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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 January 2014 (16.01.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/011705 A1

(51) International Patent Classification:
A61K 38/17 (2006.01) *A61K 47/48* (2006.01)
A61K 38/16 (2006.01) *A61P 37/00* (2006.01)

(21) International Application Number:
PCT/US2013/049816

(22) International Filing Date:
9 July 2013 (09.07.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/669,568 9 July 2012 (09.07.2012) US

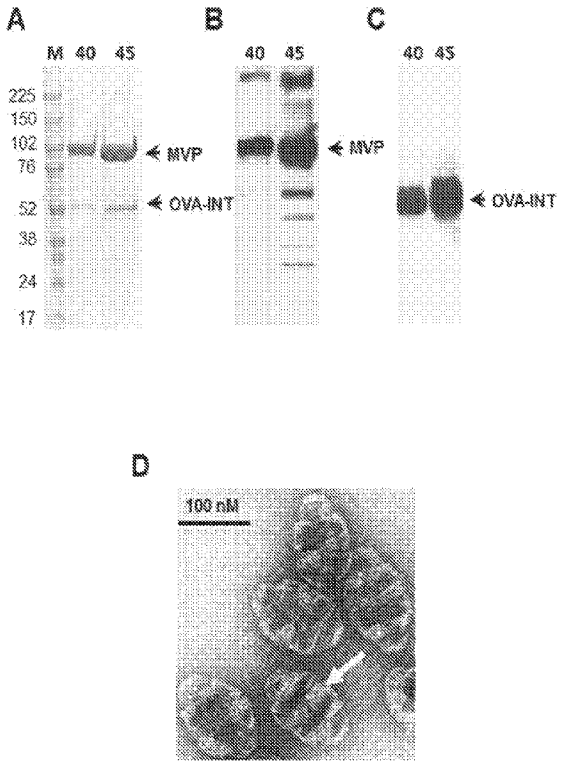
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[Continued on next page]

(54) Title: VAULT IMMUNOTHERAPY

Figure 1



(57) Abstract: The invention relates to compositions of vault complexes for use as adjuvants for stimulating a cellular immune response to an antigen, for example a tumor antigen, and methods of using the vault complexes in the treatment of diseases, such as cancer.

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, 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, 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))*

VAULT IMMUNOTHERAPY

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0001] This invention was made with support from the Government under Grant No. AI079004 awarded by the National Institutes of Health. The Government has certain rights in this invention.

RELATED APPLICATIONS

[0002] This application claims the benefit of U.S. Provisional Application 61/669,568 filed July 9, 2013, which is hereby incorporated by reference in its entirety for all purposes.

BACKGROUND OF THE INVENTION

Field of the invention

[0003] The present invention relates generally to the use of vault compositions as adjuvants for stimulating a cellular immune response to one or more antigens, for example, tumor antigens or cancer biomarkers. Also included in the invention is the use of the compositions for the treatment of diseases, such as cancer.

Introduction

[0004] With ongoing disease threats and the promise of emerging immunotherapies, demand for new vaccine technologies is growing. Developing effective and potent vaccines remains one of the most cost-effective strategies for preventing infectious diseases and cancers [1,2]. Vaccines containing killed or inactivated intact microbes elicit strong immune responses but also produce considerable inflammation at the site of vaccination [3-5]. Furthermore, engineered live vaccines, such as non-replicating recombinant viruses have been developed and also induce robust immune responses [6-8]. However, the potential for break-through replication of live vectors and anti-vector immunity further discourage the development of live vector vaccines due to safety concerns [9,10]. To further vaccine development, non-replicating adjuvants are needed which induce robust immunity with minimal inflammation.

[0005] The immune-promoting activity of any given vaccination strategy is determined by the presence of the relevant antigenic components in the vaccine formulation, enhanced by

the addition of suitable adjuvants capable of activating and promoting an efficient immune response against infectious agents or cancers [1,2]. One approach for tailoring vaccines to elicit certain types of immune responses while avoiding inflammation is to develop subunit vaccines by combining non-living or synthetic antigens with adjuvants [9]. This type of vaccine can deliver defined antigens with reduced inflammatory cytokine production but is dependent on the adjuvant formulation to stimulate cell-mediated immune responses and protection from infectious challenge or prevent tumor growth [11,12]. Most licensed vaccines promote immunity by eliciting humoral immune responses and weak cellular immune responses. Current efforts are directed to producing adjuvants which elicit cell-mediated immunity [13,14].

[0006] A major limiting factor in the development of subunit vaccines is engineering immune adjuvants to induce cell-mediated immunity and encourage $CD8^+$ T cell responses through major histocompatibility complex (MHC) class I presentation (MHC-I, cross presentation). Previous work has shown that it is difficult to achieve antigen presentation through MHC-I molecules unless the antigen is specifically targeted to the MHC-I processing machinery [15-17]. A wide range of approaches has been explored including CpG-DNA or toll-like receptor (TLR) ligands, recombinant viral vectors, fusion with bacterial toxins and others [18,19]. Adjuvants can also be designed to elicit specific immunity, such as promoting cellular immunity which is important for protection against many pathogens [20]. Currently none have been successfully developed for use in humans.

[0007] Nanoparticle pharmaceutical carriers can be engineered to elicit various types of immunity and are increasingly investigated as adjuvants for vaccines. Different types of nanocarriers, such as polymers (polymeric nanoparticles, micelles, or dendrimers), lipids (liposomes), viruses (viral nanoparticles), and organometallic compounds (carbon nanotubes) have been employed for immunotherapeutic applications [21-23]. We have engineered vaults using a recombinant technique to function as a nanocarrier. Natural vaults are barrel-shaped, hollow, 13mDa ribonucleoprotein particles that exist in nearly all eukaryotic cells [24,25]. Their precise function is unknown but they have been associated with multidrug resistance, cell signaling, nuclear-cytoplasmic transport and innate immunity [26]. We have shown that recombinant vaults can be produced to contain a bacterial antigen and induce adaptive immune responses and protective immunity following immunization [27]. In addition, vault nanocapsules can also be engineered to promote anti-tumor responses [28]. These studies

show that recombinant vault nanocapsules act as adjuvants, are versatile for eliciting various types of immunity and have outstanding potential for compound encapsulation, protection, and delivery.

Description of the Related Art

[0008] Vaults are cytoplasmic ubiquitous ribonucleoprotein particles first described in 1986 that are found in all eukaryotic cells (Kedersha et al., J Cell Biol, 103(3):699-709 (1986)). Native vaults are 12.9±1 MDa ovoid spheres with overall dimensions of approximately 40 nm in width and 70 nm in length (Kong et al., Structure, 7(4):371-379 (1999); Kedersha et al., J Cell Biol, 112(2):225-235 (1991)), present in nearly all-eukaryotic organisms with between 10^4 and 10^7 particles per cell (Suprenant, Biochemistry, 41(49):14447-14454 (2002)). Despite their cellular abundance, vault function remains elusive although they have been linked to many cellular processes, including the innate immune response, multidrug resistance in cancer cells, multifaceted signaling pathways, and intracellular transport (Berger et al., Cell Mol Life Sci, 66(1):43-61 (2009)).

[0009] Vaults are highly stable structures *in vitro*, and a number of studies indicate that the particles are non-immunogenic (Champion et al., PLoS One, 4(4):e5409 (2009)). Vaults can be engineered and expressed using a baculovirus expression system and heterologous proteins can be encapsulated inside of these recombinant particles using a protein-targeting domain termed INT for vault INTeraction. Several heterologous proteins have been fused to the INT domain (e.g. fluorescent and enzymatic proteins) and these fusion proteins are expressed in the recombinant vaults and retain their native characteristics, thus conferring new properties onto these vaults (Stephen et al., J Biol Chem, 276(26):23217-23220 (2001); Kickhoefer et al., Proc Natl Acad Sci U S A, 102(12):4348-4352 (2005)).

[0010] Vaults are generally described in U.S. Patent No. 7,482,319, filed on Mar. 10, 2004; U.S. Application No. 12/252,200, filed on Oct. 15, 2008; International Application No. PCT/US2004/007434, filed on Mar. 10, 2004; U.S. Provisional Application No. 60/453,800, filed on Mar. 20, 2003; U.S. Patent No. 6,156,879, filed on Jun. 3, 1998; U.S. Patent No. 6,555,347, filed on Jun. 28, 2000; U.S. Patent No. 6,110,740, filed on Mar. 26, 1999; International Application No. PCT/US1999/06683, filed on Mar. 26, 1999; U.S. Provisional App. No. 60/079,634, filed on Mar. 27, 1998; and International Application No. PCT/US1998/011348, filed on Jun. 3, 1998. Vault compositions for immunization against chlamydia genital infection are described in U.S. Application No. 12/467,255, filed on May

15, 2009. The entire contents of these applications are incorporated by reference in their entirety for all purposes.

SUMMARY OF THE INVENTION

[0011] As shown herein, we have characterized the types of immune responses elicited by engineered vault nanopcapsules compared to another type of nanocarrier, liposomes, using a well-characterized model antigen, ovalbumin (OVA). Ovalbumin is a highly immunogenic antigen and has often been used as a proof of principle for numerous vaccination strategies [29,30]. We show that immunization of mice with OVA encapsulated in vault nanocapsules efficiently stimulates the immune response to elicit robust $CD8^+$, $CD4^+$ memory T cell responses and antibody titers to OVA. Accordingly, as also shown herein, vault nanocapsules can be used as subunit vaccines which can generate both cellular and humoral immunity against antigens for human pathogens and cancer, which we have demonstrated for a number of tumor associated antigens.

[0012] In one aspect, the present invention provides a method for stimulating a cellular immune response in a subject, comprising administering to the subject an effective amount of an antigenic peptide or an antigenic fragment or variant thereof incorporated within a vault complex.

[0013] In a second aspect, the present invention provides a pharmaceutical composition for preventing or treating a subject for cancer, comprising a tumor antigen or an antigenic fragment or variant thereof incorporated within a vault complex, and optionally at least one pharmaceutically acceptable excipient, sufficient to stimulate a cellular immune response.

[0014] In a yet third aspect, the present invention provides a method of preventing or treating cancer in a subject, comprising administering to the subject an effective amount of a tumor antigen or an antigenic fragment or variant thereof incorporated within a vault complex, sufficient to stimulate a cellular immune response. In some embodiments, the administering reduces tumor volume or tumor growth.

[0015] In various embodiments of the above aspects, the antigenic peptide is a tumor antigen. In other embodiments, the vault complex comprises two or more vault complexes, in which each vault complex comprises two or more different antigenic peptides or antigenic fragments or variants.

[0016] In other embodiments, one or multiple copies of the antigenic peptide can be fused to INT or MVP. If fused to MVP, the antigenic peptide can be fused to the N-terminus of MVP or to the C-terminus of MVP. In some embodiments, the INT comprises the amino acid sequence of SEQ ID NO: 2.

[0017] In further embodiments, the vault complex comprises MVP, in which the number of MVP is 1-78. In some embodiments, the number of MVP is 78.

[0018] In additional embodiments, the vault complex further comprises VPARP or modified VPARP, or a portion of VPARP, or a modified portion of VPARP.
In particular embodiments, the cellular immune response is induction of $CD8^+$ and $CD4^+$ memory T-cells. In other embodiments, the cellular immune response is production of INFγ.

[0019] Further embodiments comprise administering to the subject a vault complex containing a chemokine, in which the chemokine can be CCL21. The administration can be with or without an antigen.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, where:

[0021] **Figure 1: Analysis of purified recombinant vault particles containing OVA-INT.** (A) Representative gel image showing co-purification of the protein species MVP and OVA-INT. Sucrose gradients of 40% to 60% run in SDS-PAGE (4%-15%). Lane: M: protein molecular weight markers, 40: 40% fractions of sucrose gradient and 45% fractions of sucrose gradient. (B) The gradient fractions were probed with either anti-MVP rabbit polyclonal antisera or (C) anti-OVA rabbit polyclonal antisera. (D) Negative stain EM of CP-OVA recombinant vaults Bar, 100 nm.

[0022] **Figure 2: Vault nanocapsules induce cross presentation to CD8 cells.** B3Z cells ($1x10^5$ cells/200 uL/well) were co cultured with DC 2.4 ($5x10^4$ cells/ 200 µL/well) in the presence or absence of CP-OVA (3.3 µg/200 uL/well) for 24 hrs. Control vaults (CP) were also used at concentration of 3.3 µg/200 uL/well. Following 24 hrs, T cell activation was analyzed by measuring IL-2 production. Data in all panels are representative of 3 independent experiments. Student's t-test was used to determine statistical significance between the CP-OVA and control CP-vaults. * $p<0.05$.

[0023] Figure 3: Vault nanocapsules induce CD4 T cell activation. T cells (2 x10^5 cells/mL) were co-cultured with DC (2x10^4 cells/mL) in the presence of PBS, recombinant OVA protein (2.5 μg/mL), control CP-vaults and CP-OVA with the indicated concentrations. DC-induced T cell proliferation was assessed by incorporation of [^{3}H] thymidine. The graphs show mean (SEM) values from a representative experiment (n=6 replicates) of three independent experiments. Student's t test was used to determine the p value by comparing appropriate control. *p <0.05.

[0024] Figure 4. Quantitation of OVA in delivery vehicles and immunization regimen. (A) Images of representative 4-15 % SDS polyacrylamide gel showing standards, CP-OVA, CPZ-OVA and OVA-liposomes. The amount of OVA incorporated into the delivery vehicles were quantitated using a Typhoon 9410 Typhoon Variable Mode Scanner of Coomassie blue stained SDS-PAGE gels. (B) Schematic representation of vaccination schedules and subcutaneous immunizations with saline (▒), unencapsulated OVA with saline (■), CP (▒), CP-OVA (▲), CPZ (▒), CPZ-OVA (♦), liposome (▒), or liposome-OVA (●). The immunization regimen involved three vaccinations (day -28, -14 and 0).

[0025] Figure 5: Vault nanocapsules enhance priming of endogenous CD8+ T cells. Mice were injected with various immunogens as shown on the x-axis; saline (▒), unencapsulated OVA with saline (■), CP (▒), CP-OVA (▲), CPZ (▒), CPZ-OVA (♦), liposome (▒), or liposome-OVA (●). Splenocytes were harvested, stained and gated on lymphocytes as described in the methods section. The frequency of CD8 subpopulations are shown on the y-axis. (A) Total CD8+ cells, (B) CD8+ memory cells (CD8+CD44^{hi}), (C) IFNγ-producing CD8+ cells, (D) Perforin-expressing CD8+ cells and (E) IL-4 producing CD8 cells. The cell populations from immunized groups were compared using one-way ANOVA and Bonferroni's post-hoc test). ***p<0.001, **p<0.01, *p<0.05. Representative of 3 independent experiments.

[0026] Figure 6. Vault nanocapsules encourage production of CD4+ T cells upon vaccination. Mice were injected with various immunogens as shown on the x-axis; saline (▒), unencapsulated OVA with saline (■), CP (▒), CP-OVA (▲), CPZ (▒), CPZ-OVA (♦), liposome (▒), or liposome-OVA (●). Splenocytes were harvested, stained and gated on lymphocytes as described in the methods section. The frequency of CD4 subpopulations are shown on the y-axis. (A) Total CD4+ cells, (B) CD4+ memory cells (CD4+CD44^{hi}), (C) IFNγ-producing CD4+ cells, (D) IL-17 producing CD4+ cells and (E) IL-

4 producing CD4 cells. The cell populations from immunized groups were compared using one-way ANOVA and Bonferroni's post-hoc test). ***p<0.001, **p<0.01, *p<0.05. Representative of 2 independent experiments.

[0027] Figure 7: Vault nanocapsules produce lower anti-OVA antibody titers. Antibody titers after vaccination schedule, composed of 3 weekly s.c. injections with control saline (▒), unencapsulated OVA in saline (■), CP-OVA (▲), CPZ-OVA (◆) or Liposome-OVA (●). (A) Total anti-OVA-IgG1 titers and (B) Total anti-OVA-IgG2c titers. Significance was determined by ANOVA ($p < 0.001$) with Bonferroni post-hoc test (***$p < 0.001$). (C) Ratio of anti-OVA IgG1 to IgG2c antibody. The ratio of Liposome-OVA immunized mice were compared to the other OVA-immunized groups using Mann Whitney t-test (*p<0.001). Data are representative of 2 independent experiments.

[0028] Figure 8: Flow cytometry gating scheme used to define cell populations. (A) A representative dotplot from a CPZ-OVA immunized mouse was gated on lymphocytes using SSC versus FSC. The percent of $CD3^+CD8^+$ memory T cells was determined from the events in the lymphocyte gate. (B) The lymphocyte gated population was further gated on $CD3^+$ T cells and $CD3^+$ T cells were separated into $CD8^+$ or $CD4^+$ T cells. Memory cell population was determined by hi expression of CD44 and a gate drawn. This was applied to all experimental mice to determine the percentage of $CD8^+$ memory cells. The scheme was applied to $CD8^+$ or $CD4^+$ T cells producing cytokines or expressing perforin by gating on the $CD3^+CD8^+$ or $CD3^+CD4^+$ population.

[0029] Figure 9: OVA-vault vaccination inhibited tumor growth. C57BL/6 mice bearing 7 day 3LL-OVA established tumors (*s.c.*) were treated with diluent normal saline (NS), control vaults (20μg) and OVA-vaults (2-20μg) by *sc* or *ip* injection. Bisecting tumor diameters were measured with calipers. Tumor growth **(9A)** and tumor weights **(9B)** were inhibited in the OVA-vault treatments compared to controls. Data; Mean ± SEM, *p<0.05 between OVA-vault and controls, n=8 mice/group.

[0030] Figure 10: OVA-vault vaccination on the contralateral flank of tumor inoculation inhibited tumor growth. C57BL/6 mice bearing 7 day 3LL-OVA established tumors (*s.c.*) were treated with diluent normal saline (NS), control vaults (40μg) and OVA-vaults (20-40μg) by *sc* injection. Bisecting tumor diameters were measured with calipers. Tumor growth was inhibited in the OVA-vault vaccination group compared to controls **(10A).** H&E of tumor sections showed that the Ova vault vaccination groups have diffuse

tumor burden with leukocytic infiltrates compared to control vaults that have solid tumor mass and few infiltrates **(10B).** Data; Mean ± SEM, *p<0.05 between OVA-vault and controls, n=8 mice/group.

[0031] Figure 11: OVA-vault, CCL21 vault, or combined CCL21vault + OVA-vault treatment on the contralateral flank of tumor inoculation inhibited tumor growth and induced systemic immune responses. C57BL/6 mice bearing 7 day 3LL-OVA established tumors (*s.c.*) were treated with diluent normal saline (NS), control vaults (20μg), OVA-vaults (20 μg), CCL21 (5 μg), and CCL21 (5 μg) + OVA (20 μg) by *sc* injection. Tumor growth was inhibited in the treatment groups compared to controls **(11A)** with 40-50% of treated mice completely rejecting tumors **(Table 2)**. Cytolysis of CFSE labeled 3LL-OVA following incubation with splenocytes from treated mice at effector to target ratio of 1:1 for 4hours showed enhanced tumor cytolysis compared to controls **(11B).** Data; Mean ± SEM, *p<0.05 between OVA-vault and controls, n=6 mice/group.

[0032] Figure 12: NYESO-vault vaccination inhibited tumor growth. C57BL/6 mice bearing 7 day 3LL-NYESO established tumors (*s.c.*) were treated with control vaults (20 μg), CCL21-vaults (5 μg) and NYESO vaults (20-40 μg) by *sc* injection on the contralateral flank. Tumor growth **(12A)** and tumor weights **(12B)** were inhibited in the CCL21 vault and NYESO-vault treatments compared to control. Data; Mean ± SEM, *p<0.05 between OVA-vault and controls, n=6 mice/group.

[0033] Figure 13: Efficient uptake of vault nanoparticles housing NYESO by dentritic cells. Fluorescent microscopy images demonstrating DC integration of INT vaults. DCs stained in green with either media alone or our vaults stained in red. At zero minutes and T60 for the media (A and B), auto-fluorescence surrounding the exterior of the DC. After 60 minutes of incubation with vaults, clear concentration of vaults within the confines of the DCs (C and D).

[0034] Figure 14: Increased dentritic cell activation and maturation as measured by CD86 expression by treatment with NYESO vaults. Flow cytometry for DC maturation following 20 hours of incubation with NY-ESO-1 vaults, vaults with a red fluorescent protein, or media alone. CD86 median fluorescence intensity for the NY-ESO-1 vaults demonstrated a statistically significant 43% increase in MFI.

[0035] Figure 15: Dendritic cells treated with GP100 vaults have demonstrated efficacy in stimulating CD8 T cells as shown by elevated levels of interferon gamma. In vitro ELISA demonstrating a significant increase in interferon gamma in T cells incubated with dendritic cells treated with GP100 vaults at 4 hours and for 24 hours.

DETAILED DESCRIPTION OF THE INVENTION

[0036] The descriptions of various aspects of the invention are presented for purposes of illustration, and are not intended to be exhaustive or to limit the invention to the forms disclosed. Persons skilled in the relevant art can appreciate that many modifications and variations are possible in light of the embodiment teachings.

[0037] It should be noted that the language used herein has been principally selected for readability and instructional purposes, and it may not have been selected to delineate or circumscribe the inventive subject matter. Accordingly, the disclosure is intended to be illustrative, but not limiting, of the scope of invention.

[0038] It must be noted that, as used in the specification, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise.

