shows the TEM image of PDD making a complex with a negatively charged liposomal drug (AmBisome®). This was shown in a series of experiments using PDD/DNA. SEM studies

revealed that only 40 minutes post-addition of PDD/DNA (or in theory other payloads) complex to human B cells (also carry MHC class II), the nanoparticle complexed with cargo was homed on the membrane of human B cells (Figure 2). The PADRE-dendrimer expands and activates macrophage and dendritic cells and enhances MHC class II expression (Daftarian et al., Cancer Res. 2011 Oct 10), dramatically boosting their recruitment to the draining lymph-nodes and spleen resulting in antigen-specific Th1 responses in the presence of a pathogen. The complex of PDD and AmBisome was also made in one step by adding calculated AmBisome drop wise to the PDD solution (in PBS). After 20 minutes incubation at room temperature, the complex was formed. PDD was able to reduce the drug dose by APC targeted delivery via subcutaneous injections.

Example 2 – Oral formulation of PDD/vaccine/drug – enteric coating and intestinal targeting

[00092] Described herein is a formulation for the oral administration of a PDD/cargo complex (Figure 3) including an enteric coating. The composition disclosed herein has an enteric coating which results in stability of a PDD/vaccine (or drug) complex in the stomach's low pH while the enteric coating (coated polymer) will degrade in the intestines. Figure 4 depicts the formulation process. PDD-drug platforms are formulated in a biocompatible pH-responsive anionic polymer, EUDRAGIT FS 30 D (Evonic, Germany) which is composed of methyl-acrylate, methyl methacrylate, and methacrylic acid with up to 50 percent of *Glycerol Monostearate*, and Polysorbate 80, and up to 50% of the cargo (dendrimer-peptide complexed with drug or vaccine). EUDRAGIT FS 30 D is the aqueous dispersion of an anionic copolymer based on methyl acrylate, methyl methacrylate and methacrylic acid. It is insoluble in acidic media, but dissolves by salt formation above pH 7.0. Apart from its enteric properties, its dissolution at a higher pH value allows targeted intestinal delivery. The best results were obtained with formulations made by two luer lock glass syringes connected by a three-way. By changing the ratio of EUDRAGIT FS 30 D and PlasAcryl, two formulations were developed i) a syrup-like formulation for oral gavage and ii) a soft solid formulation that is chewable.

[00093] In general, oral drug administration is known to result in rapid drug absorption/PK and yet less toxicity when compared with that of i.v. injections. Therefore, a new formulation as described herein having an enteric coating and intestinal targeting characteristics provides an improved delivery vehicle for drugs and vaccines, including an embodiment in which the drug to

be delivered is engrafted in a liposome. Moreover, this new formulation of oral delivery and formulation has shown drastic immune enhancing in the data described here. These data includes suppression of the bacterial growth, enhancement of local IFN- γ and IL-12 p40.

[00094] Female C57BL mice in groups of 8 were deprived of food for 3 hours before they received orally the PDD/ovaDNA in a final volume of 200 μ l, once. The positive control group received two biweekly interaperitoneal (IP) injections while negative control groups received either no injections, or an oral administration of a negative control dendrimer/ovaDNA. Anti-OVA antibodies were measured using sera of mice in an ELISA. As shown in the Figure 5, a sole oral administration of ORAL formulation of PDD/ova-DNA (20 ug of the plasmid DNA encoding ovalbumin), resulted in significant anti-OVA antibodies in day 21 post oral gavage. The titers of anti-OVA antibodies in the group that received a single oral gavage of the oral formulation of PDD were comparable with that of the group that received 2 IP immunizations (positive control).

Example 3 – PDD intrinsic immunoenhancing effect

[00095] A preliminary optimization demonstrated effective delivery of PDD/siRNA targeted to dendritic cells in peyer's patches. This novel oral formulation was used for the delivery of Poly:IC and PDD and were compared for their priming intestinal host defense mechanism against Gram-negative *Yersinia enterocolitica* (Figure 10). Furthermore, indications of an immunoenhancing capability of PDD have been shown by a transient enlargement of spleens or draining lymph nodes when mice received injections of PDD/AmB. C57BL mice received s.c. injections of PDD/AmB or ScrDD/AmB on the day 5 post-treatments, spleens or draining lymph nodes were removed and were compared for their size. A transient enlargement of the spleens and draining lymph nodes of mice receiving PPD/AmB was consistent. Previously conducted experiments clearly showed that the dendritic cells expansion/recruitment is partially responsible for the larger size and that this transient size change has been reported for other strong adjuvants such as CpG-DNA.

Example 4 - One oral dose of “Enteric Coated PDD/OVA-DNA” vaccine rejects B16 melanoma tumor

[00096] Oral vaccine *in vivo* efficacy was assessed using a B16 made as followed. Ova DNA (15 ug) was complexed with PDD (105 ug) and was enteric-coated using the formulation described above. This vaccine was given orally to five mice where the final volume was 0.2 ml once. Mice in group of five also received either PBS alone or a positive control vaccine composed of two subcutaneous injections (an immunization and a booster immunization 2 week after) of PDD/ova-DNA (15 ug of ova-DNA and 105 ug of the PDD in PBS). Two immunizations with PDD/ova-DNA were required to result in the rejection of the B16/ova melanoma tumor as was shown before (Daftarian et al., Cancer Res. 2011 Oct 10). Mice received subcutaneous (subQ) injections of 50,000 B16/ova melanoma cells in their left flanks, 2 weeks post last immunization. As shown in Figure 6, all mice which received PBS developed large tumors by day 14 and the tumor take in unimmunized mice was 100%. However, in groups which received PDD/ova-DNA (subQ or oral) vaccines, tumor cells were rejected. These data demonstrates that one dose of the oral formulation of the PDD vaccine was able to elicit immune responses that could prevent tumor growth. Additionally, a single oral PDD/ovaDNA was as effective as 2 subQ immunizations while in each immunization the same DNA and PDD dose was administered. This is unprecedented and calls for a different application of the platform that is re-formulation of existing vaccines in a PDD enteric-coated platform as described herein, which may result in lowering the dose and the frequency of immunizations. This is a burning issue in implementing immunization of some vaccines such as hepatitis B where a need for booster immunizations faces compliance issues.

Example 5 – Oral Administration of PDD for delivery of RNA

[00097] In the following *in vivo* experiments, mice received oral administration of control formulations or the Fluorescent-labeled dsRNA or Poly IC (a toll like 3 ligand) complexed with PDD and coated with methyl-acrylate, methyl methacrylate, and methacrylic acid, at a weight ratio of 7:3:1; this whole formulation is referred to as “EC” for enteric coated. The following *in vitro* studies were done using poly (IC) alone or complexed with PDD.

[00098] Referring to FIG. 7, 8-12 weeks-old mice (4 each) were given fluorescein-conjugated random dsRNA, or Enteric Coated (methyl-acrylate, methyl methacrylate, and methacrylic acid at a weight ratio of 7:3:1 or EC) fluorescein-conjugated random dsRNA, or EC coated PDD only, or EC coated PDD-encapsulated fluorescein-conjugated random dsRNA, after neutralizing

stomach acid by 10% sodium bicarbonate. Peyer's patches were isolated after 24 hours of administration and fluorescein signal in CD11c+ dendritic cells was detected by flow cytometry. FIG. 8 shows % fluorescein positive cells in CD11c+ cell population (the data shown in FIG. 7).

[00099] Referring to FIG. 9, 8-12 weeks-old mice (3 each) were given nothing, or EC-coated poly I:C, or EC-coated PDD only, or EC-coated PDD-encapsulated poly I:C, after neutralizing stomach acid by 10% sodium bicarbonate. Serum IL-12p40 was measured by ELISA after 48 hours. Data showed were all lower than detectable levels. The fact that the levels of pro-inflammatory cytokines are low and in the normal range indicates that the enteric coated targeted delivery of a potent toll like receptor ligand, Poly(IC), does not induce systemic inflammation and the biological effect is limited to micro-environmental space of DCs in PP.

[000100] Referring to FIG. 10, 8-12 weeks-old mice were given SF30D-coated poly I:C, or SF30D-coated PDD only, or SF30D-coated PDD-encapsulated poly I:C, after neutralizing stomach acid by 10% sodium bicarbonate. Single cell suspension was taken from Peyer's patches after 48 hours. 5×10^5 cells/500ul per well were infected with *Yersinia enterocolitica* (MOI=1) in a 48 well plate. Plate was spun after 2 hours, and media was discarded and replaced with 500ul of ID water *Yersinia* contents in supernatant (50ul) was counted after 48 hours culture.

[000101] Referring to FIG. 11, 8-12 weeks-old mice (3 each) were given EC-coated poly I:C, or EC-coated PDD only, or EC-coated PDD-encapsulated poly I:C, after neutralizing stomach acid by 10% sodium bicarbonate. Single cell suspension was taken from the mesenteric nodes after 48 hours. Cells were stimulated with heat-killed *Yersinia* for 24 hours and cytokine secretion was measured by ELISA.

[000102] Referring to FIG. 12, 8-12 weeks-old mice (3 each) were given SF30D-coated poly I:C, or EC-coated PDD only, or EC-coated PDD-encapsulated poly I:C, after neutralizing stomach acid by 10% sodium bicarbonate. RNA was isolated from Peyer's patches after 8 hours. Cytokine expression was analyzed by real-time PCR.

[000103] Referring to FIG. 13, 8-12 weeks-old mice (3 each) were given SF30D-coated poly I:C, or EC-coated PDD only, or EC-coated PDD-encapsulated poly I:C, after neutralizing stomach acid by 10% sodium bicarbonate. RNA was isolated from Peyer's patches after 8 hours. Cytokine expression was analyzed by real-time PCR.

Other Embodiments

[0100] Any improvement may be made in part or all of the compositions, kits, platforms, and method steps. All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended to illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. For example, the peptide-derivatized dendrimer (PDD) nanocarrier platform described herein can be complexed with a polypeptide or a peptide rather than a compound or nucleic acid for delivering a vaccine or drug. Any statement herein as to the nature or benefits of the invention or of preferred embodiments is not intended to be limiting, and the appended claims should not be deemed to be limited by such statements. More generally, no language in the specification should be construed as indicating any non-claimed element as being essential to the practice of the invention. This invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contraindicated by context.

What is claimed is

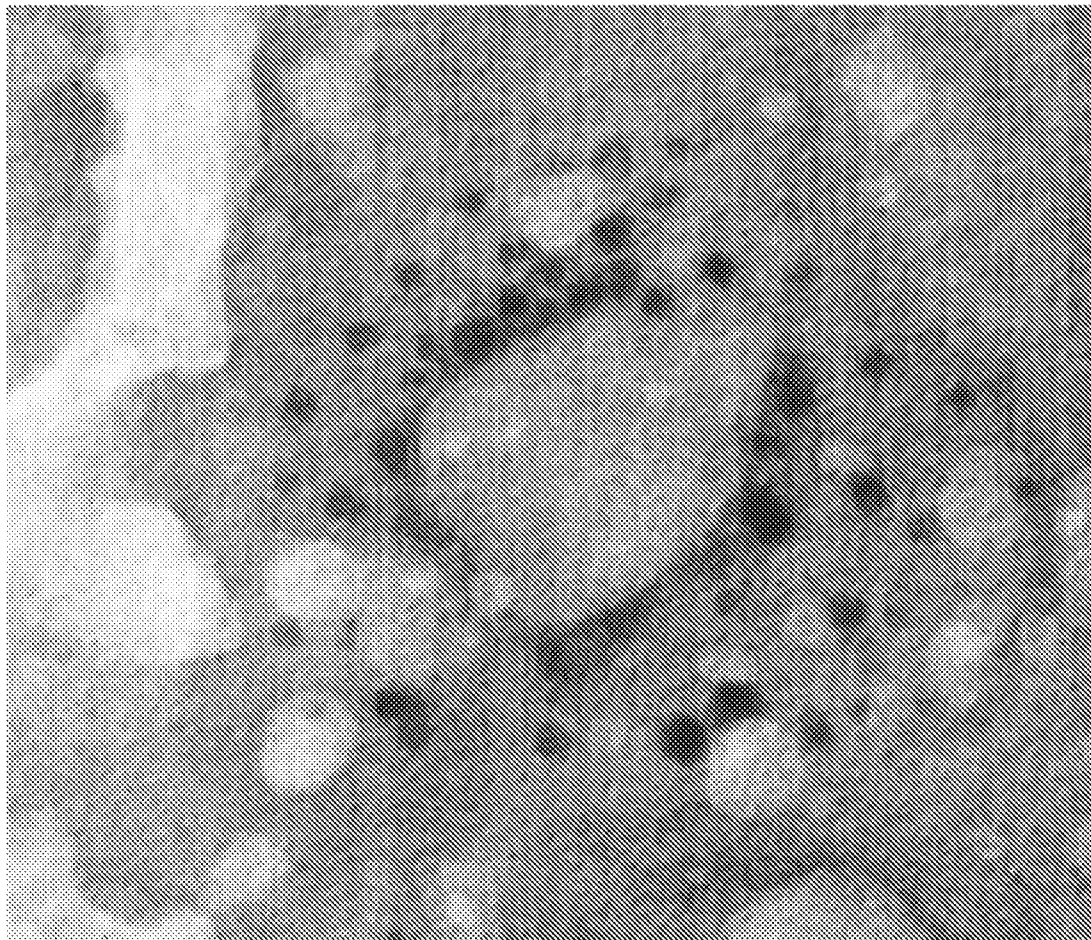
1. A composition comprising: a) at least one dendrimer having conjugated thereto: i) at least one MHC class II targeting peptide conjugated to the exterior surface of the at least one dendrimer, and ii) at least one therapeutic agent; and b) a polymer emulsion encapsulating the dendrimer, the MHC class II targeting peptide, and the at least one therapeutic agent, wherein the polymer emulsion is resistant to a pH of about 7.0 or less and degrades in a pH of about 7.1 or higher.
2. The composition of claim 1, wherein the at least one MHC class II targeting peptide specifically binds to APCs in a subject's gastrointestinal tract.
3. The composition of claim 1, wherein the therapeutic agent consists of a nucleic acid encoding an antigen, and the composition is a vaccine.
4. The composition of claim 1, wherein the therapeutic agent consists of a drug.
5. The composition of claim 1, wherein the therapeutic agent consists of a liposome encapsulating a drug.
6. The composition of claim 1, further comprising a pharmaceutically acceptable carrier.