[0039] Any terms not directly defined herein shall be understood to have the meanings commonly associated with them as understood within the art of the invention. Certain terms are discussed herein to provide additional guidance to the practitioner in describing the compositions, devices, methods and the like of embodiments of the invention, and how to make or use them. It will be appreciated that the same thing can be said in more than one way. Consequently, alternative language and synonyms can be used for any one or more of the terms discussed herein. No significance is to be placed upon whether or not a term is elaborated or discussed herein. Some synonyms or substitutable methods, materials and the like are provided. Recital of one or a few synonyms or equivalents does not exclude use of other synonyms or equivalents, unless it is explicitly stated. Use of examples, including examples of terms, is for illustrative purposes only and does not limit the scope and meaning of the embodiments of the invention herein.

Definitions

[0040] Terms used in the claims and specification are defined as set forth below unless otherwise specified.

[0041] As used herein, the term "vault" or "vault particle" refers to a large cytoplasmic ribonucleoprotein (RNP) particle found in eukaryotic cells. The vault or vault particle is composed of MVP, VPARP, and/or TEP1 proteins and one or more untranslated vRNA molecules.

[0042] As used herein, the term "vault complex" refers to a vault or recombinant vault that encapsulates a small molecule or protein of interest. A vault complex can include all the components of a vault or vault particle or just a subset. A vault complex with just a subset of the components found in vaults or vault particles can also be termed a "vault-like particle". Examples of vault-like particles include: 1) MVP without VPARP, TEP1 and vRNA; 2) MVP and either VPARP or a portion of VPARP, without TEP1 and vRNA; 3) MVP and TEP1 or a portion of TEP1 with or without the one or more than one vRNA, and without VPARP; 4) MVP without VPARP, TEP1 and vRNA, where the MVP is modified to attract a specific substance within the vault-like particle, or modified to attract the vault complex to a specific tissue, cell type or environmental medium, or modified both to attract a specific substance within the vault complex and to attract the vault particle to a specific tissue, cell type or environmental medium; and 5) MVP, and either VPARP or a portion of VPARP, or TEP1 or a portion of TEP1 with or without the one or more than one vRNA, or with both VPARP or a portion of VPARP, and TEP1, with or without the one or more than one vRNA, where one or more than one of the MVP, VPARP or portion of VPARP and TEP1 is modified to attract a specific substance within the vault-like particle, or modified to attract the vault particle to a specific tissue, cell type or environmental medium, or modified both to attract a specific substance within the vault complex and to attract the vault complex to a specific tissue, cell type or environmental medium. As used herein, a vault complex is sometimes referred to as a "vault nanoparticle".

[0043] As used herein, the term "vault targeting domain" or "vault interaction domain" is a domain that is responsible for interaction or binding of a heterologous fusion protein with a vault protein, or interaction of a VPARP with a vault protein, such as a MVP. As used herein, the term "INT domain" is a vault interaction domain from a vault poly ADP-ribose polymerase (VPARP) that is responsible for the interaction of VPARP with a major vault protein (MVP). The term "INT domain" refers to a major vault protein (MVP) interaction domain comprising amino acids 1563 – 1724 of VPARP.

[0044] As used herein, the term "MVP" is major vault protein. The term "cp-MVP" is a cysteine-rich peptide major vault protein.

[0045] The term "VPARP" refers to a vault poly ADP-ribose polymerase.

[0046] As used herein, the term "TEP-1" is a telomerase/vault associated protein 1.

[0047] As used herein, the term "vRNA" is an untranslated RNA molecule found in vaults.

[0048] As used herein, the term "vector" is a DNA or RNA molecule used as a vehicle to transfer foreign genetic material into a cell. The four major types of vectors are plasmids, bacteriophages and other viruses, cosmids, and artificial chromosomes. Vectors can include an origin of replication, a multi-cloning site, and a selectable marker.

[0049] As used herein, a "cell" includes eukaryotic and prokaryotic cells.

[0050] As used herein, the terms "organism", "tissue" and "cell" include naturally occurring organisms, tissues and cells, genetically modified organisms, tissues and cells, and pathological tissues and cells, such as tumor cell lines *in vitro* and tumors *in vivo*.

[0051] As used herein, the term "extracellular environment" is the environment external to the cell.

[0052] As used herein, the term "in vivo" refers to processes that occur in a living organism.

[0053] A "subject" referred to herein can be any animal, including a mammal (e.g., a laboratory animal such as a rat, mouse, guinea pig, rabbit, primates, etc.), a farm or commercial animal (e.g., a cow, horse, goat, donkey, sheep, etc.), a domestic animal (e.g., cat, dog, ferret, etc.), an avian species, or a human.

[0054] The term "mammal" as used herein includes both humans and non-humans and include but is not limited to humans, non-human primates, canines, felines, murines, bovines, equines, and porcines.

[0055] As used herein, the term "human" refers to "*Homo sapiens*."

[0056] As used herein, the term "sufficient amount" is an amount sufficient to produce a desired effect, e.g., an amount sufficient to stimulate a cellular immune response.

[0057] As used herein, the term "therapeutically effective amount" is an amount that is effective to ameliorate a symptom of a disease, such as cancer.

[0058] A "prophylactically effective amount" refers to an amount that is effective for prophylaxis.

[0059] As used herein, the term "stimulating" refers to activating, increasing, or triggering a molecular, cellular or enzymatic activity or response in a cell or organism, e.g. a cellular immune response.

[0060] As used herein, the term "inhibiting" refers to deactivating, decreasing, or shutting down a molecular, cellular or enzymatic activity or response in a cell or organism.

[0061] As used herein, the term "administering" includes any suitable route of administration, as will be appreciated by one of ordinary skill in the art with reference to this disclosure, including direct injection into a solid organ, direct injection into a cell mass such as a tumor, inhalation, intraperitoneal injection, intravenous injection, topical application on a mucous membrane, or application to or dispersion within an environmental medium, and a combination of the preceding.

[0062] As used herein, the term "treating" or "treatment" refers to the reduction or elimination of symptoms of a disease, e.g., cancer.

[0063] As used herein, the term "preventing" or "prevention" refers to the reduction or elimination of the onset of symptoms of a disease, e.g., cancer.

[0064] As used herein, the term "regressing" or "regression" refers to the reduction or reversal of symptoms of a disease after its onset, e.g., cancer remission.

[0065] As used in this disclosure, the term "modified" and variations of the term, such as "modification," means one or more than one change to the naturally occurring sequence of MVP, VPARP or TEP1 selected from the group consisting of addition of a polypeptide sequence to the C-terminal, addition of a polypeptide sequence to the N-terminal, deletion of between about 1 and 100 amino acid residues from the C-terminal, deletion of between about 1 and 100 amino acid residues from the N-terminal, substitution of one or more than one amino acid residue that does not change the function of the polypeptide, as will be appreciated by one of ordinary skill in the art with reference to this disclosure, such as for example, an alanine to glycine substitution, and a combination of the preceding.

[0066] As used herein, the term percent "identity," in the context of two or more nucleic acid or polypeptide sequences, refers to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (e.g., BLASTP and BLASTN or other algorithms available to persons of skill) or by visual inspection. Depending on the application, the percent "identity" can exist over a region of the sequence being compared, e.g., over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared.

[0067] For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0068] Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al., infra).

[0069] One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/).

[0070] As used in this disclosure, the term “comprise” and variations of the term, such as “comprising” and “comprises,” are not intended to exclude other additives, components, integers or steps.

[0071] It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

Compositions of the invention

[0072] As described in more detail below, the invention includes compositions and methods of using vault complexes. An embodiment of the invention has recombinant vaults having a MVP and an antigen, e.g., a tumor antigen. The vault complex can be used as an adjuvant for stimulating a cellular immune response to the antigen.

Vaults and Vault complexes

[0073] The compositions of the invention comprise a vault complex. A vault complex is a recombinant particle that encapsulates a small molecule (drug, sensor, toxin, etc.), or a protein of interest, e.g., a peptide, or a protein, including an endogenous protein, a heterologous protein, a recombinant protein, or recombinant fusion protein. Vault complexes of the invention can include a tumor antigen.

[0074] Vaults, e.g., vault particles are ubiquitous, highly conserved ribonucleoprotein particles found in nearly all eukaryotic tissues and cells, including dendritic cells (DCs), endometrium, and lung, and in phylogeny as diverse as mammals, avians, amphibians, the slime mold *Dictyostelium discoideum*, and the protozoan *Trypanosoma brucei* (Izquierdo et al., *Am. J. Pathol.*, 148(3):877-87 (1996)). Vaults have a hollow, barrel-like structure with two protruding end caps, an invaginated waist, and regular small openings surround the vault cap. These openings are large enough to allow small molecules and ions to enter the interior of the vault. Vaults have a mass of about 12.9 ± 1 MDa (Kedersha et al., *J. Cell Biol.*, 112(2):225-35 (1991)) and overall dimensions of about 42 x 42 x 75 nm (Kong et al., *Structure*, 7(4):371-9 (1999)). The volume of the internal vault cavity is approximately 50 $x10^3$ nm^3, which is large enough to enclose an entire ribosomal protein.

[0075] Vaults comprise three different proteins, designated MVP, VPARP and TEP1, and comprise one or more different untranslated RNA molecules, designated vRNAs. The number of vRNA can vary. For example, the rat *Rattus norvegicus* has only one form of vRNA per vault, while humans have three forms of vRNA per vault. The most abundant protein, major vault protein (MVP), is a 95.8 kDa protein in *Rattus norvegicus* and a 99.3 kDa protein in humans which is present in 96 copies per vault and accounts for about 75 % of

the total protein mass of the vault particle. The two other proteins, the vault poly-ADP ribose polymerase, VPARP, a 193.3 kDa protein in humans, and the telomerase/vault associated protein 1, TEP1, a 292 kDa protein in *Rattus norvegicus* and a 290 kDa protein in humans, are each present in between about 2 and 16 copies per vault.

VPARP, INT domain, and INT fusion proteins

[0076] A vault poly ADP-ribose polymerase (VPARP) includes a region of about 350 amino acids that shares 28% identity with the catalytic domain of poly ADP-ribosyl polymerase, PARP, a nuclear protein that catalyzes the formation of ADP-ribose polymers in response to DNA damage. VPARP catalyzes an NAD-dependent poly ADP-ribosylation reaction, and purified vaults have poly ADP-ribosylation activity that targets MVP, as well as VPARP itself. VPARP includes a INT domain (major vault protein (MVP) interaction domain). The INT domain is responsible for the interaction of VPARP with a major vault protein (MVP).

[0077] A vault complex of the invention can include a INT domain. The INT domain, also referred to as mINT domain for minimal INT domain, is responsible for interaction of a protein of interest with a vault protein such as a MVP. In some embodiments, the INT domain is expressed as a fusion protein with a protein of interest. Alternatively, a protein of interest can be covalently or non-covalently attached. The INT of the vault complexes of the invention are derived from VPARP sequences. Exemplary VPARP sequences and INT sequences can be found in Table 1. One of skill in the art understands that the INT can have the entire naturally occurring sequence or portions of the sequence or fragments thereof. In other embodiments, the INT has at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to any of the VPARP and/or INT sequences disclosed in Table 1.

[0078] In one embodiment, the INT is derived from a human VPARP, SEQ ID NO:3, GenBank accession number AAD47250, encoded by the cDNA, SEQ ID NO:5, GenBank accession number AF158255. In some embodiments, the vault targeting domain comprises or consists of the INT domain corresponding to residues 1473-1724 of human VPARP protein sequence (full human VPARP amino acid sequence is SEQ ID NO:3). In other embodiments, the vault targeting domain comprises or consists of the mINT domain comprising residues 1563-1724 (SEQ ID NO: 2) of the human VPARP protein sequence. In certain embodiments, the vault targeting domain is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 2 or 3.

[0079] In alternative embodiments, as with VPARP, a major vault protein (MVP) interaction domain can be derived from TEP1 sequences. Such interaction domains can be termed, for example INT2, to distinguish them from a VPARP interaction domain. One of skill in the art understands that the INT can have the entire naturally occurring sequence of the vault interaction domain in TEP1 or portions of the sequence or fragments thereof.

MVP

[0080] A vault complex of the invention can include an MVP. Exemplary MVP sequences can be found in Table 1. One of skill in the art understands that the MVP can have the entire naturally occurring sequence or portions of the sequence or fragments thereof. In other embodiments, the MVP has at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to any of the MVP sequences disclosed in Table 1.

[0081] In one embodiment, the MVP is human MVP, SEQ ID NO:6, GenBank accession number CAA56256, encoded by the cDNA, SEQ ID NO:7, GenBank accession number X79882. In other embodiments, the MVP is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the MVP sequences described herein.

[0082] In one embodiment, there is provided a vault complex comprising, consisting essentially of, or consisting of an MVP modified by adding a peptide to the N-terminal to create a one or more than one of heavy metal binding domains. In a preferred embodiment, the heavy metal binding domains bind a heavy metal selected from the group consisting of cadmium, copper, gold and mercury. In a preferred embodiment, the peptide added to the N-terminal is a cysteine-rich peptide (CP), such as for example, SEQ ID NO:8, the MVP is human MVP, SEQ ID NO:6, and the modification results in CP-MVP, SEQ ID NO:9, encoded by the cDNA, SEQ ID NO:10. These embodiments are particularly useful because vault particles consisting of CP-MVP are stable without the presence of other vault proteins.

[0083] Any of the vault complexes described herein can include MVPs or modified MVPs disclosed herein.

TEP1

[0084] In some embodiments, a vault complex of the invention can include a TEP1 protein. Exemplary TEP1 sequences can be found in Table 1. One of skill in the art understands that the TEP1 can have the entire naturally occurring sequence or portions of the sequence or fragments thereof. In other embodiments, the TEP1 has at least 50%, 60%, 70%,

80%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to any of the TEP1 sequences disclosed in Table 1.

[0085] The TEP1 can be human TEP1, SEQ ID NO:11, GenBank accession number AAC51107, encoded by the cDNA, SEQ ID NO:12, GenBank accession number U86136. Any of the vault complexes described herein can include TEP1 or modifications thereof.

vRNA

[0086] A vault complex of the invention can include a vRNA. Exemplary vRNA sequences can be found in Table 1. One of skill in the art understands that the vRNA can have the entire naturally occurring sequence or portions of the sequence or fragments thereof. In other embodiments, the vRNA has at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to any of the vRNA sequences disclosed in Table 1.

[0087] In one embodiment, the vRNA can be a human vRNA, SEQ ID NO:13, GenBank accession number AF045143, SEQ ID NO:14, GenBank accession number AF045144, or SEQ ID NO:15, GenBank accession number AF045145, or a combination of the preceding.

[0088] As will be appreciated by one of ordinary skill in the art with reference to this disclosure, the actual sequence of any of MVP, VPARP, TEP1 and vRNAs can be from any species suitable for the purposes disclosed in this disclosure, even though reference or examples are made to sequences from specific species. Further, as will be appreciated by one of ordinary skill in the art with reference to this disclosure, there are some intraspecies variations in the sequences of MVP, VPARP, TEP1 and vRNAs that are not relevant to the purposes of the present invention. Therefore, references to MVP, VPARP, TEP1 and vRNAs are intended to include such intraspecies variants.

Isolated Nucleic Acids and Vectors

[0100] Suitable expression vectors generally include DNA plasmids or viral vectors. Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can be used to produce recombinant constructs for the expression of an iRNA as described herein. Eukaryotic cell expression vectors are well known in the art and are available from a number of commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired nucleic acid segment. Delivery of expression vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by

reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

[0101] Plasmids expressing a nucleic acid sequence can be transfected into target cells as a complex with cationic lipid carriers (*e.g.,* Oligofectamine) or non-cationic lipid-based carriers (*e.g.,* Transit-TKO^{TM}). Successful introduction of vectors into host cells can be monitored using various known methods. For example, transient transfection can be signaled with a reporter, such as a fluorescent marker, such as Green Fluorescent Protein (GFP). Stable transfection of cells *ex vivo* can be ensured using markers that provide the transfected cell with resistance to specific environmental factors (*e.g.*, antibiotics and drugs), such as hygromycin B resistance.

[0102] Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors, including but not limited to lentiviral vectors, moloney murine leukemia virus, etc.; (c) adeno- associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, e.g., vaccinia virus vectors or avipox, e.g. canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct may be incorporated into vectors capable of episomal replication, e.g., EPV and EBV vectors. Constructs for the recombinant expression of a nucleic acid encoding a fusion protein will generally require regulatory elements, e.g., promoters, enhancers, etc., to ensure the expression of the fusion nucleic acid in target cells. Other aspects to consider for vectors and constructs are further described below.

[0103] Vectors useful for the delivery of a nucleic acid can include regulatory elements (promoter, enhancer, etc.) sufficient for expression of the nucleic acid in the desired target cell or tissue. The regulatory elements can be chosen to provide either constitutive or regulated/inducible expression. A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the transgene.

[0104] In a specific embodiment, viral vectors that contain the recombinant gene can be used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the

correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding a fusion protein are cloned into one or more vectors, which facilitates delivery of the nucleic acid into a patient. More detail about retroviral vectors can be found, for example, in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993). Lentiviral vectors contemplated for use include, for example, the HIV based vectors described in U.S. Patent Nos. 6,143,520; 5,665,557; and 5,981,276, which are herein incorporated by reference.

[0105] Adenoviruses are also contemplated for use in delivery of isolated nucleic acids encoding fusion proteins into a cell. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia or for use in adenovirus-based delivery systems such as delivery to the liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143-155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). A suitable AV vector for expressing a nucleic acid molecule featured in the invention, a method for constructing the recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia H *et al.* (2002), *Nat. Biotech.* 20: 1006-1010.

[0106] Use of Adeno-associated virus (AAV) vectors is also contemplated (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Pat. No. 5,436,146). Suitable AAV vectors for expressing the dsRNA featured in the invention, methods for constructing the recombinant AV vector, and methods for delivering the vectors into target cells are described in Samulski R *et al.* (1987), J. Virol. 61: 3096-3101; Fisher K J *et al.* (1996), J. Virol, 70:

520-532; Samulski R *et al.* (1989), J. Virol. 63: 3822-3826; U.S. Pat. No. 5,252,479; U.S. Pat. No. 5,139,941; International Patent Application No. WO 94/13788; and International Patent Application No. WO 93/24641, the entire disclosures of which are herein incorporated by reference.

[0107] Another preferred viral vector is a pox virus such as a vaccinia virus, for example an attenuated vaccinia such as Modified Virus Ankara (MVA) or NYVAC, an avipox such as fowl pox or canary pox.

[0108] The pharmaceutical preparation of a vector can include the vector in an acceptable diluent, or can include a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

[0109] Examples of additional expression vectors that can be used in the invention include pFASTBAC expression vectors and E. coli pET28a expression vectors.

[0110] Generally, recombinant vectors capable of expressing genes for recombinant fusion proteins are delivered into and persist in target cells. The vectors or plasmids can be transfected into target cells by a transfection agent, such as Lipofectamine. Examples of cells useful for expressing the nucleic acids encoding the fusion proteins of the invention include Sf9 cells or insect larvae cells. Recombinant vaults based on expression of the MVP protein alone can be produced in insect cells. Stephen, A.G. et al. (2001). *J. Biol. Chem.* 276:23217:23220; Poderycki, M.J., et al. (2006). *Biochemistry* (Mosc). 45: 12184-12193.

Pharmaceutical compositions of the invention

[0111] In one embodiment, the invention provides methods using pharmaceutical compositions comprising the vault complexes of the invention. These compositions can comprise, in addition to one or more of the vault complexes, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material can depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes.

[0112] In certain embodiments, the pharmaceutical compositions that are injected intra-tumorally comprise an isotonic or other suitable carrier fluid or solution.

[0113] For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives can be included, as required.

[0114] In other embodiments, pharmaceutical compositions for oral administration can be in tablet, capsule, powder or liquid form. A tablet can include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol can be included.

[0115] In some embodiments, administration of the pharmaceutical compositions may be topical, pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intraparenchymal, intrathecal or intraventricular, administration. Formulations for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives. Formulations may be reconstituted from freeze-dried (lyophilized) preparations. For intravenous use, the total concentration of solutes should be controlled to render the preparation isotonic.

Methods of use

[0116] Vault complexes described herein can be used to deliver a protein of interest (e.g., a tumor antigen) to a cell, a tissue, an environment outside a cell, a tumor, an organism or a subject. In one embodiment, the vault complex comprises a tumor antigen, and the vault complex is introduced to the cell, tissue, or tumor. In some embodiments, the vault complex is introduced into the extracellular environment surrounding the cell. In other embodiments, the vault complex is introduced into an organism or subject. Delivery of the vault complex of

the invention can include administering the vault complex to a specific tissue, specific cells, an environmental medium, or to the organism.

[0117] The methods of the invention comprise delivering a biomolecule to a cell by contacting the cell with any of the vault complexes described herein. Cells of the invention can include, but are not limited to, any eukaryotic cell, mammalian cell, or human cells, including tumor cells.

[0118] Methods of the invention include delivery of the vault complex to a subject. The delivery of a vault complex to a subject in need thereof can be achieved in a number of different ways. In vivo delivery can be performed directly by administering a vault complex to a subject. Alternatively, delivery can be performed indirectly by administering one or more vectors that encode and direct the expression of the vault complex or components of the vault complex. In one embodiment, the vault complex is administered to a mammal, such as a mouse or rat. In another embodiment, the vault complex is administered to a human.

[0119] In another embodiment, the methods of delivery of the invention include systemic injection of vaults. In other embodiments, the methods of delivery of the invention include oral ingestion of vaults.

Methods of treatment

[0120] The invention features a method of treating or managing disease, such as cancer, by administering the vault complex of the invention to a subject (e.g., patient). In some embodiments, the method of the invention comprises treating or cancer in a subject in need of such treatment or management, comprising administering to the subject a therapeutically effective amount of the vault complexes described herein.

[0121] The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. For any compound used in the methods featured in the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range of the vault complex. Such information can be used to more accurately determine useful doses in humans.