7. The composition of claim 1, wherein the at least one MHC class II targeting peptide is a Pan-DR T helper epitope (PADRE) or an epitope of influenza virus haemagglutinin molecule, and the at least one dendrimer is a poly(amidoamine) (PAMAM) dendrimer.
8. The composition of claim 4, wherein the at least one MHC class II targeting peptide is CLIP.
9. The composition of claim 1, wherein the polymer emulsion is an enteric formulation comprising methyl-acrylate, methyl methacrylate, and methacrylic acid mixed with glycerol monostearate, triethyl citrate, and polysorbate.
10. The composition of claim 3, wherein the dendrimer, the MHC class II targeting peptide, and the nucleic acid encoding an antigen are in an amount effective for inducing an immune response against the antigen when administered to a subject.
11. The composition of claim 1, wherein the dendrimer, the MHC class II targeting peptide, and the at least one therapeutic agent are in an amount effective for preventing or decreasing growth of a cancerous tumor when administered to a subject having a cancerous tumor or at risk of developing a cancerous tumor.
12. A method of delivering a therapeutic agent specifically to professional APCs in a subject, the method comprising administering to the subject a therapeutically effective amount of the composition of claim 1.

13. The method of claim 12, wherein the subject is a human, and the therapeutic agent consists of a nucleic acid encoding an antigen, and the composition is a vaccine.
14. The method of claim 12, wherein the subject is a human, and the therapeutic agent consists of a liposome or a liposome encapsulating a drug.
15. The method of claim 12, wherein the composition further comprises a pharmaceutically acceptable carrier.
16. The method of claim 12, wherein the at least one MHC class II targeting peptide is a PADRE or an epitope of influenza virus haemagglutinin molecule, and the at least one dendrimer is a PAMAM dendrimer.
17. The method of claim 14, wherein the at least one MHC class II targeting peptide is CLIP and the at least one dendrimer is a PAMAM dendrimer.
18. The method of claim 12, wherein the polymer emulsion is an enteric formulation comprising methyl-acrylate, methyl methacrylate, and methacrylic acid mixed with glycerol monostearate, triethyl citrate, and polysorbate.
19. The method of claim 13, wherein administering the composition to the subject results in an immune response against the antigen in the subject.

20. The method of claim 12, wherein the subject has a cancerous tumor or is at risk of developing a cancerous tumor and administering the composition to the subject results in prevention of or decreased growth of the cancerous tumor.
21. The method of claim 12, wherein administering the composition to the subject results in internalization of the therapeutic agent by professional APCs in the subject's gastrointestinal tract.
22. The method of claim 12, wherein the composition is administered orally, and subsequently degrades in the subject's intestines.
23. A kit for delivering a drug or vaccine to a subject, the kit comprising:
- a) the composition of claim 1;
 - b) a pharmaceutically acceptable carrier;
 - c) instructions for use; and
 - d) packaging.

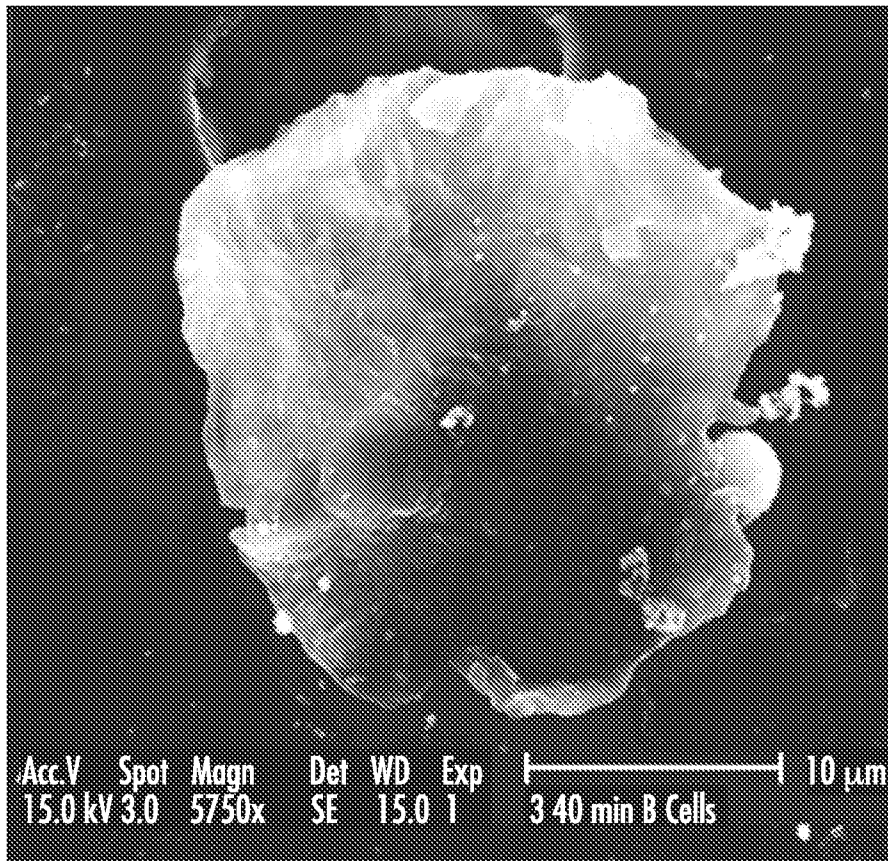
1/13



TEM OF PDD/AmBISOME COMPLEX. PDD WAS
ADDED TO AmBISOME AT A 7:1 RATIO. AFTER A TWENTY-MINUTE
INCUBATION AT ROOM TEMPERATURE THE TEM IMAGE WAS OBTAINED.

FIG. 1

2/13



PDD TARGETS HUMAN
APC. DNA/PPD
PPD WAS LOADED
WITH pcDNA3 AND
THE COMPLEX WAS
INCUBATED FOR 40
MINUTES AT RT WITH
MAGNETICALLY PURIFIED
HUMAN B-CELLS. CELLS
WERE WASHED, FIXED
AND IMAGED IN THE
SEM. PDD/CARGO IS
SEEN ON 40 MIN POST
CO-INCUBATION.

FIG. 2

3/13

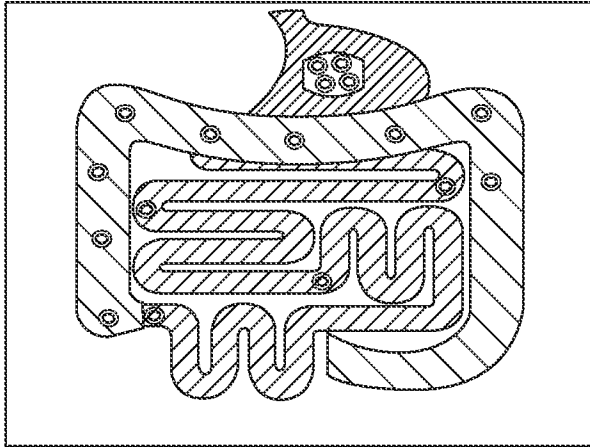


FIG. 3

4/13

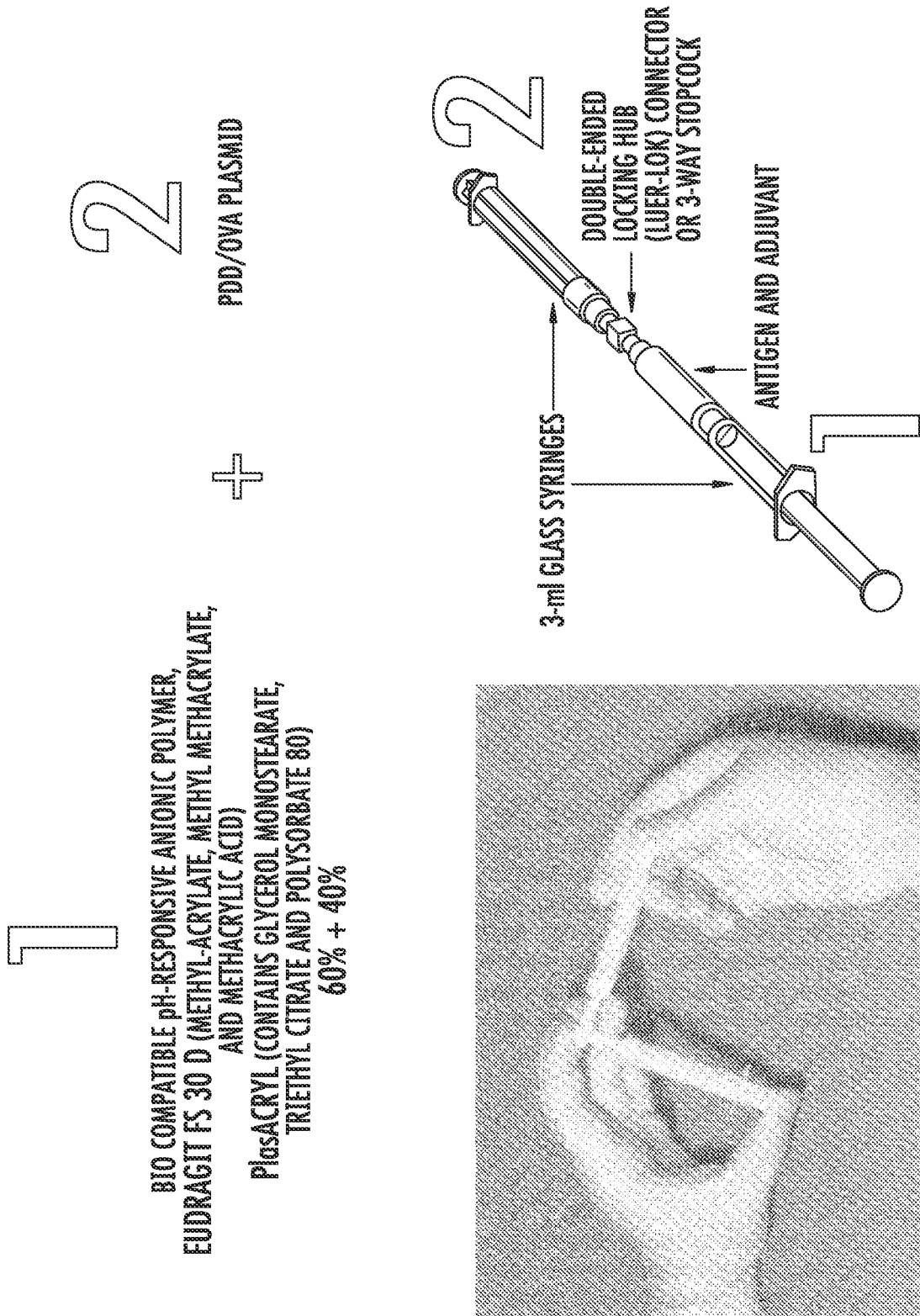


FIG. 4

5/13

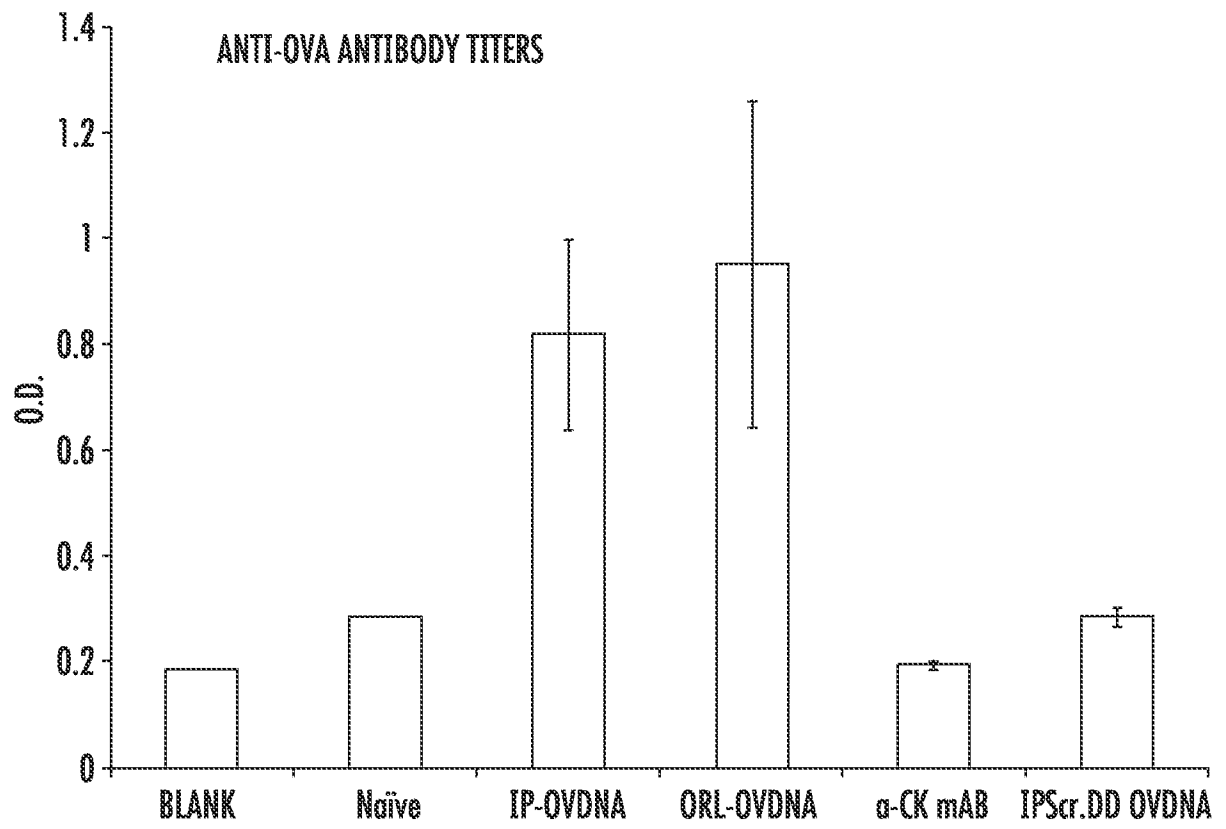


FIG. 5

6/13