[0122] The pharmaceutical composition according to the present invention to be given to a subject, administration is preferably in a "therapeutically effective amount" or "prophylactically effective amount" (as the case can be, although prophylaxis can be

considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of protein aggregation disease being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980. A composition can be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

[0123] In certain embodiments, the dosage of vault complexes is between about 0.1 and 10,000 micrograms per kilogram of body weight or environmental medium. In another embodiment, the dosage of vault complexes is between about 1 and 1,000 micrograms per kilogram of body weight or environmental medium. In another embodiment, the dosage of vault complexes is between about 10 and 1,000 micrograms per kilogram of body weight or environmental medium. For intravenous injection and intraperitoneal injection, the dosage is preferably administered in a final volume of between about 0.1 and 10 ml. For inhalation the dosage is preferably administered in a final volume of between about 0.01 and 1 ml. As will be appreciated by one of ordinary skill in the art with reference to this disclosure, the dose can be repeated a one or multiple times as needed using the same parameters to effect the purposes disclosed in this disclosure.

[0124] For instance, the pharmaceutical composition may be administered once to a subject, or the vault complex may be administered as two, three, or more sub-doses or injections at appropriate intervals. In that case, the vault complexes can be injected in sub-doses in order to achieve the total required dosage.

[0125] The vault complexes featured in the invention can be administered in combinations of vault complexes containing different tumor antigens, or in combination with other known agents effective in treatment of cancer. An administering physician can adjust the amount and timing of vault complex administration or injection on the basis of results observed using standard measures of efficacy known in the art or described herein. The skilled artisan will also appreciate that certain factors may 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.

Methods of preparing vault complexes

[0126] The methods of the invention include preparing the vault complexes described herein.

[0127] In one embodiment, the vault complexes are derived or purified from natural sources, such as mammalian liver or spleen tissue, using methods known to those with skill in the art, such as for example tissue homogenization, differential centrifugation, discontinuous sucrose gradient fractionation and cesium chloride gradient fractionation. In another embodiment, the vault complexes are made using recombinant technology.

[0128] In some embodiments, a target of interest, i.e., protein of interest, is selected for packaging in the vault complexes. The target of interest may be selected from the group consisting of an enzyme, a pharmaceutical agent, a plasmid, a polynucleotide, a polypeptide, a sensor and a combination of the preceding. In a preferred embodiment, the target of interest is a recombinant protein, e.g., a cell adhesion modifying substance, e.g., an RGD-containing peptide.

[0129] Preferably, if the target of interest is a recombinant protein, the polynucleotide sequences encoding the recombinant protein are used to generate a bacmid DNA, which is used to generate a baculovirus comprising the sequence. The baculovirus is then used to infect insect cells for protein production using an *in situ* assembly system, such as the baculovirus protein expression system, according to standard techniques, as will be appreciated by one of ordinary skill in the art with reference to this disclosure. Advantageously, the baculovirus protein expression system can be used to produce milligram quantities of vault complexes, and this system can be scaled up to allow production of gram quantities of vault complexes according to the present invention.

[0130] In another embodiment, the target of interest is incorporated into the provided vaults. In one embodiment, incorporation is accomplished by incubating the vaults with the target of interest at an appropriate temperature and for an appropriate time, as will be appreciated by one of ordinary skill in the art with reference to this disclosure. The vaults containing the protein of interest are then purified, such as, for example sucrose gradient

fractionation, as will be appreciated by one of ordinary skill in the art with reference to this disclosure.

[0131] In other embodiments, the vaults comprising the target of interest are administered to an organism, to a specific tissue, to specific cells, or to an environmental medium. Administration is accomplished using any suitable route, as will be appreciated by one of ordinary skill in the art with reference to this disclosure.

[0132] In one embodiment, the method comprises preparing the composition of the invention by a) mixing a INT or INT fusion protein generated in insect Sf9 cells with a MVP or MVP fusion protein generated in insect Sf9 cells to generate a mixture; b) incubating the mixture for a sufficient period of time to allow formation of vault complexes, thereby generating the composition. For example, Sf9 cells are infected with pVI-MVP encoding recombinant baculoviruses. Lysates containing recombinant tumor antigen-INT and rat MVP generated in Sf-9 cells can be mixed to allow the formation of a macromolecular vault complex containing the tumor antigen-INT fusion protein.

EXAMPLES

[0133] Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

[0134] The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., T.E. Creighton, *Proteins: Structures and Molecular Properties* (W.H. Freeman and Company, 1993); A.L. Lehninger, *Biochemistry* (Worth Publishers, Inc., current addition); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); Carey and Sundberg *Advanced Organic Chemistry 3rd Ed.* (Plenum Press) Vols A and B(1992).

Example 1: Preparation of recombinant vaults packaged with chicken ovalbumin

[0135] Recombinant vaults were produced using a baculovirus expression system in Sf9 insect cells that express a stabilized form of recombinant vaults (CP) and contain a cysteine rich peptide on the N terminus to increase stability [31]. Cryoelectron microscopy imaging of recombinant and tissue derived vaults revealed the localization of the MVP interacting domain, INT [31]. Another form of recombinant vaults (CPZ) contains a 33 amino acid mimic of the Ig binding domain of staphylococcal protein A (Z) in addition to the CP peptide [32]. CPZ vaults were shown to bind antibody and may direct uptake thorough FcRs [27]. These vaults (CP or CPZ) were packaged with chicken ovalbumin by fusion of OVA protein to the vault-targeting protein, INT to form OVA-INT. The OVA-containing vaults were purified and the majority of particles were found in the 40% and 45% sucrose fraction as previously described [33]. Analysis of these fractions by SDS- PAGE and Western blotting (Figure 1) shows the co-purification of MVP and OVA-INT (Figure 1 A). The identity of the components was confirmed by Western analysis with either an anti-MVP polyclonal antibody (Figure 1B) or an anti-OVA antibody (Figure 1C). Purified CP-OVA recombinant vaults were evaluated by negative stain electron microscopy (Figure 1D). The addition of the OVA-INT protein to CP or CPZ did not alter recombinant vault morphology as compared to empty CP vaults when evaluated by transmission electron microcopy (data not shown) and as shown previously [27]. The presence of additional protein density or lighter staining area (arrow) near the waist of the vault barrel, which based on earlier structural studies, is the expected location of OVA-INT [34]. We used these CP and CPZ-vaults containing OVA-INT in vaccinations, henceforth referred to as CP-OVA and CPZ-OVA.

Example 2: Ovalbumin packaged inside vault nanocapsules can induce a MHC-I restricted response

[0136] Dendritic cells (DCs) possess the unique ability to process particulate antigens efficiently into the MHC-I pathway, in a process known as cross-priming. Several approaches have been used to encourage cross priming such as adding exogenous antigenic proteins or peptides with adjuvants to stimulate cytotoxic T lymphocytes (CTLs) [35]. Therefore, we investigated whether recombinant vaults engineered to express OVA could be efficiently internalized, processed and presented by DC in an MHC-I restricted manner to activate $CD8^+$ T cells. To this end, the DC2.4 cell line ($H\text{-}2K^b$) was pulsed with CP-OVA and secretion of IL-2 was measured as an activation marker of the OVA-responsive $CD8^+$ T cell hybridoma

B3Z (H-2K^b). The combination of DC2.4 cells, B3Z cells and CP that did not contain OVA-INT could not effectively stimulate IL-2 secretion. However, CP-OVA (produced by combining CP + OVA-INT) incubated with both DC2.4 cells and B3Z hybridoma cells induced secretion of IL-2 (Figure 2). We examined different concentrations of CP-OVA vaults and determined that 3.3 µg CP-OVA vaults per 200 µL per well gave us the greatest IL-2 secretion (data not shown). Additional controls included the B3Z CD8+ T cell hybridoma incubated with CP-OVA alone which induced modest IL-2 levels and suggests that vaults interact with T cells and participate in autopresentation of MHC-I responses [36]. Finally, incubation of CP-OVA vaults with the DC2.4 cell line only produced baseline levels of IL-2. We concluded that exogenous antigen packaged within vault nanocapsules could be delivered and presented by the MHC-I pathway in DCs and possibly through autopresentation to enhance T cell responses.

Example 3: Ovalbumin packaged inside vault nanocapsules can induce a MHC-II restricted response

[0137] We also examined the MHC class II pathway using bone-marrow–derived DCs from syngeneic BALB/c (H-2 IA/E^d) mice pulsed with CP-OVA for 24 hours. These DCs were then used to stimulate naive OVA-responsive CD4$^+$ T cells from DO11.10 (H-2 A/E^d) mice. D11.10 cells are transgenic for the TCR recognizing the amino acid 323-339 peptide of OVA on MHC-II. As shown in Figure 3, DC induced significant proliferation in the presence of OVA. However, OVA encased in vault nanoparticles at two concentrations; 2.5 µg and 10.0 µg, stimulated a greater degree of T cell proliferation at both concentrations compared to recombinant OVA protein alone and were not statistically different from each other (Figure 3). These data show that OVA encased in vault nanocapsules was more effective at inducing CD4$^+$ T cell proliferation than soluble OVA.

Example 4: Vaccination of mice with OVA packaged vault nanocapsules induces CD8$^+$ and CD4$^+$ T cells *in vivo*

[0138] We characterized cell- and antibody-mediated immune responses to OVA encapsulated in vault nanocapsules and liposomes *in vivo* following subcutaneous administration. To evaluate the type of immune response we immunized mice with either CP-OVA or CPZ-OVA vaults containing equal amounts of endotoxin-free OVA (see material and methods). Liposomes where chosen as a control delivery method since they are a class of nanocarriers and have been utilized as delivery systems for drugs, peptides, proteins and DNA [29,37]. Liposomes are microscopic vesicles consisting of phospholipid bilayers which

surround aqueous compartments and were prepared in this study by encapsulating OVA in DOTAP/DOPE as described in the methods section [38]. The amount of OVA within the vaults and liposomes was quantitated by SDS gel quantitation (Figure 4A). Mice were immunized with equal amounts of delivery vehicle and OVA and the immunization regimen is described in Figure 4B. The percentage of T cells responsive to the OVA CD8 peptide (SIINFEKL) or the OVA CD4 peptide 256-280 (TEWTSSNVMEERKIKV) were documented by surface, intracellular cytokine or perforin staining and FACS analysis after stimulation with each OVA peptide in C57BL/6 mice ($H2^b$ background) as described in the methods section. We also examined the anti-OVA-antibody responses following immunization by ELISA.

[0139] CD8+ T cells play a critical role in protection against viral and intracellular bacterial and protozoan infections and are important in tumor and graft rejection [39]. After activation, naive antigen (Ag)-responsive $CD8^+$ T cells are able to proliferate quickly and differentiate into potent effector cells capable of rapid cytokine production and cytolytic killing of target cells [40,41]. We wanted to see if entrapment of OVA in vault nanocapsules facilitated cross-presentation of Ag to the MHC-I pathway, resulting in activation of a potent $CD8^+$ T cell immunity *in vivo* as we observed previously *in vitro*. We evaluated induction of $CD8^+$ T-cell responses among mice immunized with OVA-vaults (CP-OVA and CPZ-OVA), empty vaults (CP and CPZ) and Liposome-OVA as shown in Figure 5. Control groups included soluble OVA and saline immunization. The induction of effector $CD8^+$ T cell responses in the spleen was measured 2 weeks after the last immunization by measuring the number of total $CD8^+$ T cells, $CD8^+$ memory T cells ($CD44^{hi}$), expression of the cytolytic marker perforin, and the production of IFNγ and IL-4 after stimulation with the $H2^b$ restricted CD8 OVA peptide, SIINFEKL. All experimental controls were elevated over their respective controls. To simplify the graphs we only show statistical results for comparison of our control immunization group (Liposome-OVA) to the other OVA immunization groups. Our "control" group was Liposome-OVA group because we were interested to learn how vault immunization differed from liposome immunization.

[0140] As shown in Figure 5A, we found a marked in increase of OVA-responsive SIINFEKL $CD8^+$ T cells in the CPZ-OVA immunized group over that found in Liposome-OVA immunized mice in the lymphoid compartment. It was surprising that total $CD8^+$ responses were only slightly elevated in the OVA and CP-OVA group and suggested that

$CD8^+$ T cell subset examination may be more revealing than examining total $CD8^+$ T cells in the lymphoid compartment. We also saw an increase in $CD8^+$ memory T cells (Figure 5B) and $CD8^+$ IFNγ producing T cells (Figure 5C) in mice immunized with OVA encased vault nanocapsules compared to OVA delivered in liposomes while OVA immunization in saline did not increase these responses compared to the Liposome-OVA group. This is consistent with previous studies finding that OVA alone and liposome delivery does not enhance memory $CD8^+$ cytotoxic T cells [42]. Although we noted an increase in the number of $CD8^+$ T cells expressing perforin in CPZ-OVA immunized mice compared to Liposome-OVA immunized mice we also found increased $CD8^+$ $perforin^+$ T cells in the OVA group but no increase in the CP-OVA immunized mice. Interestingly, the number of IL-4 producing cells in CP-OVA immunized mice had markedly lower numbers compared to other OVA immunized groups. As expected, vaccination with OVA in any delivery vehicle or dissolved in saline significantly increased SINFEKL-responsive $CD8^+$ T cells over control groups for all immunization groups (Figure 5). These findings demonstrate that immunization of antigen encased within vaults is cross-presented *in vivo* and stimulates a $CD8^+$ T cell response characterized by memory T cells and IFNγ producing T cells.

[0141] It has been documented that $CD4^+$ T cell help is important for $CD8^+$ T cell function. Since we observed increased numbers of OVA-responsive $CD8^+$ memory and IFNγ producing T cells in CP- and CPZ-OVA immunized mice, we investigated if the number of $CD4^+$ T cells was also increased following vault immunization. To address this issue, splenocytes from each group were stimulated *ex vivo* with the class II peptide, OVA 265-280 and the $CD4^+$ T cell response was characterized by FACS.

[0142] We found that immunization with CPZ-OVA but not CP-OVA vault nanocapsules induced a significant amount of total $CD4^+$ T cells in the lymphoid compartment of the spleen when compared to Liposome-OVA group (Figure 6A). Also, immunization with both forms of vault nanocapsules significantly elevated the number of $CD4^+$ memory T cells compared to Liposome-OVA immunized mice (Figure 6B). We did not see a significant increase in IFNγ or IL-17 producing $CD4^+$ T cells over that seen in Liposome-OVA immunized mice following vault or liposome immunization of OVA (Figures 6C & D). However, CPZ-OVA but not CP-OVA immunization induced similar numbers of IL-4 producing CD4+ T cells as mice immunized with Liposome-OVA (Figure 6E). We also noted significant increases in subsets as well as total $CD4^+$ T cells in all immunized groups when

compared to control groups as expected (Figure 6). Taken together, these data show that immunization with CPZ-OVA induces $CD4^+$ T cells characterized by memory cells and IL-4 producing cells. Immunization with CPZ vaults results in the combination $CD8^+$ T cells and $CD4^+$ helper T cells.

Example 5: Vault nanocapsules can be modified to induce select antibody Ig isotypes

[0143] Co-operation of $CD4^+$ T helper cells with antigen specific B cells is crucial for inducing long-lived neutralizing antibody responses for protective immunity followed by vaccination [43]. We investigated whether OVA delivered in vault nanocapsules also induced anti-OVA antibody since they were capable of inducing $CD4^+$ T cell memory and IL-4 producing cells. The serum titers of OVA-responsive IgG1 and IgG2c in each group were measured after immunization by ELISA. We found that mice immunized with Liposome-OVA induced significantly greater levels of anti-OVA IgG1 and IgG2c compared to CP-OVA, CPZ-OVA or OVA immunized mice (Figures 7A & B) indicating that liposomes induce high levels of anti-OVA antibody [44-46]. Further inspection revealed that the addition of the "Z" domain reduced mean anti-OVA IgG2c titers by 0.5 to 1 log in comparison to CP-OVA and OVA groups while IgG1 remained comparable. Comparison of the ratio of anti-OVA IgG1:IgG2c revealed that Liposome-OVA immunized mice produced equal levels of IgG1 and IgG2c resulting in a ratio near one while immunization with CP-OVA, CPZ-OVA or OVA increased the ratio of IgG1:IgG2. Moreover, mice immunized with vault nanocapsules modified to express the "Z" domain (CPZ-OVA) had a significantly increased this ratio compared to Liposome-OVA immunized group. In contrast, the OVA and CP-OVA groups were not significantly different compared to the Liposome-OVA group (Figure 7C). As expected all OVA immunization groups induced significant IgG1 and IgG2c serum antibody titers compared to the corresponding controls (Figure 7). These data show that modification of the vault body by addition of the "Z" domain modifies the antibody isotype and suggests that the vault nanocapsule can be modified to alter the humoral responses.

Example 6: Use of vault particles as an adjuvant to deliver an antigen

[0144] When the vault particle is used as an adjuvant to deliver the model antigen ovalbumin (OVA) to mice harboring the solid tumor produced from Lewis lung carcinoma cells engineered to express ovalbumin, a cellular immune response directed against the tumor

is induced resulting in immune attack on the tumor itself leading to reduction in the tumor size. This antitumor immune response can be induced with a contralateral subcutaneous injection of the vault encapsulated ovalbumin with equal efficacy. See Figures 9 A and B and 10 A and B.

Example 7: Use of CCL21 chemokine containing vault particles to activate an antitumor response

[0145] The antitumor immune response to the vault adjuvant engineered to deliver specific antigens can be further activated by vault particles containing the CCL21 chemokine. See Figure 11 A and B and Table 2.

[0146] As one embodiment of this invention, the CCL21-vault can be combined with one or more than one vault containing tumor antigens to increase the cellular immune response induced toward the tumor. See Figure 11A and Table 2.

Example 8: Use of vault particles to deliver the tumor antigens

[0147] When the vault particle is used as an adjuvant to deliver the tumor antigen NYESO1 to mice harboring the solid tumor produced from Lewis lung carcinoma cells engineered to express NYESO1, immune responses directed against the tumor are induced resulting in immune attack on the tumor itself. This antitumor immune response can be induced with a contralateral subcutaneous injection of the vault encapsulated NYESO. See Figure 12 A and B.

[0148] These results have also been extended to glioblastoma by packaging the glioblastoma associated antigens (GAA): GP100, EGFRv3, NY-ESO, and TRP-2 onto the INT domain. All GAA-INT fusion proteins have been packaged into CP, CPZ, or pVIZ vaults. Vault nanoparticles housing NY-ESO have been shown efficient uptake by dendritic cells. See Figure 13.

[0149] Furthermore, dendritic cell activation and maturation as measured by CD86 expression has also been shown to be significantly increased by treatment with NY-ESO vaults. See Figure 14.

[0150] Additionally, dendritic cells treated with GP100 vaults have demonstrated efficacy in stimulating CD8 T cells shown by elevated levels of interferon gamma. See Figure 15.

Example 9: Use of vault particle delivery of tumor antigens for personalized therapeutics

[0151] The compositions and methods disclosed herein can be utilized for personalized therapeutics directed against a wide variety of tumors. For example a biopsy of a particular tumor (lung glioblastoma etc.) can analyzed using existing procedures to determine the presence of common tumor antigens (biomarkers). Vault particles can be produced and engineered to contain individual tumor antigens and a mixture of these particles can be formulated based on the biopsy results of an individual tumor. This mixture of vault particles can then be used to immunize the patient and stimulate a specific cellular immune response that will be directed against the patient's particular tumor.

[0152] In other words, in lung cancer there are approximately 10 to 15 different antigens (tumor biomarkers) that are primarily expressed in nearly 99% of all lung tumors. Each of these 10 to 15 different antigens can be produced as fusion proteins with the vault packaging domain INT (antigen 1-INT, antigen 2-INT, antigen 3-INT etc.). These 10 to 15 different antigens-INT fusion proteins can be expressed, purified and stored either separately or mixed with recombinant vaults to form individual vault adjuvant antigen preparations that can be stored. Following biopsy, an individual's lung tumor can be analyzed for expression of the presence of the common biomarkers (the 10 to 15 different antigens) that are present in that tumor, thus allowing for tailored treatments for tumor eradication. For this example we will assume that antigens 3, 5 and 9 are present in an individual's tumor. A formulation of three different vault preparations (vaults containing antigen 3-INT, plus vaults containing antigen 5-INT plus vaults containing antigen 9-INT) can then be administered by subcutaneous injection to induce a cellular immune response to the individual tumor.

Example 10: Methods and materials

[0153] Expression and Purification of Recombinant vaults

[0154] Recombinant baculoviruses were generated using the Bac-to-Bac protocol (Invitrogen, Carlsbad, CA). The 385 amino acid coding region of ovalbumin was fused to major vault protein interaction domain (INT) derived from VPARP (amino acids 1563–1724) by PCR ligation[52,53]. Two PCR reactions were carried out: first = OVA-forward :CCCCACTAGTCCATGGGCTCCATCGG and OVA-INT reverse: TCCTGCCAGTGTTGTGTGCAGCTAGCAGGGGAAACACATCTGCC using plasmid pMFG-OVA as the template (plasmid pMFG-OVA was a kind gift from Dr Carlo Heirman,

Laboratory of Molecular and Cellular Therapy, Department of Physiology–Immunology, Medical School of the Vrije Universiteit Brussel, Brussels, Belgium). The second PCR reaction with primer OVA-INT forward: TTGGCAGATGTGTTTCCCCTGCTAGCTGC ACACAACACTGGCAGGA and INT reverse: GGGCTCGAGTTAGCCTTGACTGTAATGGAG using INT in pET28 as the template. The PCR reactions were purified on a Qiagen column and a second round of PCR was carried out using the OVA-forward x INT reverse. The resultant PCR product containing the fused OVA-INT was purified on a Qiagen column, digested with Spe I and Xho I, gel purified, and ligated to pFastBac to form a pFastBac vector containing OVA-INT. Construction of cp-MVP-z, or cp-MVP in pFastBac has been described previously [32].