PROTECTS AGAINST TUMOR
B16/OVA TUMOR CHALLENGE POST IMMUNIZATION

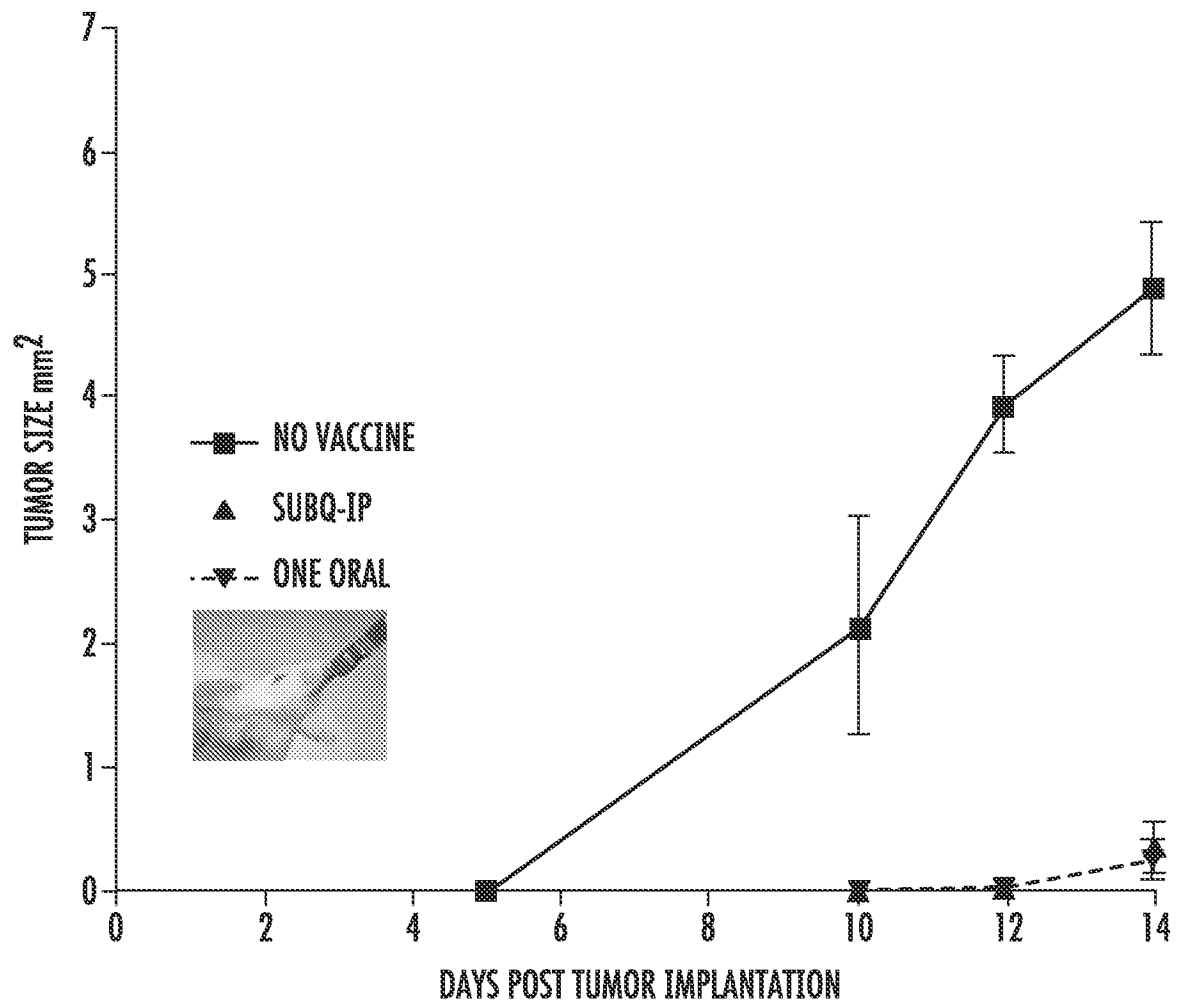
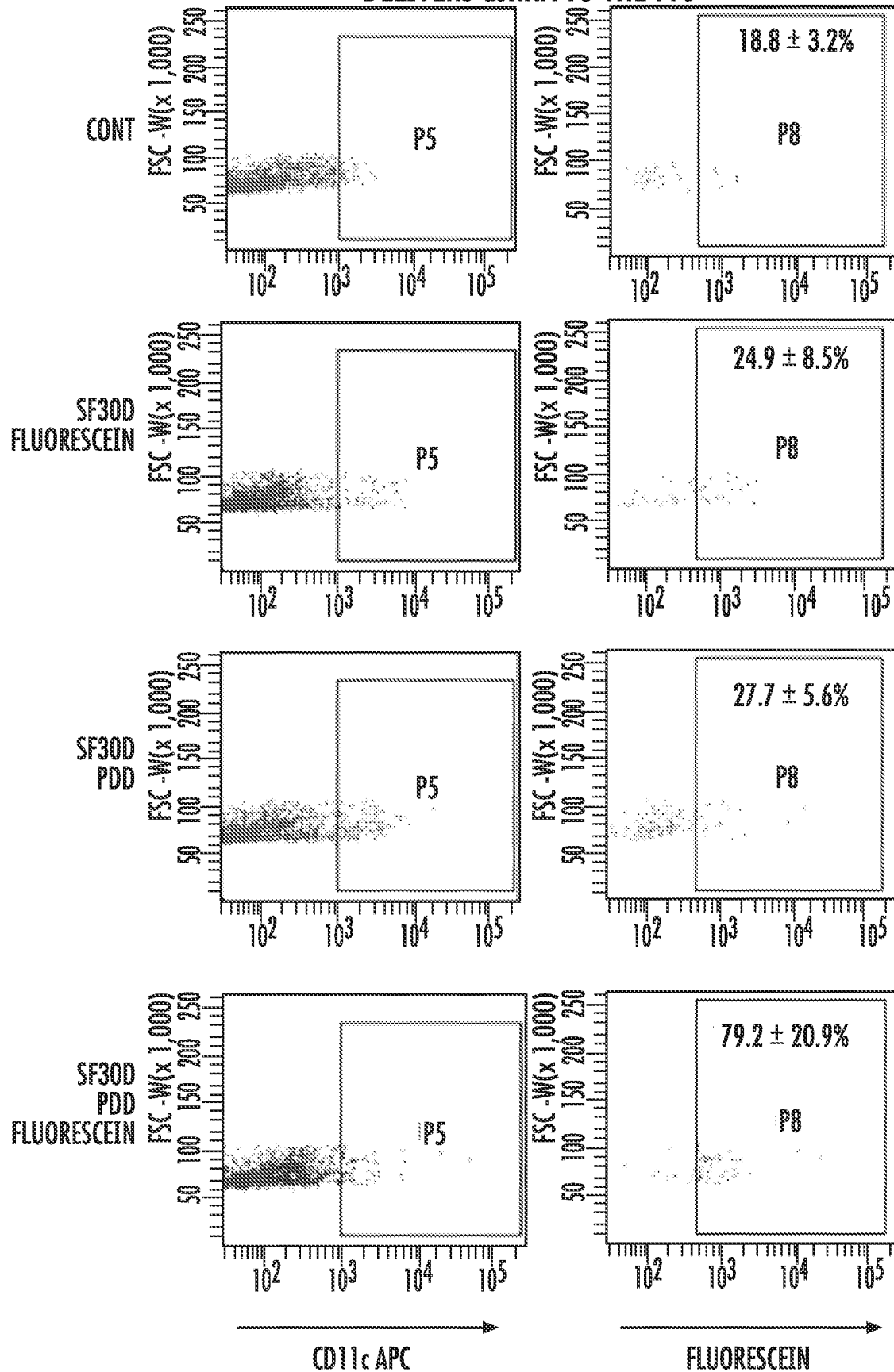
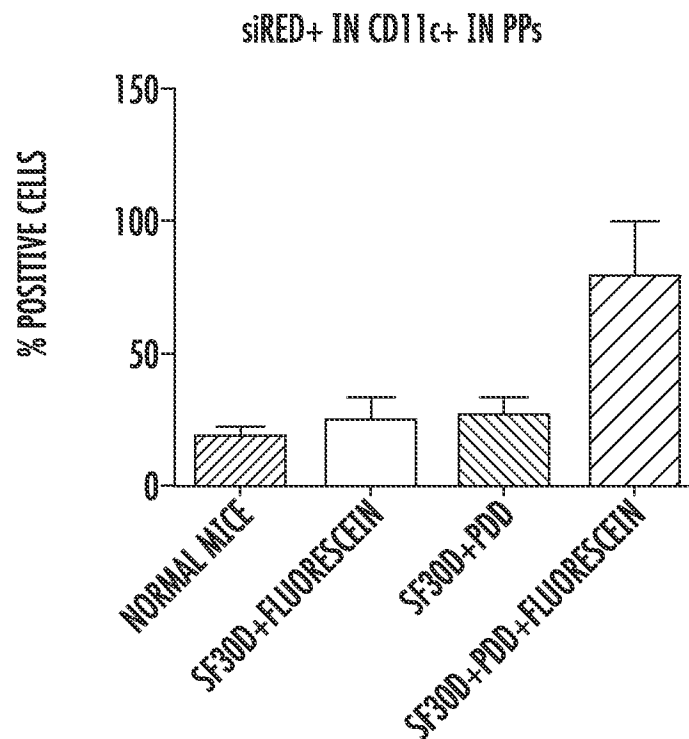


FIG. 6

7/13

**ORALLY ADMINISTERED PDD SUCCESSFULLY
DELIVERS dsRNA TO THE PP5****FIG. 7**

8/13

**ORALLY ADMINISTERED PDD SUCCESSFULLY
DELIVERS dsRNA TO THE PP_s****FIG. 8**

9/13

ORALLY ADMINISTERED POLY I:C-PDD DOES
NOT INDUCE SYSTEMIC PRO-INFLAMMATORY RESPONSE

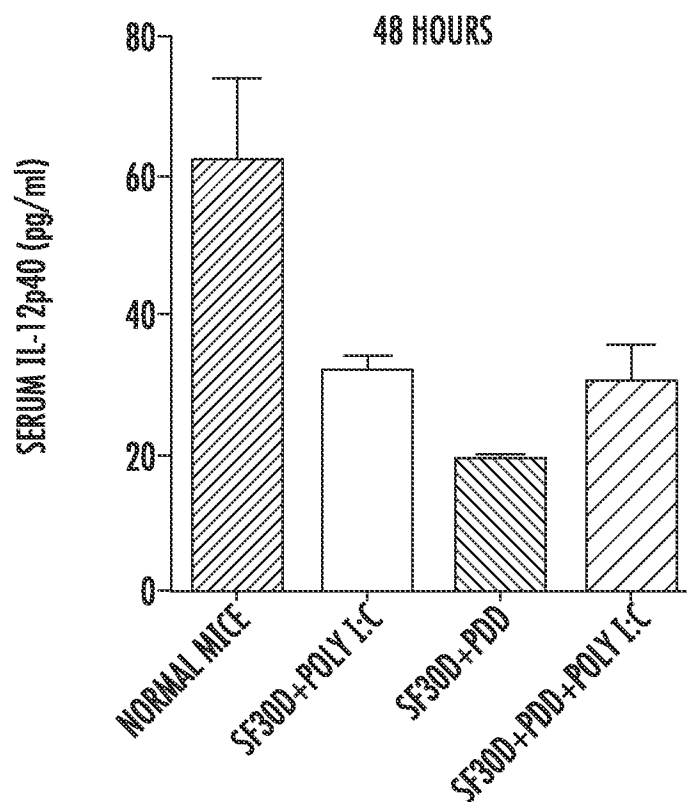


FIG. 9

10/13