[0155] Sf9 cells were infected with Ova-INT, cp-MVP-z, or cp-MVP recombinant baculoviruses at a multiplicity of infection (MOI) of 0.01 for approximately 65 h and then pelleted and lysed on ice in buffer A [50 mM Tris-HCl (pH 7.4), 75 mM NaCl, and 0.5 mM MgCl2] with 1% Triton X-100, 1 mM dithiothreitol, 0.5 mM `μg/ml chymostatin, 5 μM leupeptin, 5 μM pepstatin) (Sigma, St. Louis, MO). Lysates containing cp-MVP-z vaults were mixed with lysates containing either OVA-INT were incubated on ice for 30 min to allow the INT fusion proteins to package inside of vaults. Recombinant vaults were purified as previously described[33] and resuspended in 100–200 μl of sterile phosphate buffered saline. The protein concentration was determined using the BCA assay (Pierce, Rockville, IL) and sample integrity was analyzed by negative stain electron microscopy and SDS-PAGE with Coomassie staining or transferred to hybond membrane (Amersham) for Western blot analysis. The density of the bands was determined by gel scanning and densitometry analysis using a 9410 Typhoon Variable Mode Scanner (GE Healthcare Life Sciences, Piscataway, NJ).

[0156] **Preparation of OVA-liposomes**

[0157] To generate OVA-liposomes, 10 mg lyophilized DOTAP/DOPE (1:1) (1,2-dioleoyl-3-trimethylammonium-propane/1,2-dioleoyl-*sn*-glycero-3-phospho-ethanolamine) (Avanti Polar Lipids, Alabaster, AL) was re-hydrated in 1 mL endotoxin-free 5% glucose and mixed slowly (rotated) overnight at room temperature. Lyophilized EndoGrade Ovalbumin (< 1EU/mg = 1 endotoxin unit has ~0.1 ng of endotoxin) (Profos AG, BioVender, LLC, Candler, NC) was reconstituted in endotoxin-free sterile saline (<0.1 EU/mL endotoxin, Sigma) to a stock solution of 10 mg/mL. Aliquots were stored frozen and thawed

immediately before use. The entrapment of OVA was generated by combining 1.25 mg of resuspended ovalbumin with 2.5 mg of swollen DOTAP/DOPE lipids and further facilitated by brief sonication. OVA-liposomes were separated from unincorporated ovalbumin by ultracentrifugation at 100,000 x g using an Optima XL-80K (Beckman Coulter, Fullerton, CA) ultracentrifuge and washed two additional times. Quantitation of encapsulated OVA was determined by subjecting OVA-liposomes (1, 2, 4 μL) to SDS-PAGE electrophoresis in parallel with known amounts of ovalbumin (0.25, 0.5, 1.0, 2.5, 5 μg) and visualized by Coomassie blue staining.

[0158] Gel Electrophoresis and Immunoblotting

[0159] Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed using the discontinuous buffer system and 4-15% acrylamide gels. Protein samples of OVA-liposome or OVA-vaults were transferred to an Immobilon-P transfer membrane (Millipore, city, Bedford, MA) and blocked with 5% (wt/vol) nonfat dry milk in PBS-0.1% Tween 20 (PBS-T). Membranes were incubated for 1 hr with anti-MVP (1:500, MAB 1023, Santa Cruz Biotechnology Inc, Santa Cruz, CA) or anti-INT followed by a 1 h incubation with the appropriate horseradish conjugate (1:5,000, Amersham Biosciences, Piscataway, NJ). Bound conjugates were detected with ECL-Plus (GE Healthcare, Life Sciences, Piscataway, NJ) and 9410 Typhoon Variable Mode Scanner (GE Healthcare Life Sciences, Piscataway, NJ).

[0160] Antigen processing and presentation assay

[0161] DC2.4 H-2Kb ($5x10^4$/well) were plated in triplicates in 96-well plates and allowed to settle at 37 °C. Then, MHC Class I restricted $CD8^+$ T cell line B3Z (10^5/well) were added, in the presence of control vaults (200 ng/mL) and OVA vaults (200 ng/mL) for 24 hrs. After 24 h incubation at 37 °C, the plate was centrifuged at 1800 rpm, and the culture supernatant was collected and assayed for IL-2 using an IL-2 ELISA kit (BD Biosciences, San Jose, CA).

[0162] DC-dependent T cell proliferation

[0163] DC cultures were generated by flushing the bone marrow (BM) from the bone shafts, washed and plated bacteriological Petri dishes (Falcon Plastics, Oxnard, CA). The cells were cultured at 2×10^5 cells/mL in RPMI 1640 culture medium (10 mM HEPES/2 mM l-glutamine/10% 0.22 um filtered FBS/50 uM β-mercaptoethanol) supplemented with mGM-CSF (20 ng/mL) and mIL-4 (20 ng/mL) in an atmosphere of 5% CO_2 at 37 °C. Fresh medium containing mGM-CSF (20 ng/mL) and mIL-4 (20 ng/mL) was added for 3–6 days after the

start of culture. To induce maturation, cells were cultured for an additional 24 h in the presence of LPS (1 μg/mL). The DC were harvested and purified with anti-CD11c magnetic beads, and suspended in complete RPMI-1640 medium and seeded at 5×10^5/mL/well on 24-well culture plates followed by incubation with 25 and 100 μg/mL of CP-OVA or recombinant OVA protein for 4 h at 37 °C, 5% CO_2. Nonadherent cells consisting of mostly immature or mature DC were harvested for all the analyses performed in this study. Responder $CD4^+$ T cells were separated from splenocytes with mouse $CD4^+$ T-cell enrichment system (StemCell Technologies, Vancouver, Canada) according to the manufacturer's instructions. $CD4^+$ T cells (2×10^4/well) were added to OVA protein or CP-OVA pulsed DC and cultured for an additional 4 days. During the last 16–18 h of the 4-day culture, cells were pulsed with 1 μCi [3H]thymidine (Amersham, Arlington, IL). The cells were harvested onto filter paper and [3H]thymidine incorporation was measured with a β-plate scintillation counter (PerkinElmer, Wellesley, MA).

[0164] Immunization Procedures

[0165] The OVA protein concentration was adjusted using endotoxin-free sterile saline (<0.1 EU/mL, 1EU has ~0.1 of endotoxin (Sigma) to 2.5 μg OVA in 20 μg of vault nanoparticles or liposomes using a Typhoon 9410 Variable Mode Scanner of Coomassie blue stained SDS-PAGE gels. The immunogens were injected into C57BL/6 mice (5-6 wk old) by subcutaneous injections at the base of the neck in 100 μl sterile saline. The mice were immunized 3 times at 2 wk intervals. The spleen and blood was obtained 2 wk after the last immunization. The splenocytes were immediately used for FACS analysis and serum samples were stored frozen at -80°C until assayed.

[0166] Measurement of anti-OVA antibody from serum

[0167] An ELISA was used to determine the level of anti-OVA antibody isotypes in the serum. Briefly 96-well microtitre plates (Nunc, Roskilde, Denmark) were coated with 75μl per well of OVA (1 μg /75 μl) in PBS and incubated over night at 4 °C. After being washed in buffer (phosphate buffered saline containing 0.05% Tween-20 (v/v) (PBS/T20) the plates were blocked with 150 μl of PBS supplemented with 5% non-fat dry milk for 2 h at room temperature. After washing, 7 μl of serum diluted from 1:40 to 1:5120 in PBS was added and incubated at 4 °C overnight. Unbound antibody was then washed away and 75 μl of goat anti-mouse IgG1- IgG2c-biotin (Southern Biotechnology Associates, Inc., Birmingham, AL),

diluted 1/10,000 in PBS, was added and the plates incubated for 4 h at room temperature. The plates were then washed and 75 µl of NeutraAvidin horse radish peroxidase diluted in PBS at 1:1000 was added for 20 min. After a final wash step, 100 mL of TetraMethylBenzidine (TMB) (Zymed Laboratories Inc., San Francisco, CA) substrate was added and incubated at room temperature, in the dark, for 20 min. The reaction was stopped with 50 µL of 2 N sulphuric acid and the plates were read at 450 nm in a microplate reader (Model 550, Bio-Rad Laboratories, Hercules, CA).

[0168] Measurement of IL-2 production

[0169] Spleens were removed and placed in RPMI media (Gibco, Grand Island, NY) supplemented with 10% heat inactivated FCS. They were macerated to release the lymphocytes which were then washed by centrifugation. The cell pellet was resuspended in fresh media at a concentration of 2 X 10^6 cells/mL and 1 mL of cells placed in each well of a 24-well plate (Nunc, Roskilde, Demark). They were restimulated with media (negative control) or OVA (100 µg/mL) for 72 h at 37°C in a humidified atmosphere with 5% CO_2. The plate was frozen until required. One hundred microlitres of the supernatants were tested for IL-2 in a sandwich ELISA following the manufacturer's instructions (PharMingen, San Diego, USA). In brief, 96-well, flat-bottomed plates were coated with 50 µL of a 2 µg/mL concentration of capture antibody (PharMingen). Plates were washed and blocked with 200 µL/well of PBS/FCS. Doubling dilutions of standards and supernatants were added and incubated at 4°C overnight. The plates were washed and 100µL of a biotin-conjugated detecting mAb (PharMingen) was added at a concentration of 1 mg/mL. The enzyme and substrate were then added and analyzed as per the serum antibody ELISA. The amount of each cytokine in the supernatant was extrapolated from the standard curve derived using recombinant IL-2 (PharMingen) standards.

[0170] Characterization of T cell populations by flow cytometry

[0171] Lymphocytes were isolated from spleens by mechanical disruption through a cell strainer. RBCs were lysed using ammonium chloride-potassium buffer. The cells were stimulated @ 37°C with OVA peptide 265-280:TEWTSSNVMEERKIKV (2 µg) to identify CD4 cells or OVA peptide: SIINFEKL (2 µg) to identify CD8 cells for 5 hr. For the last 4h, cells were incubated in the presence of Brefeldin A (BioLegend) at 1 µg/mL. At the end of culture, the cells were stained using fluorochrome-conjugated MAbs against CD3, CD8, CD4, CD44, CCR7 and CD62L (BioLegend, San Diego, CA) in staining buffer (PBS with

2% fetal bovine serum and 0.1% sodium azide) and then treated with Fix/Perm (BioLegend). After permeabilization, the cells were further stained with fluorochrome-conjugated antibodies against IFN-γ, IL-4, IL-17 and perforin. Data were collected on LSR II (BD Biosciences, San Jose, CA) and analyzed using FCS Express (De Novo Software, Los Angeles, CA). $CD8^+$ and $CD4^+$ T cells were determined by gating on lymphocytes (FSC vs SSC) and $CD8^+$ or $CD4^+$ memory, cytokine producing or perforin expressing T cells were determined by gating on either $CD3^+CD8^+$ or $CD3^+CD4^+$ T cells as shown in Figure S1.

[0172] Statistical analysis

[0173] Statistical analysis was performed using Prism 5 (GraphPad, San Diego, CA). Data are presented as mean for each group and statistical significance for IL-2 secretion, proliferation, flow cytometry and Ig titers were determined by one way analysis of variance (ANOVA) with Bonferroni's Multiple Comparison Test. The ratio of isotypes was compared by Kruskal-Walis and Dunn's post-test.

[0174] Discussion

[0175] The work presented here illustrates the potential of engineered vault nanocapsules to act as potent adjuvants for the induction of combined cellular and humoral immune responses. Overall, our results demonstrate that immunization of OVA encased in vault nanocapsules, was more effective at generating greater cellular immunity characterized by increased numbers of OVA responsive memory $CD8^+$ and $CD4^+$ T cells. Also, modification of the vault body, by addition of the "Z" domain, altered the level of anti-OVA Ig subclass as shown by an increased IgG1:IgG2C ratio. These findings show that immune responses against OVA induced by vault nanoparticles differ compared to those induced by liposomes.

[0176] An important feature of vault nanocapsules as adjuvants is the robust induction of $CD8^+$ and $CD4^+$ memory T cells. The delivery of antigens to antigen presenting cells, especially DC, is a critical step for initiating and regulating the adaptive immune responses and we have shown that DC efficiently internalize vault nanocapules [27,41]. We have also shown that vaults containing immunogenic proteins activate inflammasomes and escape into the cytoplasm [unpublished data, [27]. This may explain induction of an OVA-responsive $CD8^+$ memory T cell response and cross-presentation. Vaults may also stimulate antigen-responsive $CD8^+$ and $CD4^+$ memory T cells by acting as intracellular depots or altering JAK/STAT signaling [47].

[0177] A potential vaccine should have the ability to induce and maintain antigen-responsive effector and/or memory T cells [7]. Our data show that immunization with vault nanocapsules was capable of inducing phenotypic markers of memory cells in $CD8^+$ and $CD4^+$ T cells. It will be interesting to extend these studies and examine memory responses *in vivo* using protection from infection or tumor models. In addition, we found enhanced production of OVA-responsive $CD8^+$ T cells that could secrete IFNγ. Surprisingly, there was not much difference between Liposome-OVA and OVA immunized groups and one questions the present of LPS. We did not measure LPS concentrations directly but all reagents used were endotoxin free and the purchased OVA was endotoxin free (see methods). However, there are differences in the amount of IFNγ produced when splenocytes are stimulated with OVA protein, CD8 or CD4 OVA peptides and whether IFNγ is measured in total splenocytes or $CD8^+$ or $CD4^+$ T cells [48].

[0178] The induction of effector $CD4^+$ T cells occurs in the same manner and with similar dynamics as is seen with the induction of effector memory $CD8^+$ T cells [43]. However, the increased $CD4^+$ memory T cells appear to be dominated by helper cells in mice immunized with CPZ-OVA vaults. Our data shows that the addition of the "Z" domain modifies antibody isotypes and supports the increased ratio of anti-OVA IgG1 over IgG2c titers. Adjuvants enhance immunity to immunogens but also steer immunity toward specific immune responses. For instance, alum is a known to promote Th2 responses [49]. The ability of vault vaccines to alter antibody isotypes suggests that modification of the vault toward certain immune responses is possible [50]. Recently, we have modified the vault by the addition of a lytic peptide derived from the adenovirus pIV protein. This modification allows those vaults to rapidly escape phagocytic vesicles [51]. Future studies will examine the *in vivo* immune responses generated by these vaults.

[0179] These results plus our previous studies with chemokines (CCL21) [28] and a chlamydial protein (MOMP) [27], supports the hypothesis that vault nanocapsules can be potent antigen delivery vehicles. Vault nanocapsules act as "smart" adjuvants that are capable of directing immunity toward desired responses with little induction of inflammatory cytokines when delivered via a mucosal route [27]. Further studies comparing immunization routes will be needed to determine the most effective route for the desired immune response. Since vaults are ubiquitous and conserved across eukaryote species, the platform has a major advantage over other delivery systems which have safety concerns associated with attenuated

bacteria or viruses. In addition, vault nanocapsules are uniform in size and are able to be produced in abundance. Combining adjuvant and carrier activity, engineered vaults enhance the response with a much lower dose of the antigen and circumvent the protein-purification requirements of traditional subunit vaccines and particulate antigen-delivery modalities. With possibilities of further engineering the surface of vaults to either target specific cells or by allowing the proteins to escape endosomes, vaults provide a uniquely tunable platform with ease of manufacture for the delivery of a wide spectrum of subunit antigens for vaccines against infectious disease or other therapeutic targets.

[0180] References

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[0181] While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

[0182] All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

Table 1. Sequences

SEQ ID NO:1	INT DNA sequence

TGC ACA CAA CAC TGG CAG GAT GCT GTG CCT TGG ACA GAA CTC CTC AGT
CTA CAG ACA GAG GAT GGC TTC TGG AAA CTT ACA CCA GAA CTG GGA CTT
ATA TTA AAT CTT AAT ACA AAT GGT TTG CAC AGC TTT CTT AAA CAA AAA
GGC ATT CAA TCT CTA GGT GTA AAA GGA AGA GAA TGT CTC CTG GAC CTA
ATT GCC ACA ATG CTG GTA CTA CAG TTT ATT CGC ACC AGG TTG GAA AAA
GAG GGA ATA GTG TTC AAA TCA CTG ATG AAA ATG GAT GAC CCT TCT ATT
TCC AGG AAT ATT CCC TGG GCT TTT GAG GCA ATA AAG CAA GCA AGT GAA
TGG GTA AGA AGA ACT GAA GGA CAG TAC CCA TCT ATC TGC CCA CGG CTT
GAA CTG GGG AAC GAC TGG GAC TCT GCC ACC AAG CAG TTG CTG GGA CTC
CAG CCC ATA AGC ACT GTG TCC CCT CTT CAT AGA GTC CTC CAT TAC AGT
CAA GGC TAA

SEQ ID NO:2	INT protein sequence (residues 1563-1724 of the human VPARP protein sequence)

CTQHWQDAVPWTELLSLQTEDGFWKLTPELGLILNLNTNGLHSFLKQKGIQSLGVKGRECLLDLIA
TMLVLQFIRTRLEKEGIVFKSLMKMDDPSISRNIPWAFEAIKQASEWVRRTEGQYPSICPRLELGN
DWDSATKQLLGLQPISTVSPLHRVLHYSQG

SEQ ID NO:3	VPARP protein sequence (Genbank #AAD47250)

Met Val Met Gly Ile Phe Ala Asn Cys Ile Phe Cys Leu Lys Val Lys Tyr Leu
Pro Gln Gln Gln Lys Lys Lys Leu Gln Thr Asp Ile Lys Glu Asn Gly Gly Lys
Phe Ser Phe Ser Leu Asn Pro Gln Cys Thr His Ile Ile Leu Asp Asn Ala Asp
Val Leu Ser Gln Tyr Gln Leu Asn Ser Ile Gln Lys Asn His Val His Ile Ala
Asn Pro Asp Phe Ile Trp Lys Ser Ile Arg Glu Lys Arg Leu Leu Asp Val Lys
Asn Tyr Asp Pro Tyr Lys Pro Leu Asp Ile Thr Pro Pro Pro Asp Gln Lys Ala
Ser Ser Ser Glu Val Lys Thr Glu Gly Leu Cys Pro Asp Ser Ala Thr Glu Glu
Glu Asp Thr Val Glu Leu Thr Glu Phe Gly Met Gln Asn Val Glu Ile Pro His
Leu Pro Gln Asp Phe Glu Val Ala Lys Tyr Asn Thr Leu Glu Lys Val Gly Met
Glu Gly Gly Gln Glu Ala Val Val Val Glu Leu Gln Cys Ser Arg Asp Ser Arg
Asp Cys Pro Phe Leu Ile Ser Ser His Phe Leu Leu Asp Asp Gly Met Glu Thr
Arg Arg Gln Phe Ala Ile Lys Lys Thr Ser Glu Asp Ala Ser Glu Tyr Phe Glu
Asn Tyr Ile Glu Glu Leu Lys Lys Gln Gly Phe Leu Leu Arg Glu His Phe Thr
Pro Glu Ala Thr Gln Leu Ala Ser Glu Gln Leu Gln Ala Leu Leu Leu Glu Glu
Val Met Asn Ser Ser Thr Leu Ser Gln Glu Val Ser Asp Leu Val Glu Met Ile
Trp Ala Glu Ala Leu Gly His Leu Glu His Met Leu Leu Lys Pro Val Asn Arg
Ile Ser Leu Asn Asp Val Ser Lys Ala Glu Gly Ile Leu Leu Leu Val Lys Ala
Ala Leu Lys Asn Gly Glu Thr Ala Glu Gln Leu Gln Lys Met Met Thr Glu Phe
Tyr Arg Leu Ile Pro His Lys Gly Thr Met Pro Lys Glu Val Asn Leu Gly Leu
Leu Ala Lys Lys Ala Asp Leu Cys Gln Leu Ile Arg Asp Met Val Asn Val Cys
Glu Thr Asn Leu Ser Lys Pro Asn Pro Pro Ser Leu Ala Lys Tyr Arg Ala Leu
Arg Cys Lys Ile Glu His Val Glu Gln Asn Thr Glu Glu Phe Leu Arg Val Arg
Lys Glu Val Leu Gln Asn His His Ser Lys Ser Pro Val Asp Val Leu Gln Ile
Phe Arg Val Gly Arg Val Asn Glu Thr Thr Glu Phe Leu Ser Lys Leu Gly Asn
Val Arg Pro Leu Leu His Gly Ser Pro Val Gln Asn Ile Val Gly Ile Leu Cys
Arg Gly Leu Leu Leu Pro Lys Val Val Glu Asp Arg Gly Val Gln Arg Thr Asp
Val Gly Asn Leu Gly Ser Gly Ile Tyr Phe Ser Asp Ser Leu Ser Thr Ser Ile
Lys Tyr Ser His Pro Gly Glu Thr Asp Gly Thr Arg Leu Leu Leu Ile Cys Asp
Val Ala Leu Gly Lys Cys Met Asp Leu His Glu Lys Asp Phe Pro Leu Thr Glu
Ala Pro Pro Gly Tyr Asp Ser Val His Gly Val Ser Gln Thr Ala Ser Val Thr
Thr Asp Phe Glu Asp Asp Glu Phe Val Val Tyr Lys Thr Asn Gln Val Lys Met
Lys Tyr Ile Ile Lys Phe Ser Met Pro Gly Asp Gln Ile Lys Asp Phe His Pro
Ser Asp His Thr Glu Leu Glu Glu Tyr Arg Pro Glu Phe Ser Asn Phe Ser Lys
Val Glu Asp Tyr Gln Leu Pro Asp Ala Lys Thr Ser Ser Ser Thr Lys Ala Gly
Leu Gln Asp Ala Ser Gly Asn Leu Val Pro Leu Glu Asp Val His Ile Lys Gly