PP CELL SUSPENSION INFECTED WITH *Y. ENTEROCOLITICA*
48 HOURS POST ADMINISTRATION

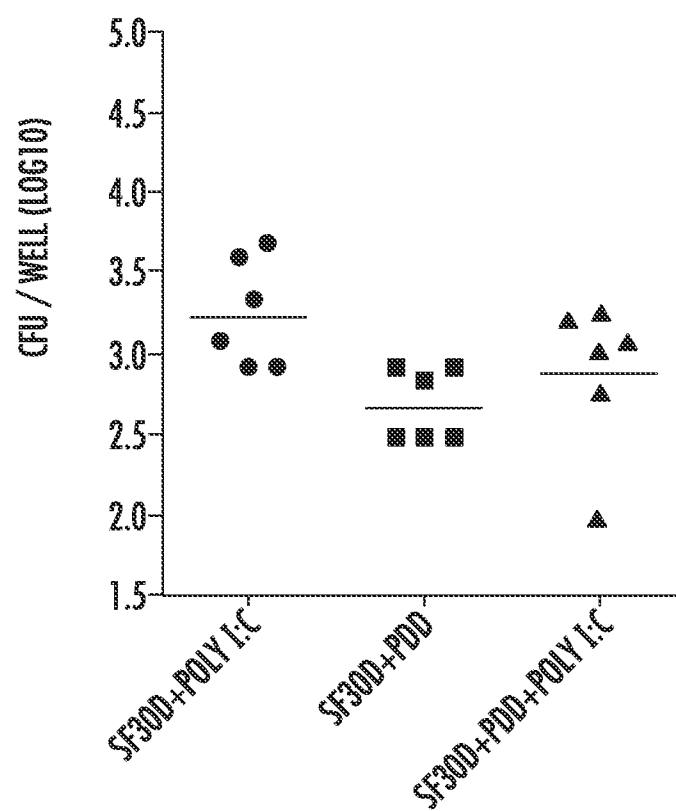


FIG. 10

11/13

**CYTOKINE PRODUCTION IN THE MLN AFTER
STIMULATION WITH HKY (10⁶g/ml)
48 HOURS POST ADMINISTRATION**

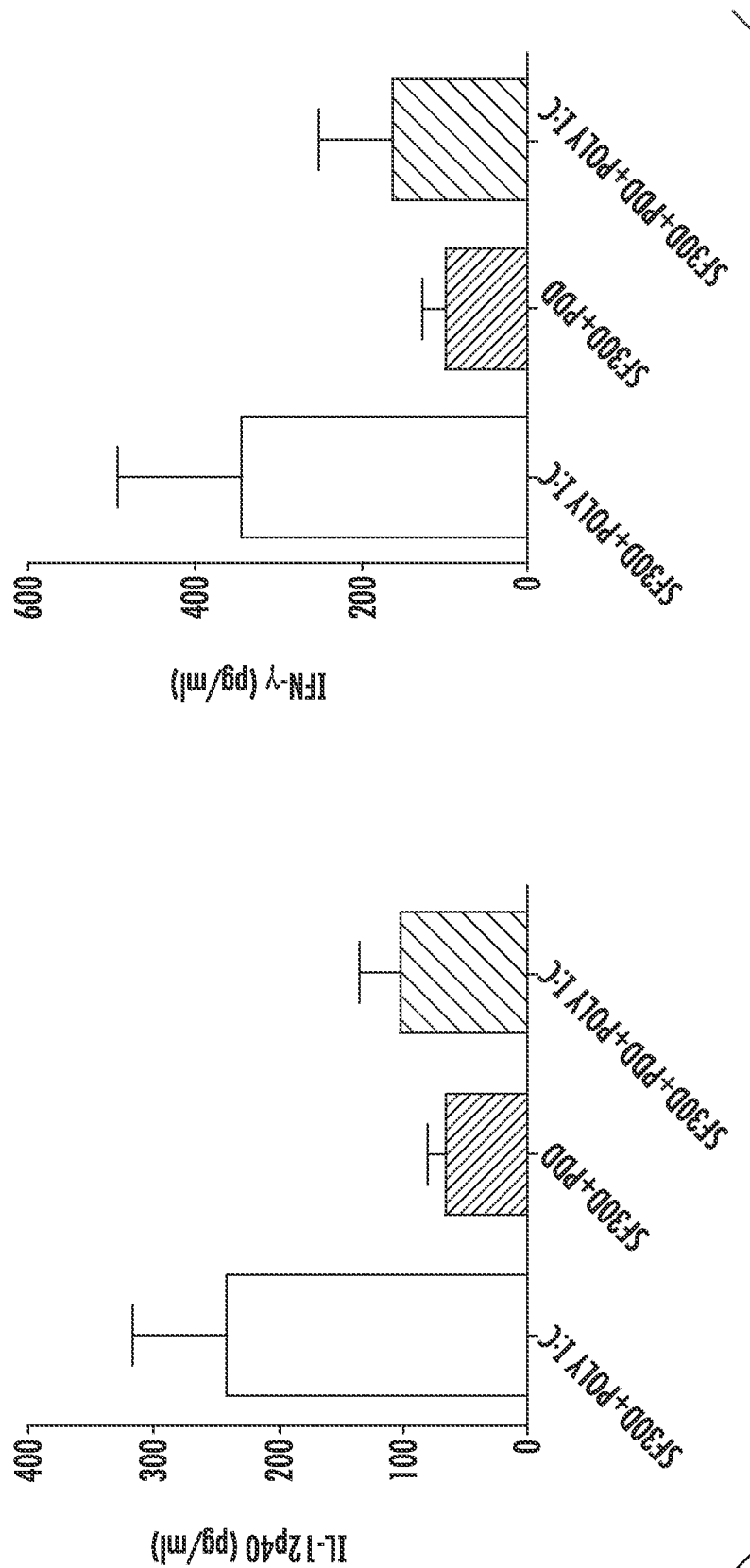
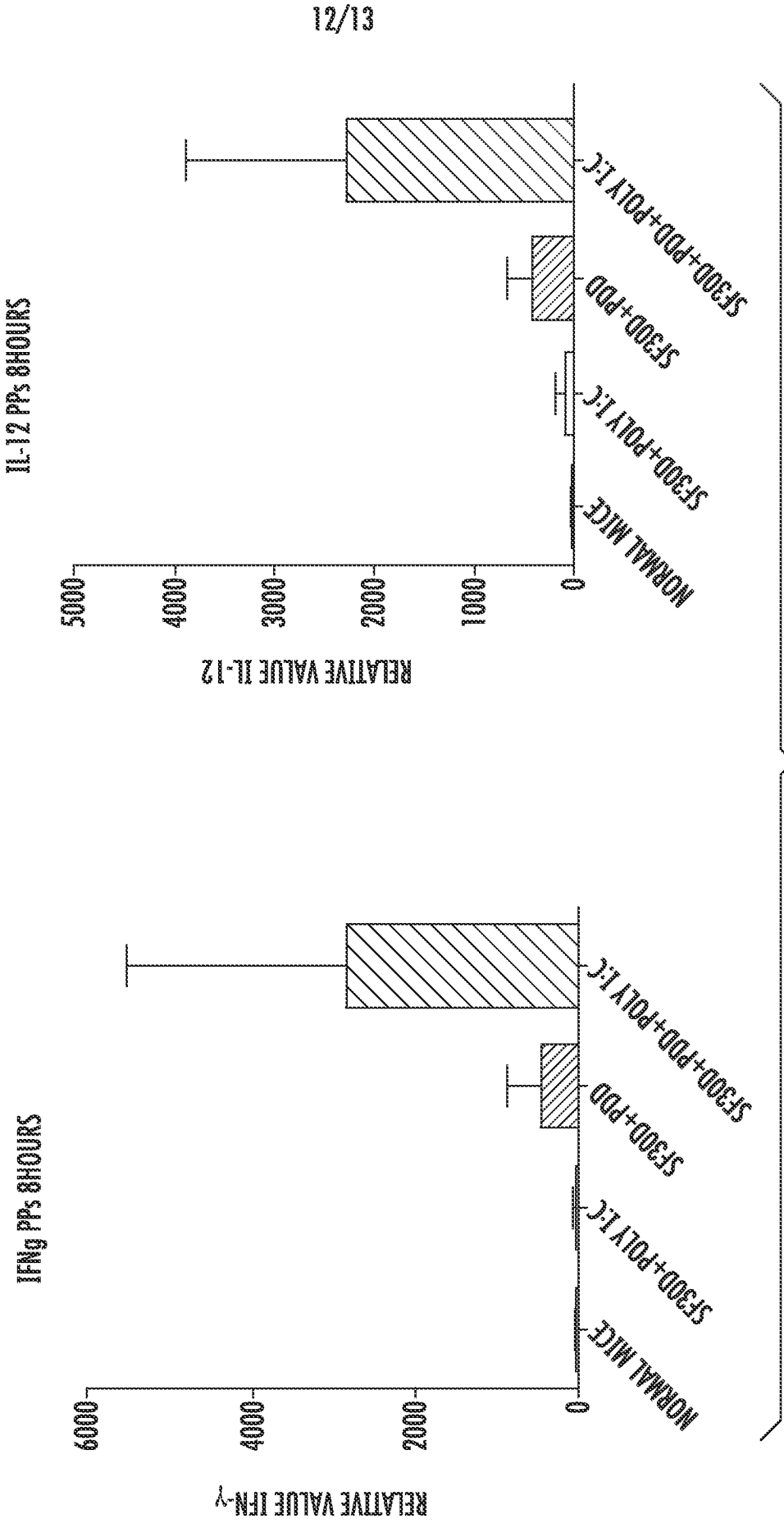