Arg Ile Ile Asp Thr Val Ala Gln Val Ile Val Phe Gln Thr Tyr Thr Asn Lys
Ser His Val Pro Ile Glu Ala Lys Tyr Ile Phe Pro Leu Asp Asp Lys Ala Ala
Val Cys Gly Phe Glu Ala Phe Ile Asn Gly Lys His Ile Val Gly Glu Ile Lys
Glu Lys Glu Glu Ala Gln Gln Glu Tyr Leu Glu Ala Val Thr Gln Gly His Gly
Ala Tyr Leu Met Ser Gln Asp Ala Pro Asp Val Phe Thr Val Ser Val Gly Asn
Leu Pro Pro Lys Ala Lys Val Leu Ile Lys Ile Thr Tyr Ile Thr Glu Leu Ser
Ile Leu Gly Thr Val Gly Val Phe Phe Met Pro Ala Thr Val Ala Pro Trp Gln
Gln Asp Lys Ala Leu Asn Glu Asn Leu Gln Asp Thr Val Glu Lys Ile Cys Ile
Lys Glu Ile Gly Thr Lys Gln Ser Phe Ser Leu Thr Met Ser Ile Glu Met Pro
Tyr Val Ile Glu Phe Ile Phe Ser Asp Thr His Glu Leu Lys Gln Lys Arg Thr
Asp Cys Lys Ala Val Ile Ser Thr Met Glu Gly Ser Ser Leu Asp Ser Ser Gly
Phe Ser Leu His Ile Gly Leu Ser Ala Ala Tyr Leu Pro Arg Met Trp Val Glu
Lys His Pro Glu Lys Glu Ser Glu Ala Cys Met Leu Val Phe Gln Pro Asp Leu
Asp Val Asp Leu Pro Asp Leu Ala Ser Glu Ser Glu Val Ile Ile Cys Leu Asp
Cys Ser Ser Ser Met Glu Gly Val Thr Phe Leu Gln Ala Lys Gln Ile Thr Leu
His Ala Leu Ser Leu Val Gly Glu Lys Gln Lys Val Asn Ile Ile Gln Phe Gly
Thr Gly Tyr Lys Glu Leu Phe Ser Tyr Pro Lys His Ile Thr Ser Asn Thr Thr
Ala Ala Glu Phe Ile Met Ser Ala Thr Pro Thr Met Gly Asn Thr Asp Phe Trp
Lys Thr Leu Arg Tyr Leu Ser Leu Leu Tyr Pro Ala Arg Gly Ser Arg Asn Ile
Leu Leu Val Ser Asp Gly His Leu Gln Asp Glu Ser Leu Thr Leu Gln Leu Val
Lys Arg Ser Arg Pro His Thr Arg Leu Phe Ala Cys Gly Ile Gly Ser Thr Ala
Asn Arg His Val Leu Arg Ile Leu Ser Gln Cys Gly Ala Gly Val Phe Glu Tyr
Phe Asn Ala Lys Ser Lys His Ser Trp Arg Lys Gln Ile Glu Asp Gln Met Thr
Arg Leu Cys Ser Pro Ser Cys His Ser Val Ser Val Lys Trp Gln Gln Leu Asn
Pro Asp Ala Pro Glu Ala Leu Gln Ala Pro Ala Gln Val Pro Ser Leu Phe Arg
Asn Asp Arg Leu Leu Val Tyr Gly Phe Ile Pro His Cys Thr Gln Ala Thr Leu
Cys Ala Leu Ile Gln Glu Lys Glu Phe Cys Thr Met Val Ser Thr Thr Glu Leu
Gln Lys Thr Thr Gly Thr Met Ile His Lys Leu Ala Ala Arg Ala Leu Ile Arg
Asp Tyr Glu Asp Gly Ile Leu His Glu Asn Glu Thr Ser His Glu Met Lys Lys
Gln Thr Leu Lys Ser Leu Ile Ile Lys Leu Ser Lys Glu Asn Ser Leu Ile Thr
Gln Phe Thr Ser Phe Val Ala Val Glu Lys Arg Asp Glu Asn Glu Ser Pro Phe
Pro Asp Ile Pro Lys Val Ser Glu Leu Ile Ala Lys Glu Asp Val Asp Phe Leu
Pro Tyr Met Ser Trp Gln Gly Glu Pro Gln Glu Ala Val Arg Asn Gln Ser Leu
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Ile Pro Phe Ser Lys Arg Lys Met Glu Leu Ser Gln Pro Glu Val Ser Glu Asp
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Ala Thr Glu Pro Leu Phe Lys Lys Val Ser Pro Trp Glu Thr Ser Thr Ser Ser
Phe Phe Pro Ile Leu Ala Pro Ala Val Gly Ser Tyr Leu Thr Pro Thr Thr Arg
Ala His Ser Pro Ala Ser Leu Ser Phe Ala Ser Tyr Arg Gln Val Ala Ser Phe
Gly Ser Ala Ala Pro Pro Arg Gln Phe Asp Ala Ser Gln Phe Ser Gln Gly Pro
Val Pro Gly Thr Cys Ala Asp Trp Ile Pro Gln Ser Ala Ser Cys Pro Thr Gly
Pro Pro Gln Asn Pro Pro Ser Ala Pro Tyr Cys Gly Ile Val Phe Ser Gly Ser
Ser Leu Ser Ser Ala Gln Ser Ala Pro Leu Gln His Pro Gly Gly Phe Thr Thr
Arg Pro Ser Ala Gly Thr Phe Pro Glu Leu Asp Ser Pro Gln Leu His Phe Ser
Leu Pro Thr Asp Pro Asp Pro Ile Arg Gly Phe Gly Ser Tyr His Pro Ser Ala
Tyr Ser Pro Phe His Phe Gln Pro Ser Ala Ala Ser Leu Thr Ala Asn Leu Arg
Leu Pro Met Ala Ser Ala Leu Pro Glu Ala Leu Cys Ser Gln Ser Arg Thr Thr
Pro Val Asp Leu Cys Leu Leu Glu Glu Ser Val Gly Ser Leu Glu Gly Ser Arg
Cys Pro Val Phe Ala Phe Gln Ser Ser Asp Thr Glu Ser Asp Glu Leu Ser Glu
Val Leu Gln Asp Ser Cys Phe Leu Gln Ile Lys Cys Asp Thr Lys Asp Asp Ser
Ile Pro Cys Phe Leu Glu Leu Lys Glu Glu Asp Glu Ile Val Cys Thr Gln His
Trp Gln Asp Ala Val Pro Trp Thr Glu Leu Leu Ser Leu Gln Thr Glu Asp Gly
Phe Trp Lys Leu Thr Pro Glu Leu Gly Leu Ile Leu Asn Leu Asn Thr Asn Gly
Leu His Ser Phe Leu Lys Gln Lys Gly Ile Gln Ser Leu Gly Val Lys Gly Arg
Glu Cys Leu Leu Asp Leu Ile Ala Thr Met Leu Val Leu Gln Phe Ile Arg Thr
Arg Leu Glu Lys Glu Gly Ile Val Phe Lys Ser Leu Met Lys Met Asp Asp Pro
Ser Ile Ser Arg Asn Ile Pro Trp Ala Phe Glu Ala Ile Lys Gln Ala Ser Glu
Trp Val Arg Arg Thr Glu Gly Gln Tyr Pro Ser Ile Cys Pro Arg Leu Glu Leu
Gly Asn Asp Trp Asp Ser Ala Thr Lys Gln Leu Leu Gly Leu Gln Pro Ile Ser
Thr Val Ser Pro Leu His Arg Val Leu His Tyr Ser Gln Gly

SEQ ID NO:5	**VPARP cDNA, Genbank #AF158255**

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SEQ ID NO:6	MVP (Genbank #CAA56256)

Met Ala Thr Glu Glu Phe Ile Ile Arg Ile Pro Pro Tyr His Tyr Ile His Val
Leu Asp Gln Asn Ser Asn Val Ser Arg Val Glu Val Gly Pro Lys Thr Tyr Ile
Arg Gln Asp Asn Glu Arg Val Leu Phe Ala Pro Met Arg Met Val Thr Val Pro
Pro Arg His Tyr Cys Thr Val Ala Asn Pro Val Ser Arg Asp Ala Gln Gly Leu
Val Leu Phe Asp Val Thr Gly Gln Val Arg Leu Arg His Ala Asp Leu Glu Ile
Arg Leu Ala Gln Asp Pro Phe Pro Leu Tyr Pro Gly Glu Val Leu Glu Lys Asp
Ile Thr Pro Leu Gln Val Val Leu Pro Asn Thr Ala Leu His Leu Lys Ala Leu
Leu Asp Phe Glu Asp Lys Asp Gly Asp Lys Val Val Ala Gly Asp Glu Trp Leu
Phe Glu Gly Pro Gly Thr Tyr Ile Pro Arg Lys Glu Val Glu Val Val Glu Ile
Ile Gln Ala Thr Ile Ile Arg Gln Asn Gln Ala Leu Arg Leu Arg Ala Arg Lys
Glu Cys Trp Asp Arg Asp Gly Lys Glu Arg Val Thr Gly Glu Glu Trp Leu Val
Thr Thr Val Gly Ala Tyr Leu Pro Ala Val Phe Glu Glu Val Leu Asp Leu Val
Asp Ala Val Ile Leu Thr Glu Lys Thr Ala Leu His Leu Arg Ala Arg Arg Asn
Phe Arg Asp Phe Arg Gly Val Ser Arg Arg Thr Gly Glu Glu Trp Leu Val Thr
Val Gln Asp Thr Glu Ala His Val Pro Asp Val His Glu Glu Val Leu Gly Val
Val Pro Ile Thr Thr Leu Gly Pro His Asn Tyr Cys Val Ile Leu Asp Pro Val
Gly Pro Asp Gly Lys Asn Gln Leu Gly Gln Lys Arg Val Val Lys Gly Glu Lys
Ser Phe Phe Leu Gln Pro Gly Glu Gln Leu Glu Gln Gly Ile Gln Asp Val Tyr
Val Leu Ser Glu Gln Gln Gly Leu Leu Leu Arg Ala Leu Gln Pro Leu Glu Glu
Gly Glu Asp Glu Glu Lys Val Ser His Gln Ala Gly Asp His Trp Leu Ile Arg
Gly Pro Leu Glu Tyr Val Pro Ser Ala Lys Val Glu Val Val Glu Glu Arg Gln
Ala Ile Pro Leu Asp Glu Asn Glu Gly Ile Tyr Val Gln Asp Val Lys Thr Gly
Lys Val Arg Ala Val Ile Gly Ser Thr Tyr Met Leu Thr Gln Asp Glu Val Leu
Trp Glu Lys Glu Leu Pro Pro Gly Val Glu Glu Leu Leu Asn Lys Gly Gln Asp
Pro Leu Ala Asp Arg Gly Glu Lys Asp Thr Ala Lys Ser Leu Gln Pro Leu Ala
Pro Arg Asn Lys Thr Arg Val Val Ser Tyr Arg Val Pro His Asn Ala Ala Val
Gln Val Tyr Asp Tyr Arg Glu Lys Arg Ala Arg Val Val Phe Gly Pro Glu Leu
Val Ser Leu Gly Pro Glu Glu Gln Phe Thr Val Leu Ser Leu Ser Ala Gly Arg
Pro Lys Arg Pro His Ala Arg Arg Ala Leu Cys Leu Leu Leu Gly Pro Asp Phe
Phe Thr Asp Val Ile Thr Ile Glu Thr Ala Asp His Ala Arg Leu Gln Leu Gln
Leu Ala Tyr Asn Trp His Phe Glu Val Asn Asp Arg Lys Asp Pro Gln Glu Thr
Ala Lys Leu Phe Ser Val Pro Asp Phe Val Gly Asp Ala Cys Lys Ala Ile Ala
Ser Arg Val Arg Gly Ala Val Ala Ser Val Thr Phe Asp Asp Phe His Lys Asn
Ser Ala Arg Ile Ile Arg Thr Ala Val Phe Gly Phe Glu Thr Ser Glu Ala Lys
Gly Pro Asp Gly Met Ala Leu Pro Arg Pro Arg Asp Gln Ala Val Phe Pro Gln
Asn Gly Leu Val Val Ser Ser Val Asp Val Gln Ser Val Glu Pro Val Asp Gln
Arg Thr Arg Asp Ala Leu Gln Arg Ser Val Gln Leu Ala Ile Glu Ile Thr Thr

Asn Ser Gln Glu Ala Ala Ala Lys His Glu Ala Gln Arg Leu Glu Gln Glu Ala
Arg Gly Arg Leu Glu Arg Gln Lys Ile Leu Asp Gln Ser Glu Ala Glu Lys Ala
Arg Lys Glu Leu Leu Glu Leu Glu Ala Leu Ser Met Ala Val Glu Ser Thr Gly
Thr Ala Lys Ala Glu Ala Glu Ser Arg Ala Glu Ala Ala Arg Ile Glu Gly Glu
Gly Ser Val Leu Gln Ala Lys Leu Lys Ala Gln Ala Leu Ala Ile Glu Thr Glu
Ala Glu Leu Gln Arg Val Gln Lys Val Arg Glu Leu Glu Leu Val Tyr Ala Arg
Ala Gln Leu Glu Leu Glu Val Ser Lys Ala Gln Gln Leu Ala Glu Val Glu Val
Lys Lys Phe Lys Gln Met Thr Glu Ala Ile Gly Pro Ser Thr Ile Arg Asp Leu
Ala Val Ala Gly Pro Glu Met Gln Val Lys Leu Leu Gln Ser Leu Gly Leu Lys
Ser Thr Leu Ile Thr Asp Gly Ser Thr Pro Ile Asn Leu Phe Asn Thr Ala Phe
Gly Leu Leu Gly Met Gly Pro Glu Gly Gln Pro Leu Gly Arg Arg Val Ala Ser
Gly Pro Ser Pro Gly Glu Gly Ile Ser Pro Gln Ser Ala Gln Ala Pro Gln Ala
Pro Gly Asp Asn His Val Val Pro Val Leu Arg

SEQ ID NO:7	MVP cDNA, Genbank #X79882

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caagctcctg gagacaacca cgtggtgcct gtactgcgct aa

SEQ ID NO:8	CP Peptide

Met Ala Gly Cys Gly Cys Pro Cys Gly Cys Gly Ala

SEQ ID NO:9	CP-MVP

Met Ala Gly Cys Gly Cys Pro Cys Gly Cys Gly Ala Met Ala Thr Glu Glu Phe
Ile Ile Arg Ile Pro Pro Tyr His Tyr Ile His Val Leu Asp Gln Asn Ser Asn
Val Ser Arg Val Glu Val Gly Pro Lys Thr Tyr Ile Arg Gln Asp Asn Glu Arg
Val Leu Phe Ala Pro Met Arg Met Val Thr Val Pro Pro Arg His Tyr Cys Thr
Val Ala Asn Pro Val Ser Arg Asp Ala Gln Gly Leu Val Leu Phe Asp Val Thr
Gly Gln Val Arg Leu Arg His Ala Asp Leu Glu Ile Arg Leu Ala Gln Asp Pro
Phe Pro Leu Tyr Pro Gly Glu Val Leu Glu Lys Asp Ile Thr Pro Leu Gln Val
Val Leu Pro Asn Thr Ala Leu His Leu Lys Ala Leu Leu Asp Phe Glu Asp Lys
Asp Gly Asp Lys Val Val Ala Gly Asp Glu Trp Leu Phe Glu Gly Pro Gly Thr
Tyr Ile Pro Arg Lys Glu Val Glu Val Val Glu Ile Ile Gln Ala Thr Ile Ile
Arg Gln Asn Gln Ala Leu Arg Leu Arg Ala Arg Lys Glu Cys Trp Asp Arg Asp
Gly Lys Glu Arg Val Thr Gly Glu Glu Trp Leu Val Thr Thr Val Gly Ala Tyr
Leu Pro Ala Val Phe Glu Glu Val Leu Asp Leu Val Asp Ala Val Ile Leu Thr
Glu Lys Thr Ala Leu His Leu Arg Ala Arg Arg Asn Phe Arg Asp Phe Arg Gly
Val Ser Arg Arg Thr Gly Glu Glu Trp Leu Val Thr Val Gln Asp Thr Glu Ala
His Val Pro Asp Val His Glu Glu Val Leu Gly Val Val Pro Ile Thr Thr Leu
Gly Pro His Asn Tyr Cys Val Ile Leu Asp Pro Val Gly Pro Asp Gly Lys Asn
Gln Leu Gly Gln Lys Arg Val Val Lys Gly Glu Lys Ser Phe Phe Leu Gln Pro
Gly Glu Gln Leu Glu Gln Gly Ile Gln Asp Val Tyr Val Leu Ser Glu Gln Gln
Gly Leu Leu Leu Arg Ala Leu Gln Pro Leu Glu Glu Gly Glu Asp Glu Glu Lys
Val Ser His Gln Ala Gly Asp His Trp Leu Ile Arg Gly Pro Leu Glu Tyr Val
Pro Ser Ala Lys Val Glu Val Val Glu Glu Arg Gln Ala Ile Pro Leu Asp Glu
Asn Glu Gly Ile Tyr Val Gln Asp Val Lys Thr Gly Lys Val Arg Ala Val Ile
Gly Ser Thr Tyr Met Leu Thr Gln Asp Glu Val Leu Trp Glu Lys Glu Leu Pro
Pro Gly Val Glu Glu Leu Leu Asn Lys Gly Gln Asp Pro Leu Ala Asp Arg Gly
Glu Lys Asp Thr Ala Lys Ser Leu Gln Pro Leu Ala Pro Arg Asn Lys Thr Arg
Val Val Ser Tyr Arg Val Pro His Asn Ala Ala Val Gln Val Tyr Asp Tyr Arg
Glu Lys Arg Ala Arg Val Val Phe Gly Pro Glu Leu Val Ser Leu Gly Pro Glu
Glu Gln Phe Thr Val Leu Ser Leu Ser Ala Gly Arg Pro Lys Arg Pro His Ala
Arg Arg Ala Leu Cys Leu Leu Leu Gly Pro Asp Phe Phe Thr Asp Val Ile Thr
Ile Glu Thr Ala Asp His Ala Arg Leu Gln Leu Gln Leu Ala Tyr Asn Trp His
Phe Glu Val Asn Asp Arg Lys Asp Pro Gln Glu Thr Ala Lys Leu Phe Ser Val
Pro Asp Phe Val Gly Asp Ala Cys Lys Ala Ile Ala Ser Arg Val Arg Gly Ala
Val Ala Ser Val Thr Phe Asp Asp Phe His Lys Asn Ser Ala Arg Ile Ile Arg
Thr Ala Val Phe Gly Phe Glu Thr Ser Glu Ala Lys Gly Pro Asp Gly Met Ala
Leu Pro Arg Pro Arg Asp Gln Ala Val Phe Pro Gln Asn Gly Leu Val Val Ser
Ser Val Asp Val Gln Ser Val Glu Pro Val Asp Gln Arg Thr Arg Asp Ala Leu
Gln Arg Ser Val Gln Leu Ala Ile Glu Ile Thr Thr Asn Ser Gln Glu Ala Ala
Ala Lys His Glu Ala Gln Arg Leu Glu Gln Glu Ala Arg Gly Arg Leu Glu Arg
Gln Lys Ile Leu Asp Gln Ser Glu Ala Glu Lys Ala Arg Lys Glu Leu Leu Glu
Leu Glu Ala Leu Ser Met Ala Val Glu Ser Thr Gly Thr Ala Lys Ala Glu Ala
Glu Ser Arg Ala Glu Ala Ala Arg Ile Glu Gly Glu Gly Ser Val Leu Gln Ala
Lys Leu Lys Ala Gln Ala Leu Ala Ile Glu Thr Glu Ala Glu Leu Gln Arg Val
Gln Lys Val Arg Glu Leu Glu Leu Val Tyr Ala Arg Ala Gln Leu Glu Leu Glu
Val Ser Lys Ala Gln Gln Leu Ala Glu Val Glu Val Lys Lys Phe Lys Gln Met
Thr Glu Ala Ile Gly Pro Ser Thr Ile Arg Asp Leu Ala Val Ala Gly Pro Glu
Met Gln Val Lys Leu Leu Gln Ser Leu Gly Leu Lys Ser Thr Leu Ile Thr Asp
Gly Ser Thr Pro Ile Asn Leu Phe Asn Thr Ala Phe Gly Leu Leu Gly Met Gly
Pro Glu Gly Gln Pro Leu Gly Arg Arg Val Ala Ser Gly Pro Ser Pro Gly Glu
Gly Ile Ser Pro Gln Ser Ala Gln Ala Pro Gln Ala Pro Gly Asp Asn His Val
Val Pro Val Leu Arg

SEQ ID NO:10	CP-MVP cDNA

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SEQ ID NO:11	TEP1, Genbank #AAC51107

Met Glu Lys Leu His Gly His Val Ser Ala His Pro Asp Ile Leu Ser Leu Glu
Asn Arg Cys Leu Ala Met Leu Pro Asp Leu Gln Pro Leu Glu Lys Leu His Gln
His Val Ser Thr His Ser Asp Ile Leu Ser Leu Lys Asn Gln Cys Leu Ala Thr
Leu Pro Asp Leu Lys Thr Met Glu Lys Pro His Gly Tyr Val Ser Ala His Pro
Asp Ile Leu Ser Leu Glu Asn Gln Cys Leu Ala Thr Leu Ser Asp Leu Lys Thr
Met Glu Lys Pro His Gly His Val Ser Ala His Pro Asp Ile Leu Ser Leu Glu
Asn Arg Cys Leu Ala Thr Leu Pro Ser Leu Lys Ser Thr Val Ser Ala Ser Pro
Leu Phe Gln Ser Leu Gln Ile Ser His Met Thr Gln Ala Asp Leu Tyr Arg Val
Asn Asn Ser Asn Cys Leu Leu Ser Glu Pro Pro Ser Trp Arg Ala Gln His Phe
Ser Lys Gly Leu Asp Leu Ser Thr Cys Pro Ile Ala Leu Lys Ser Ile Ser Ala
Thr Glu Thr Ala Gln Glu Ala Thr Leu Gly Arg Trp Phe Asp Ser Glu Glu Lys
Lys Gly Ala Glu Thr Gln Met Pro Ser Tyr Ser Leu Ser Leu Gly Glu Glu Glu
Glu Val Glu Asp Leu Ala Val Lys Leu Thr Ser Gly Asp Ser Glu Ser His Pro
Glu Pro Thr Asp His Val Leu Gln Glu Lys Lys Met Ala Leu Leu Ser Leu Leu
Cys Ser Thr Leu Val Ser Glu Val Asn Met Asn Asn Thr Ser Asp Pro Thr Leu
Ala Ala Ile Phe Glu Ile Cys Arg Glu Leu Ala Leu Leu Glu Pro Glu Phe Ile
Leu Lys Ala Ser Leu Tyr Ala Arg Gln Gln Leu Asn Val Arg Asn Val Ala Asn
Asn Ile Leu Ala Ile Ala Ala Phe Leu Pro Ala Cys Arg Pro His Leu Arg Arg
Tyr Phe Cys Ala Ile Val Gln Leu Pro Ser Asp Trp Ile Gln Val Ala Glu Leu

Tyr Gln Ser Leu Ala Glu Gly Asp Lys Asn Lys Leu Val Pro Leu Pro Ala Cys
Leu Arg Thr Ala Met Thr Asp Lys Phe Ala Gln Phe Asp Glu Tyr Gln Leu Ala
Lys Tyr Asn Pro Arg Lys His Arg Ala Lys Arg His Pro Arg Arg Pro Pro Arg
Ser Pro Gly Met Glu Pro Pro Phe Ser His Arg Cys Phe Pro Arg Tyr Ile Gly
Phe Leu Arg Glu Glu Gln Arg Lys Phe Glu Lys Ala Gly Asp Thr Val Ser Glu
Lys Lys Asn Pro Pro Arg Phe Thr Leu Lys Lys Leu Val Gln Arg Leu His Ile
His Lys Pro Ala Gln His Val Gln Ala Leu Leu Gly Tyr Arg Tyr Pro Ser Asn
Leu Gln Leu Phe Ser Arg Ser Arg Leu Pro Gly Pro Trp Asp Ser Ser Arg Ala
Gly Lys Arg Met Lys Leu Ser Arg Pro Glu Thr Trp Glu Arg Glu Leu Ser Leu
Arg Gly Asn Lys Ala Ser Val Trp Glu Glu Leu Ile Glu Asn Gly Lys Leu Pro
Phe Met Ala Met Leu Arg Asn Leu Cys Asn Leu Leu Arg Val Gly Ile Ser Ser
Arg His His Glu Leu Ile Leu Gln Arg Leu Gln His Gly Lys Ser Val Ile His
Ser Arg Gln Phe Pro Phe Arg Phe Leu Asn Ala His Asp Ala Ile Asp Ala Leu
Glu Ala Gln Leu Arg Asn Gln Ala Leu Pro Phe Pro Ser Asn Ile Thr Leu Met
Arg Arg Ile Leu Thr Arg Asn Glu Lys Asn Arg Pro Arg Arg Arg Phe Leu Cys
His Leu Ser Arg Gln Gln Leu Arg Met Ala Met Arg Ile Pro Val Leu Tyr Glu
Gln Leu Lys Arg Glu Lys Leu Arg Val His Lys Ala Arg Gln Trp Lys Tyr Asp
Gly Glu Met Leu Asn Arg Tyr Arg Gln Ala Leu Glu Thr Ala Val Asn Leu Ser
Val Lys His Ser Leu Pro Leu Leu Pro Gly Arg Thr Val Leu Val Tyr Leu Thr
Asp Ala Asn Ala Asp Arg Leu Cys Pro Lys Ser Asn Pro Gln Gly Pro Pro Leu
Asn Tyr Ala Leu Leu Leu Ile Gly Met Met Ile Thr Arg Ala Glu Gln Val Asp
Val Val Leu Cys Gly Gly Asp Thr Leu Lys Thr Ala Val Leu Lys Ala Glu Glu
Gly Ile Leu Lys Thr Ala Ile Lys Leu Gln Ala Gln Val Gln Glu Phe Asp Glu
Asn Asp Gly Trp Ser Leu Asn Thr Phe Gly Lys Tyr Leu Leu Ser Leu Ala Gly
Gln Arg Val Pro Val Asp Arg Val Ile Leu Leu Gly Gln Ser Met Asp Asp Gly
Met Ile Asn Val Ala Lys Gln Leu Tyr Trp Gln Arg Val Asn Ser Lys Cys Leu
Phe Val Gly Ile Leu Leu Arg Arg Val Gln Tyr Leu Ser Thr Asp Leu Asn Pro
Asn Asp Val Thr Leu Ser Gly Cys Thr Asp Ala Ile Leu Lys Phe Ile Ala Glu
His Gly Ala Ser His Leu Leu Glu His Val Gly Gln Met Asp Lys Ile Phe Lys
Ile Pro Pro Pro Pro Gly Lys Thr Gly Val Gln Ser Leu Arg Pro Leu Glu Glu
Asp Thr Pro Ser Pro Leu Ala Pro Val Ser Gln Gln Gly Trp Arg Ser Ile Arg
Leu Phe Ile Ser Ser Thr Phe Arg Asp Met His Gly Glu Arg Asp Leu Leu Leu
Arg Ser Val Leu Pro Ala Leu Gln Ala Arg Ala Ala Pro His Arg Ile Ser Leu
His Gly Ile Asp Leu Arg Trp Gly Val Thr Glu Glu Glu Thr Arg Arg Asn Arg
Gln Leu Glu Val Cys Leu Gly Glu Val Glu Asn Ala Gln Leu Phe Val Gly Ile
Leu Gly Ser Arg Tyr Gly Tyr Ile Pro Pro Ser Tyr Asn Leu Pro Asp His Pro
His Phe His Trp Ala Gln Gln Tyr Pro Ser Gly Arg Ser Val Thr Glu Met Glu
Val Met Gln Phe Leu Asn Arg Asn Gln Arg Leu Gln Pro Ser Ala Gln Ala Leu
Ile Tyr Phe Arg Asp Ser Ser Phe Leu Ser Ser Val Pro Asp Ala Trp Lys Ser
Asp Phe Val Ser Glu Ser Glu Glu Ala Ala Cys Arg Ile Ser Glu Leu Lys Ser
Tyr Leu Ser Arg Gln Lys Gly Ile Thr Cys Arg Arg Tyr Pro Cys Glu Trp Gly
Gly Val Ala Ala Gly Arg Pro Tyr Val Gly Gly Leu Glu Glu Phe Gly Gln Leu
Val Leu Gln Asp Val Trp Asn Met Ile Gln Lys Leu Tyr Leu Gln Pro Gly Ala
Leu Leu Glu Gln Pro Val Ser Ile Pro Asp Asp Asp Leu Val Gln Ala Thr Phe
Gln Gln Leu Gln Lys Pro Pro Ser Pro Ala Arg Pro Arg Leu Leu Gln Asp Thr
Val Gln Gln Leu Met Leu Pro His Gly Arg Leu Ser Leu Val Thr Gly Gln Ser
Gly Gln Gly Lys Thr Ala Phe Leu Ala Ser Leu Val Ser Ala Leu Gln Ala Pro
Asp Gly Ala Lys Val Ala Pro Leu Val Phe Phe His Phe Ser Gly Ala Arg Pro
Asp Gln Gly Leu Ala Leu Thr Leu Leu Arg Arg Leu Cys Thr Tyr Leu Arg Gly
Gln Leu Lys Glu Pro Gly Ala Leu Pro Ser Thr Tyr Arg Ser Leu Val Trp Glu
Leu Gln Gln Arg Leu Leu Pro Lys Ser Ala Glu Ser Leu His Pro Gly Gln Thr
Gln Val Leu Ile Ile Asp Gly Ala Asp Arg Leu Val Asp Gln Asn Gly Gln Leu
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CLAIMS

1. A method for stimulating a cellular immune response in a subject, comprising administering to the subject an effective amount of an antigenic peptide or an antigenic fragment or variant thereof incorporated within a vault complex.

2. The method of claim 1, wherein the antigenic peptide is a tumor antigen.

3. The method of claim 1, wherein the vault complex comprises two or more vault complexes, wherein each vault complex comprises two or more different antigenic peptides or antigenic fragments or variants.

4. The method of claim 1, wherein the antigenic peptide is fused to INT.

5. The method of claim 4, wherein the INT comprises the amino acid sequence of SEQ ID NO: 2.

6. The method of claim 1, wherein the antigenic peptide is fused to MVP.

7. The method of claim 6, wherein the antigenic peptide is fused to the N-terminus of MVP.

8. The method of claim 1, wherein the vault complex comprises MVP.

9. The method of claim 8, wherein the number of MVP is 1-78.

10. The method of claim 8, wherein the number of MVP is 78.

11. The method of claim 8, wherein the vault complex further comprises VPARP or modified VPARP, or a portion of VPARP, or a modified portion of VPARP.

12. The method of claim 1, wherein the cellular immune response is induction of $CD8^+$ and $CD4^+$ memory T-cells.

13. The method of claim 1, wherein the cellular immune response is production of INFγ.

14. The method of claim 1, further comprising administering to the subject a vault complex containing a chemokine.

15. The method of claim 14, wherein the chemokine is CCL21.

16. A pharmaceutical composition for preventing or treating a subject for cancer, comprising a tumor antigen or an antigenic fragment or variant thereof incorporated within a vault complex, and at least one pharmaceutically acceptable excipient, sufficient to stimulate a cellular immune response.

17. The pharmaceutical composition of claim 16, wherein tumor antigen is fused to INT.

18. The method of claim 17, wherein the INT comprises the amino acid sequence of SEQ ID NO: 2.

19. The pharmaceutical composition of claim 16, wherein the antigenic peptide is fused to MVP.

20. The pharmaceutical composition of claim 16, wherein the vault complex comprises MVP.

21. The pharmaceutical composition of claim 20, wherein the number of MVP is 1-78.

22. The pharmaceutical composition of claim 20, wherein the number of MVP is 78.

23. The pharmaceutical composition of claim 20, wherein the vault complex further comprises VPARP or modified VPARP, or a portion of VPARP, or a modified portion of VPARP.

24. The pharmaceutical composition of claim 15, wherein the cellular immune response is induction of $CD8^+$ and $CD4^+$ memory T-cells.

25. The pharmaceutical composition of claim 15, wherein the cellular immune response is production of INFγ.

26. The pharmaceutical composition of claim 15, further comprising a vault complex containing a chemokine.

27. The pharmaceutical composition of claim 26, wherein the chemokine is CCL21.

28. A method of preventing or treating cancer in a subject, comprising administering to the subject an effective amount of a tumor antigen or an antigenic fragment or variant thereof incorporated within a vault complex, sufficient to stimulate a cellular immune response.

29. The method of claim 28, wherein tumor antigen is fused to INT.

30. The method of claim 4, wherein the INT comprises the amino acid sequence of SEQ ID NO: 2.

31. The method of claim 28, wherein the antigenic peptide is fused to MVP.

32. The method of claim 28, wherein the vault complex comprises MVP.

33. The method of claim 32, wherein the number of MVP is 1-78.

34. The method of claim 32, wherein the number of MVP is 78.

35. The method of claim 32, wherein the vault complex further comprises VPARP or modified VPARP, or a portion of VPARP, or a modified portion of VPARP.

36. The method of claim 28, wherein the cellular immune response is induction of $CD8^+$ and $CD4^+$ memory T-cells.

37. The method of claim 28, wherein the cellular immune response is production of INFγ.

38. The method of claim 28, further comprising administering to the subject a vault complex containing a chemokine.

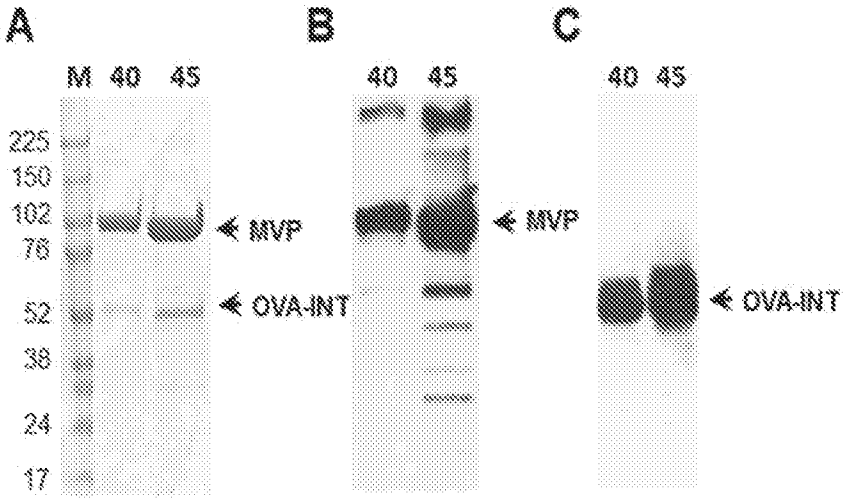
39. The method of claim 38, wherein the chemokine is CCL21.

40. The method of claims 28-39, wherein the administering reduces tumor volume.

41. The method of claims 28-39, wherein the administering reduces tumor growth.

42. A method of preparing a vault complex comprising a) mixing an INT or INT fusion protein generated in insect Sf9 cells with a MVP or MVP fusion protein generated in insect Sf9 cells to generate a mixture; b) incubating the mixture for a sufficient period of time to allow formation of vault complexes, thereby generating the vault complex of claims 1-41.

Figure 1


A
B
C
M
40
45
40
45
40
45
225
150
102
76
52
38
24
17
MVP
OVA-INT
MVP
OVA-INT

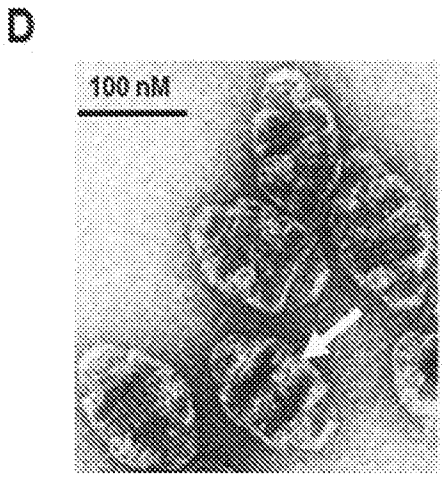

D
100 nM

Figure 2

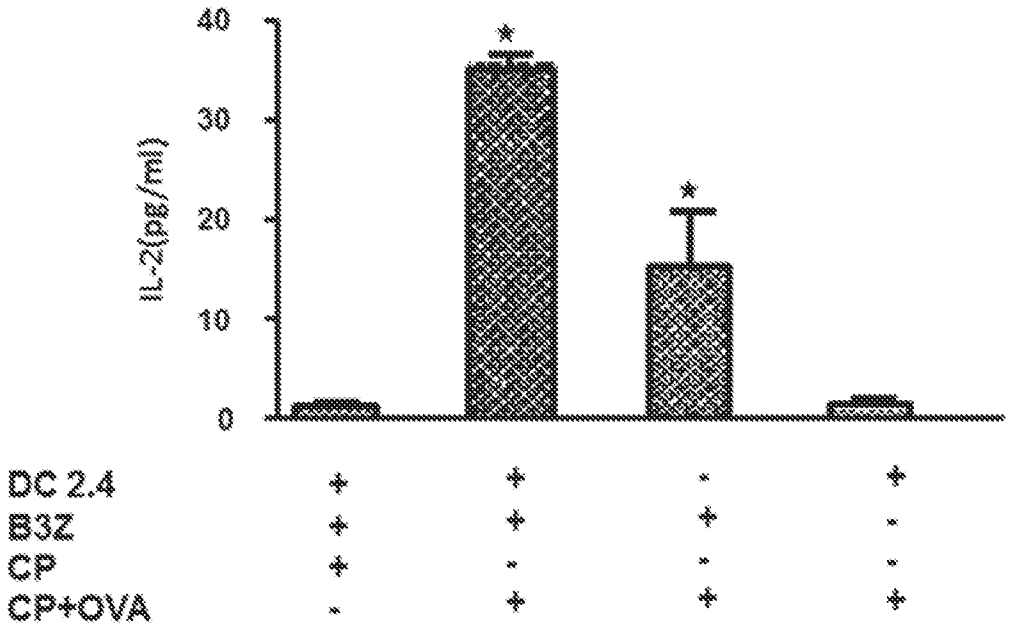
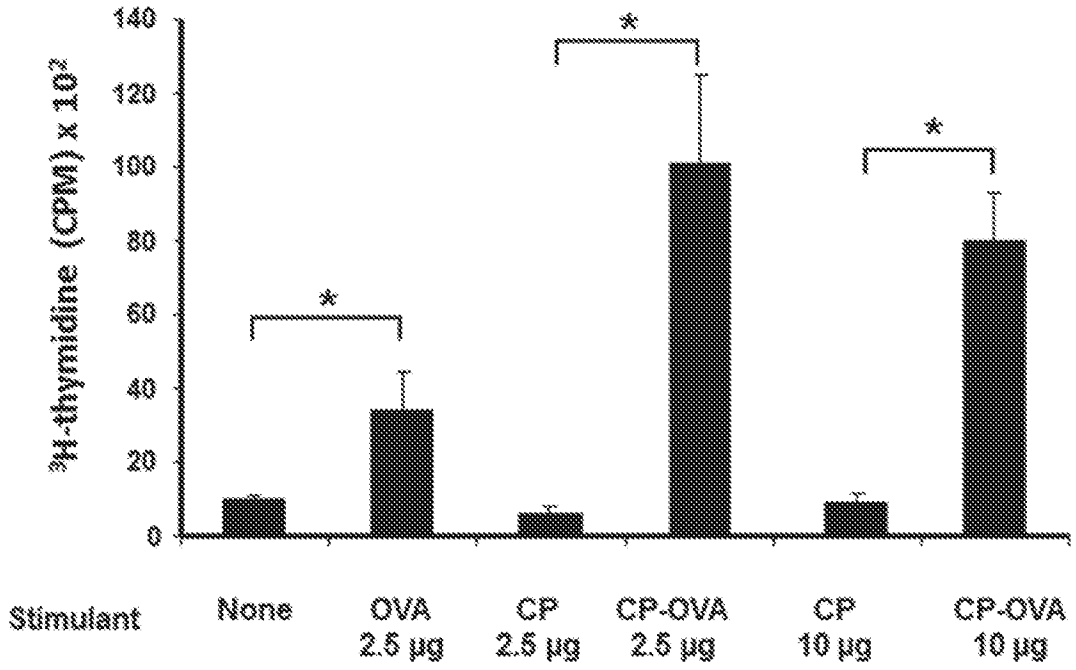


Figure 3


^{3}H-thymidine (CPM) x 10^2
140
120
100
80
60
40
20
0
*
*
*
Stimulant
None
OVA 2.5 µg
CP 2.5 µg
CP-OVA 2.5 µg
CP 10 µg
CP-OVA 10 µg