FIG. 11

CYTOKINE EXPRESSION IN THE PPs
8 HOURS POST ADMINISTRATION



12/13

13/13

ABILITY OF KILLING SALMONELLA
PP CELL SUSPENSION (8 HOURS POST ORAL DELIVERY)

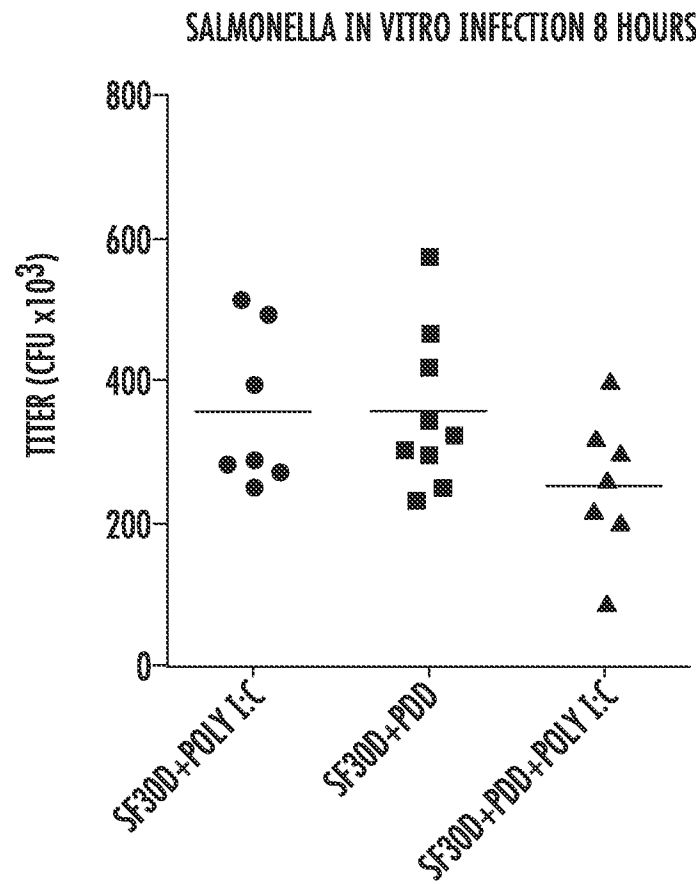


FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US14/13647

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - A61K 39/12, 39/00, 39/38 (2014.01) USPC - 424/186.1, 184.1, 190.1, 450, 400, 204.1, 209.1, 210.1 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(8): A61K 39/12, 39/00, 39/38 (2014.01) USPC: 424/186.1, 184.1, 190.1, 450, 400, 204.1, 209.1, 210.1 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) MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; ProQuest; PubMed; dendrimer; 'PAMAM Dendrimer,' conjugated, affixed, coupled, linked, bound, joined, 'MHC class II targeting peptide,' 'PADRE,' 'CLIP,' 'therapeutic agent,' vaccine, drug; encapsulated, enclosed, covered, enveloped, surrounded, wrapped		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2010/115046 A2 (DAFTARIAN, PM et al.) October 7, 2010; abstract; paragraphs [0014], [0015], [0017], [0019], [0023], [0051], [0053], [0075], [0088], [0091], [00113], figure 1	1-23
Y	EP 1117386 B1 (COLE, ET et al.) December 1, 2004; paragraphs [0001], [0013], [0017], [0023], [0024]	1-23
Y	WO 2012/094495 A2 (NEWELL, MK et al.) July 12, 2012; abstract; page 2, lines 27-32; page 3, lines 4-8	2
Y	DAFTARIAN, P. Adjuvanted And APC Opsonized Nanovaccines. 2012 [retrieved on 2014-03-18]. Retrieved from the Internet; <URL: http://ncnr.org/wp-content/uploads/2012/10/1350-Daftarian.pdf ; Slide 26.	5, 14, 17
Y	CA 2762586 A1 (DAFTARIAN, PM et al.) November 25, 2010; abstract; paragraph [0073], [0131]	8, 17
Y	EP 2283830 A1 (ACTOGENIX, N.V.) February 16, 2011; paragraphs [0004], [0009], [0010], [0012], [0016], [0020]	9, 18
Y	WO 2010/042876 A9 (SHI, J et al.) April 15, 2010; paragraphs [00135], [00511], [00533]	21
☐ Further documents are listed in the continuation of Box C. ☐		
* 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 07 April 2014 (07.04.2014)		Date of mailing of the international search report 24 APR 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201		Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774