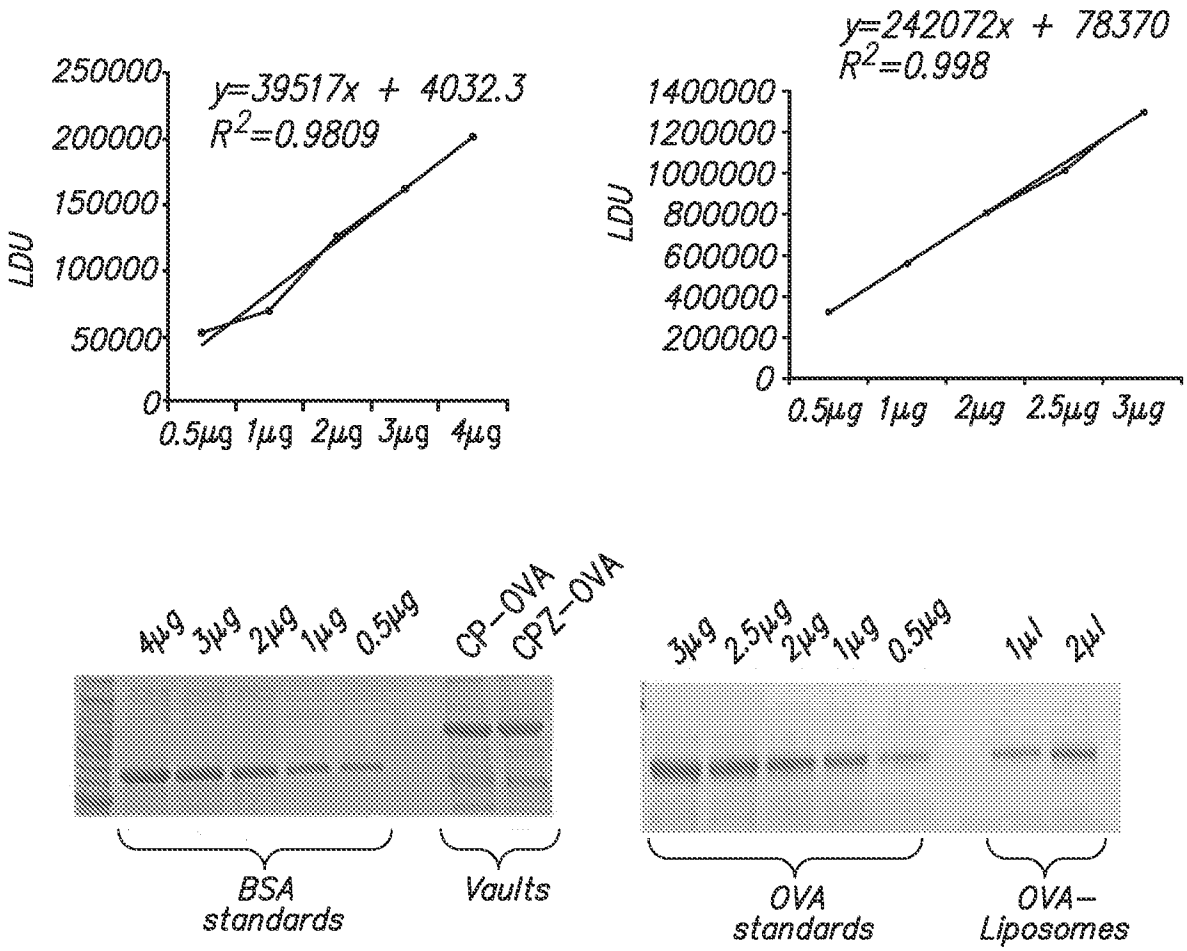
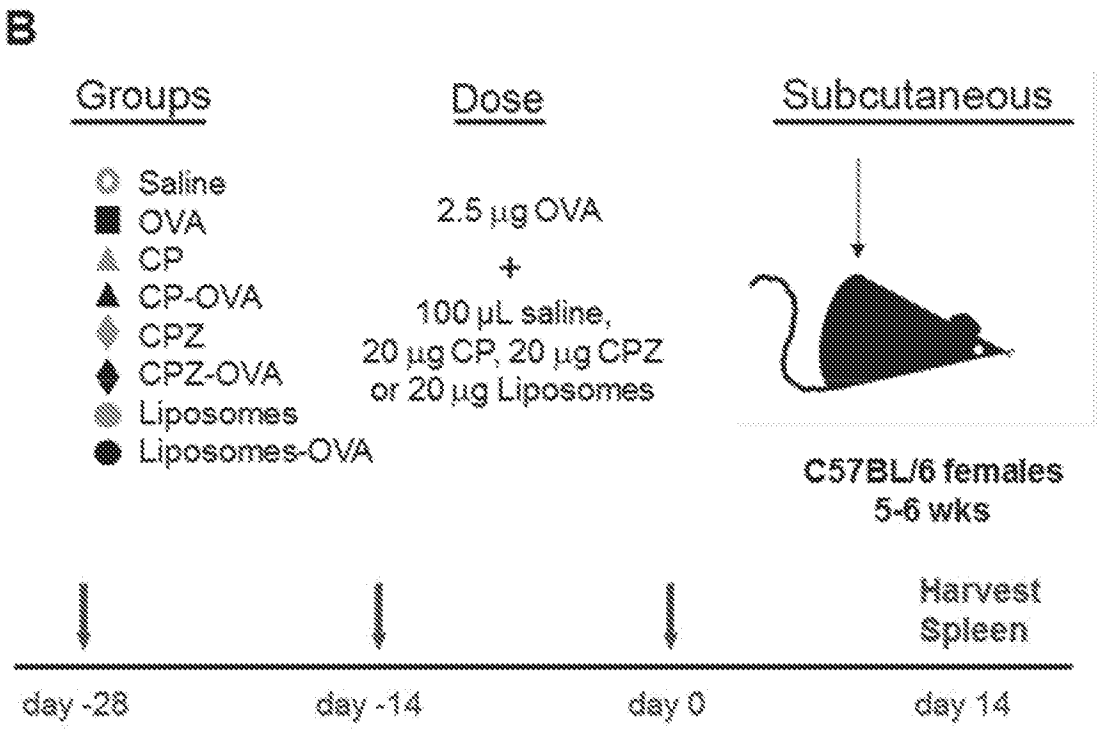

y=39517x + 4032.3
R2=0.9809
LDU
250000
200000
150000
100000
50000
0
0.5µg 1µg 2µg 3µg 4µg
y=242072x + 78370
R2=0.998
LDU
1400000
1200000
1000000
800000
600000
400000
200000
0
0.5µg 1µg 2µg 2.5µg 3µg
4µg 3µg 2µg 1µg 0.5µg
CP-OVA
CPZ-OVA
BSA standards
Vaults
3µg 2.5µg 2µg 1µg 0.5µg
1µl 2µl
OVA standards
OVA-Liposomes

FIG. 4A

Figure 4


B
Groups
Dose
Subcutaneous
Saline
OVA
CP
CP-OVA
CPZ
CPZ-OVA
Liposomes
Liposomes-OVA
2.5 µg OVA
+
100 µL saline,
20 µg CP, 20 µg CPZ
or 20 µg Liposomes
C57BL/6 females
5-6 wks
Harvest
Spleen
day -28
day -14
day 0
day 14

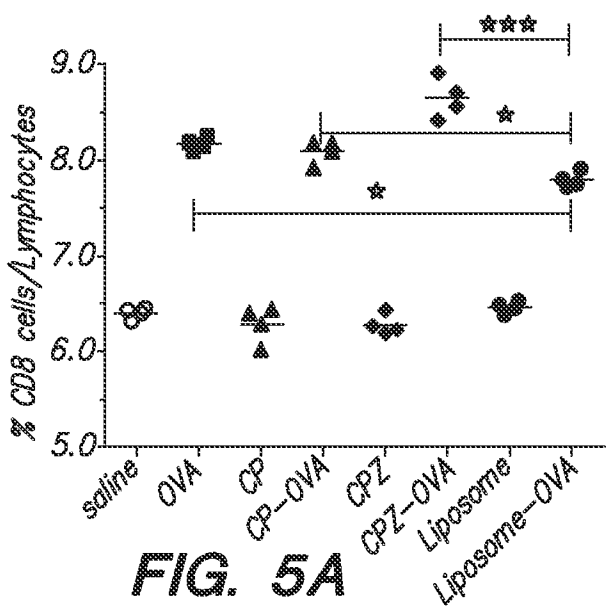


FIG. 5A

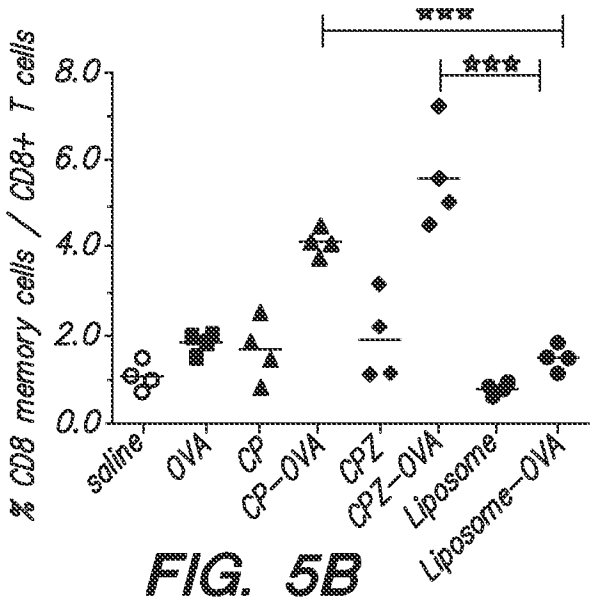


FIG. 5B

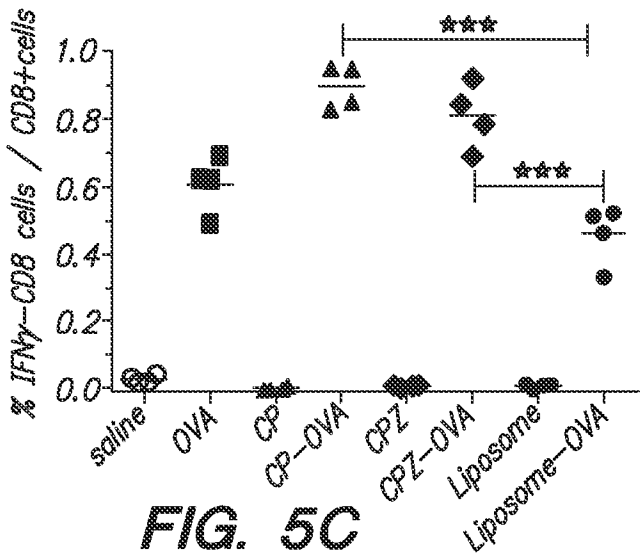


FIG. 5C

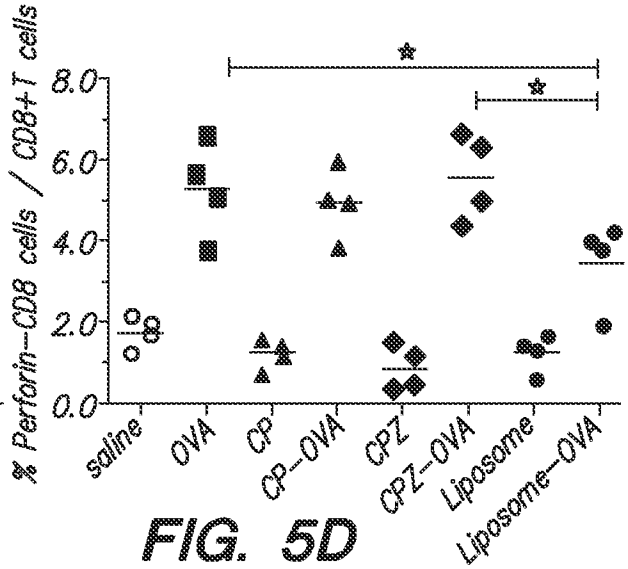


FIG. 5D

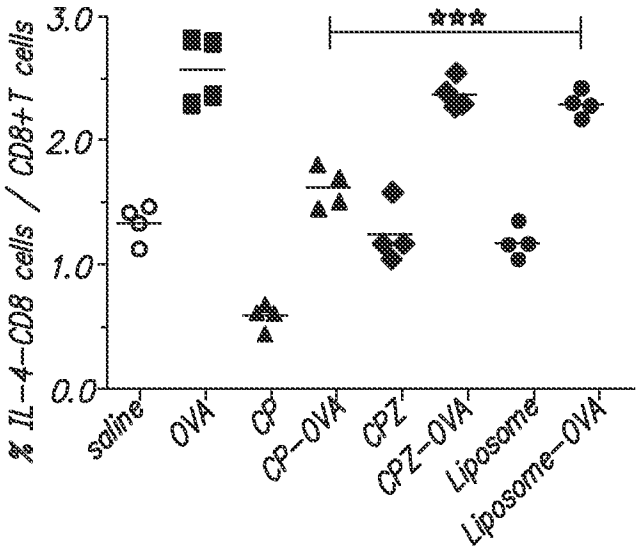


FIG. 5E

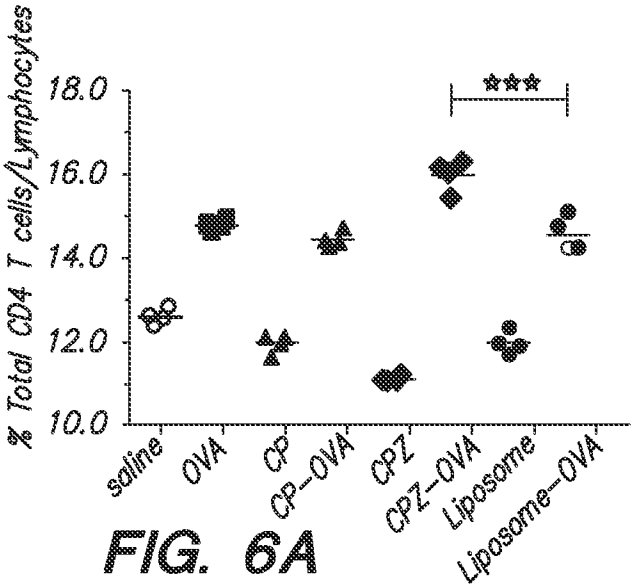


FIG. 6A

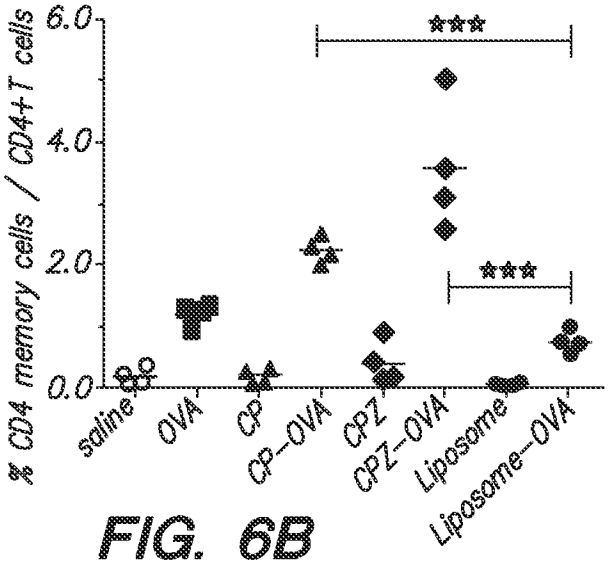


FIG. 6B

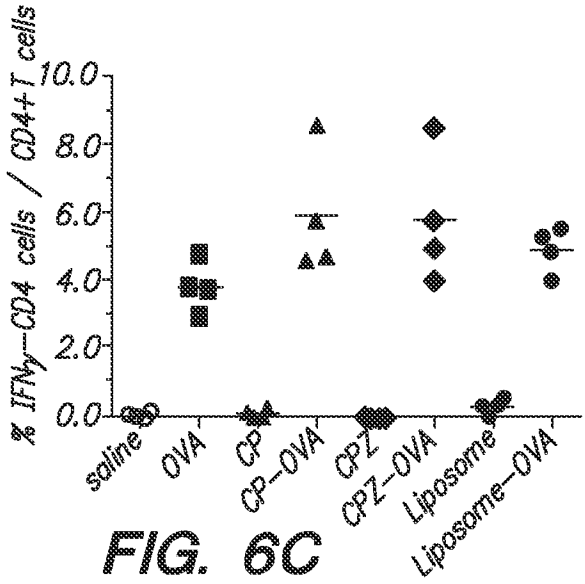


FIG. 6C

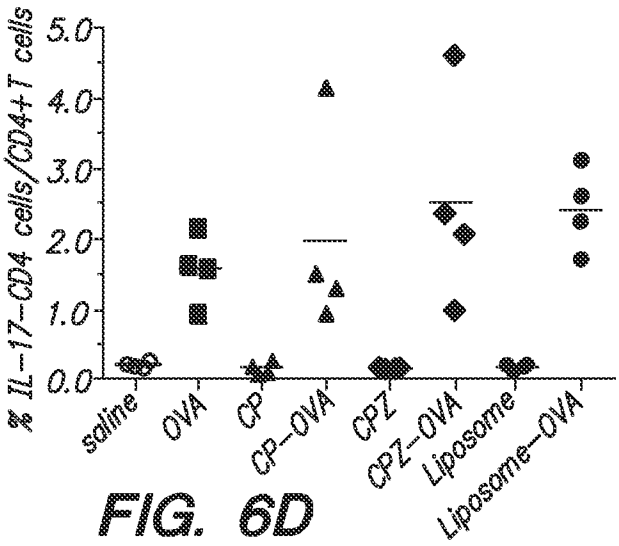


FIG. 6D

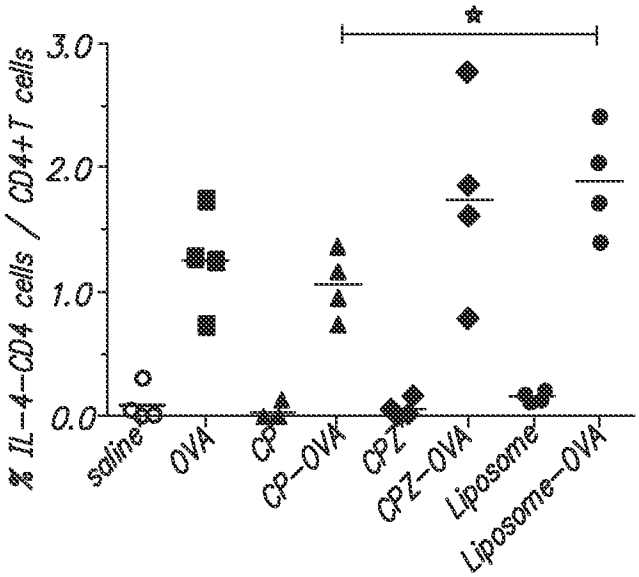


FIG. 6E

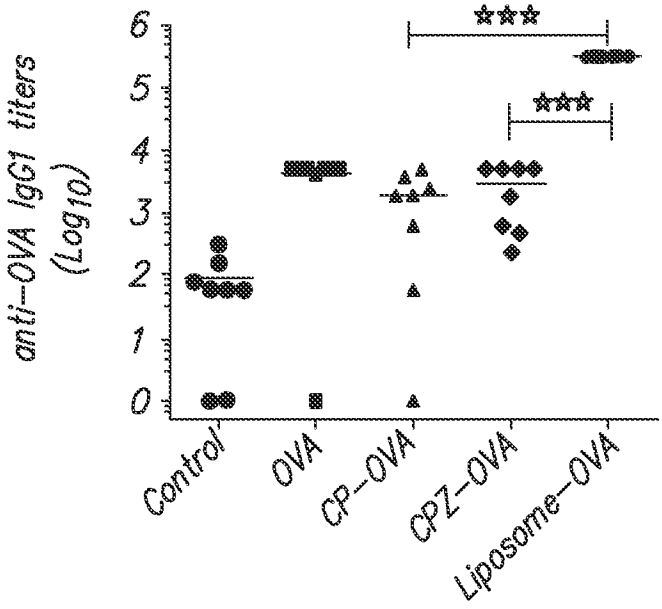
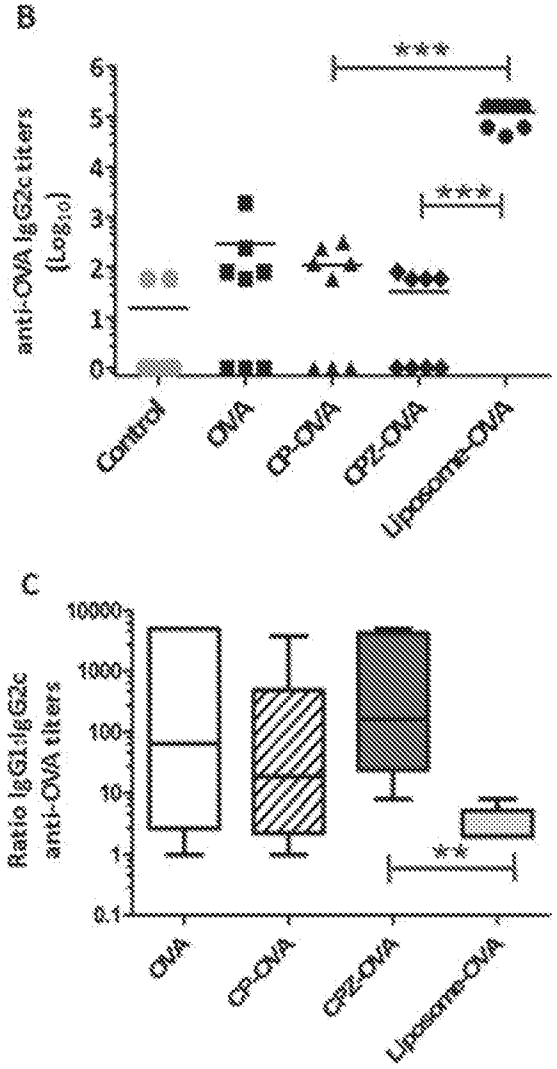

anti-OVA IgG1 titers
(Log10)
0
1
2
3
4
5
6
***
***
Control
OVA
CP-OVA
CPZ-OVA
Liposome-OVA

FIG. 7A

Figure 7


B
anti-OVA IgG2c titers
(Log10)
0
1
2
3
4
5
6
***
***
Control
OVA
CP-OVA
CPZ-OVA
Liposome-OVA
C
Ratio IgG1/IgG2c
anti-OVA titers
0.1
1
10
100
1000
10000
**
OVA
CP-OVA
CPZ-OVA
Liposome-OVA

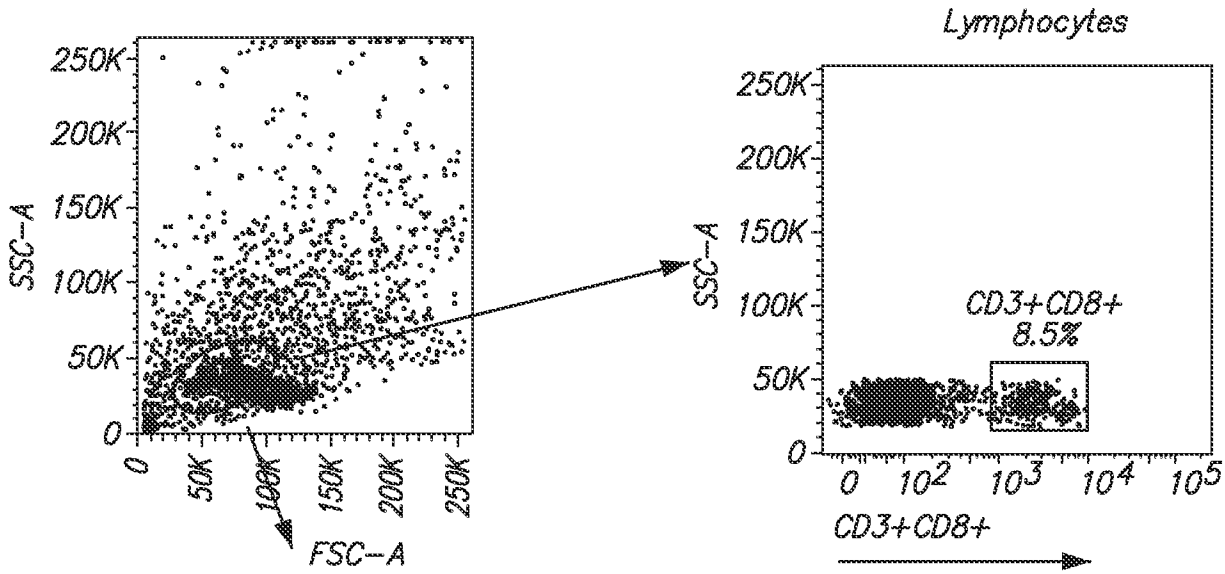

Lymphocytes
250K
200K
150K
100K
50K
0
SSC-A
FSC-A
CD3+CD8+
8.5%
10^2
10^3
10^4
10^5
CD3+CD8+

FIG. 8A

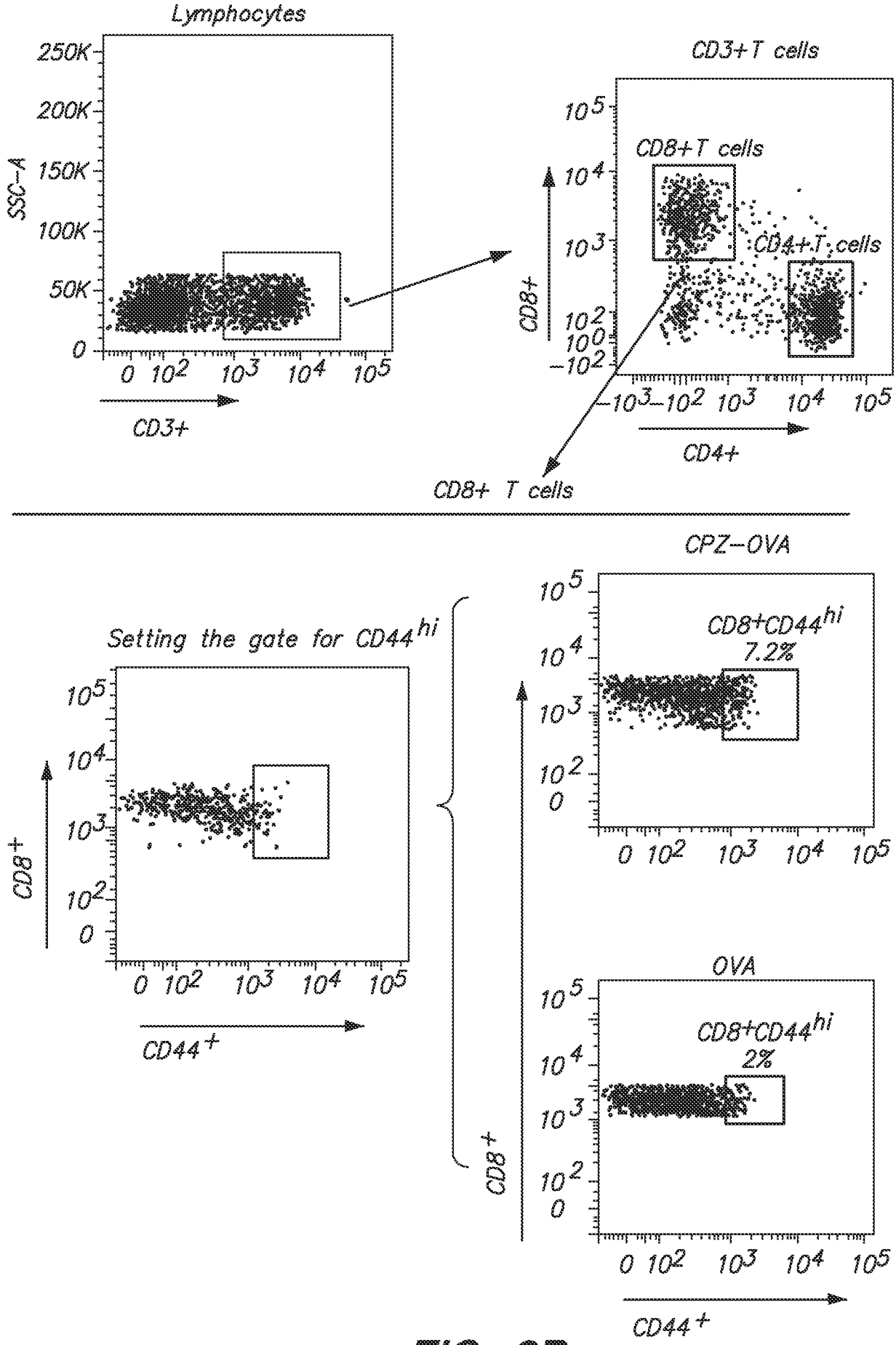
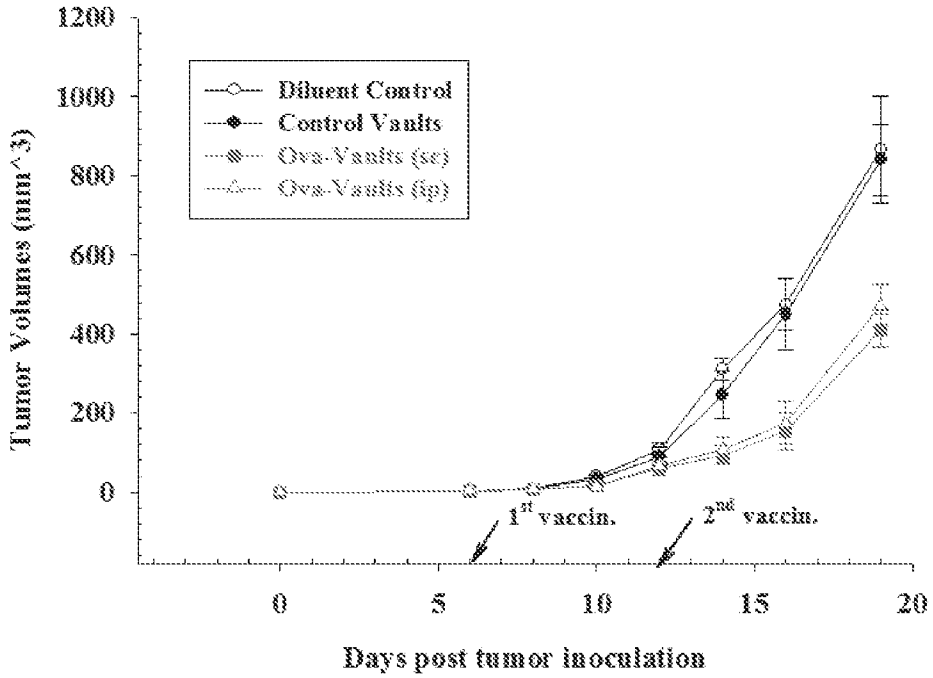

Lymphocytes
SSC-A
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5
CD3+
CD3+T cells
CD8+T cells
CD4+T cells
CD8+
10^5
10^4
10^3
10^2
10^0
-10^2
-10^3 -10^2 10^3 10^4 10^5
CD4+
CD8+ T cells
Setting the gate for CD44^hi
CD8+
CD44+
CPZ-OVA
CD8+CD44^hi
7.2%
OVA
CD8+CD44^hi
2%

FIG. 8B

Figure 9 IP or SC routes of Therapeutic Vaccination with OVA-vaults inhibits 3LL-OVA tumor growth

A



B

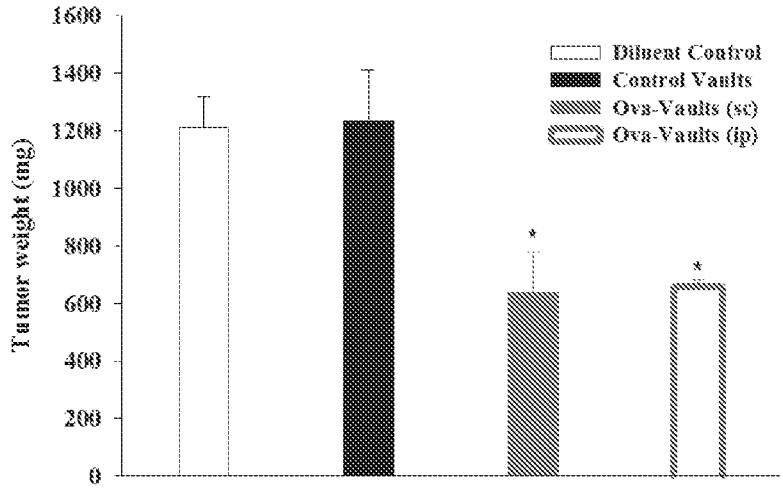
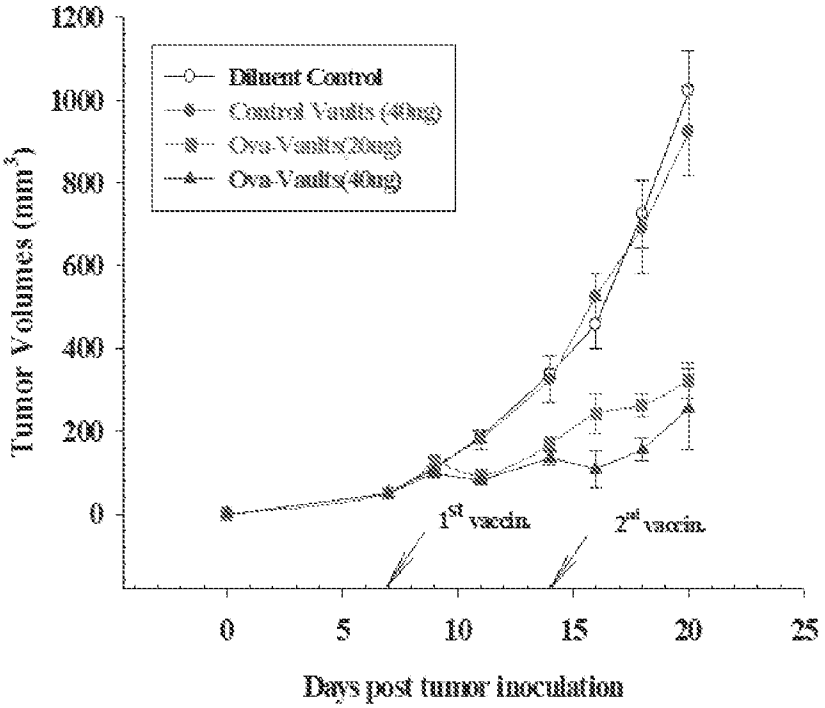
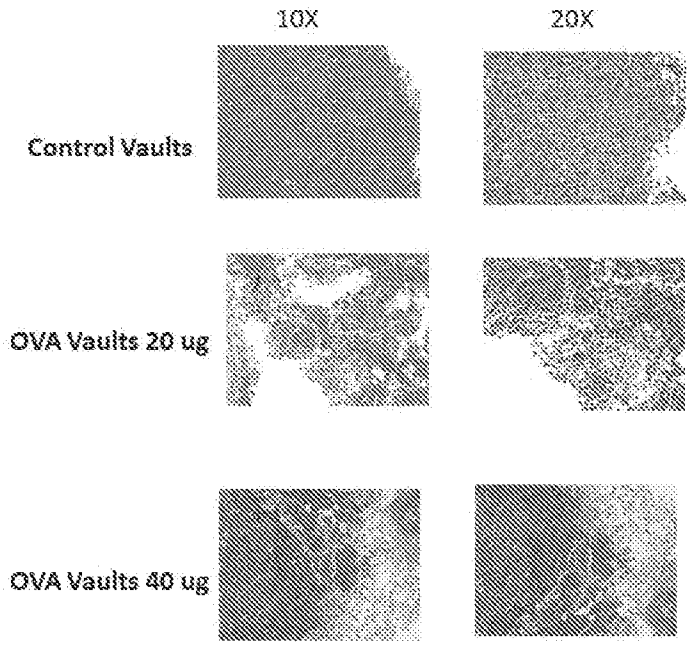


Figure 10 ***SC*** **therapeutic vaccination with OVA-vault on the contralateral flank inhibits 3LL-OVA tumor growth**

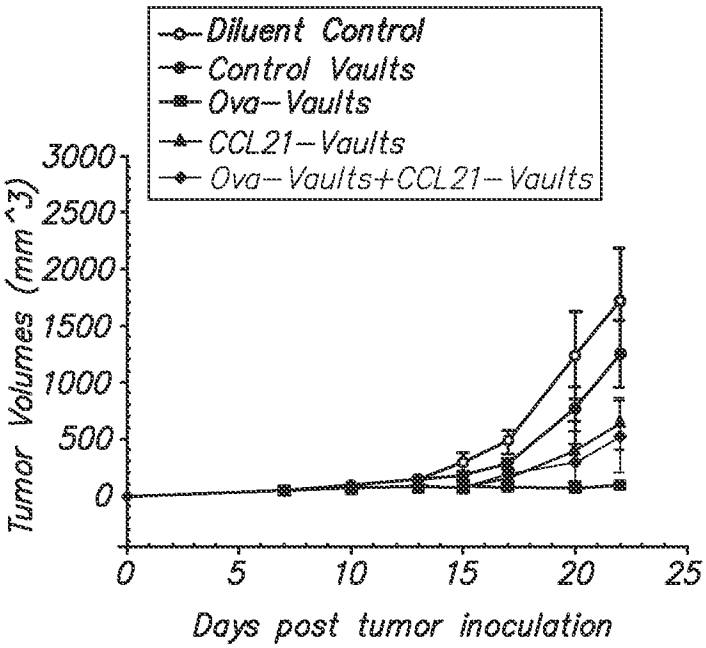
A



B



Therapeutic vaccination with OVA-vaults or CCL21 vaults or combination of both vaults leads to tumor rejection and systemic immune responses



Groups (n-6)	# of mice with complete rejection
Diluent Control	0
Control Vaults	0
Ova-Vaults	3
CCL21-Vaults	2
Ova-Vaults+ CCL21-Vaults	3

Treatment administered s.c on contralateral flank of the tumor on days 7, 10 and 14.

FIG. 11A

Figure 11

Table 2

B

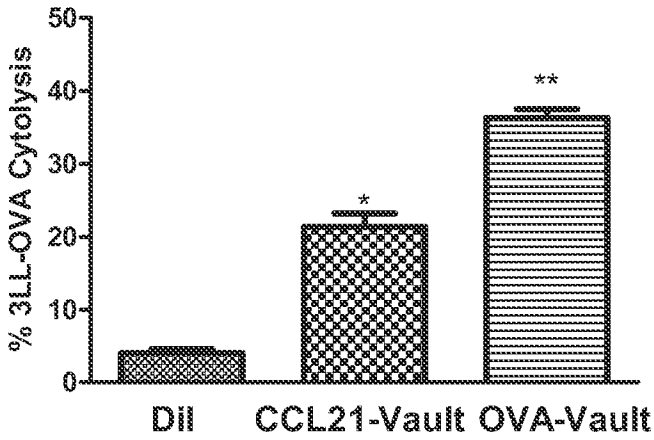
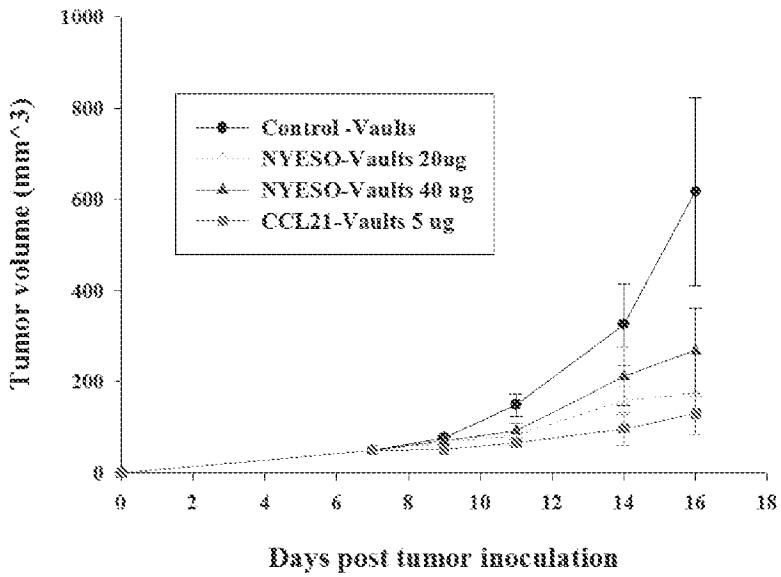


Figure 12 Therapeutic Vaccination with NYESO-vaults inhibits 3LL-NYESO tumor growth

A



(Day 7,11 & 14 :Vaccination by *s.c* injection on the contra-lateral flank of tumor)

B

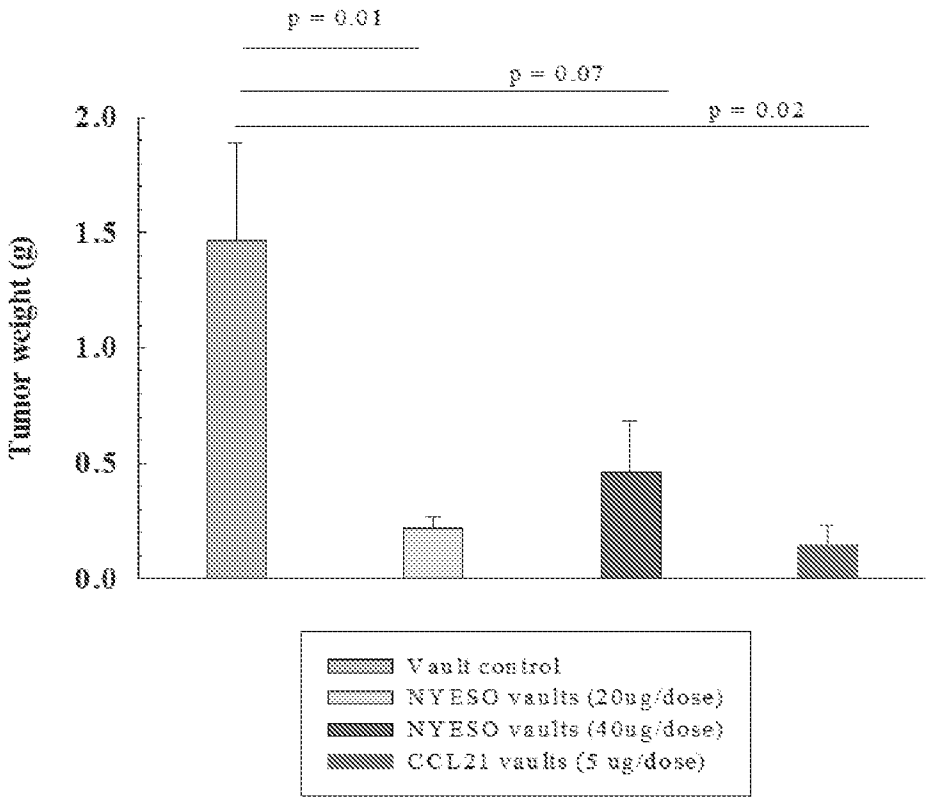


Figure 13

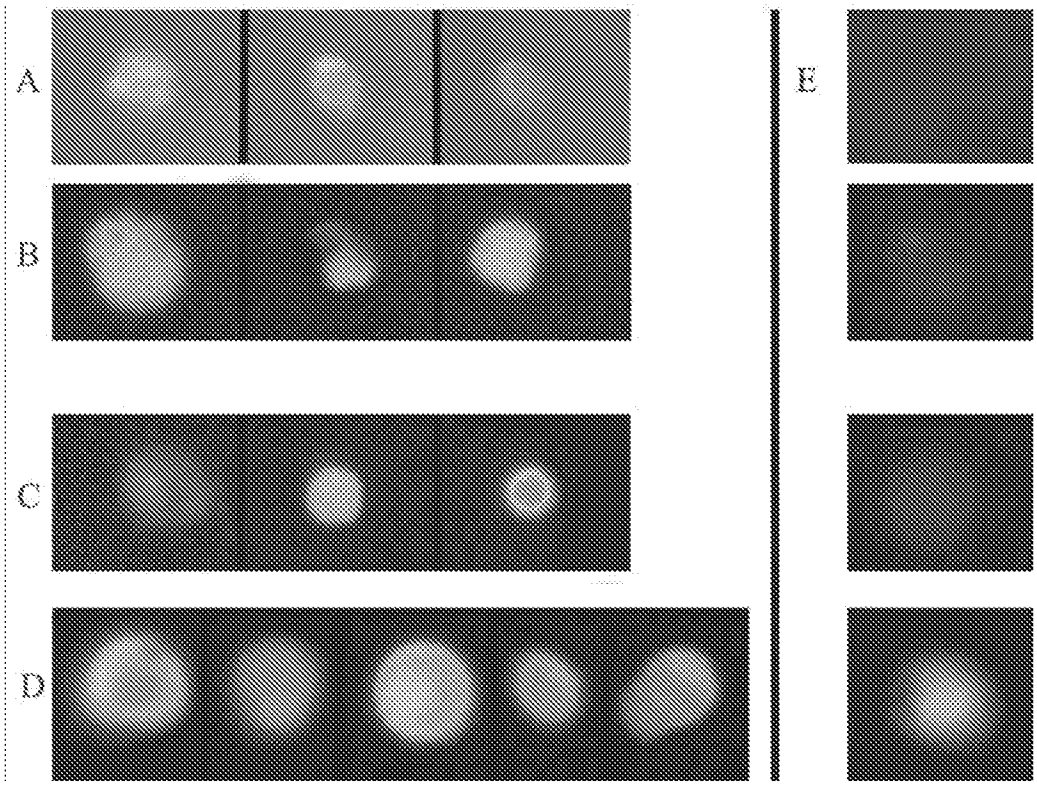


Figure 14

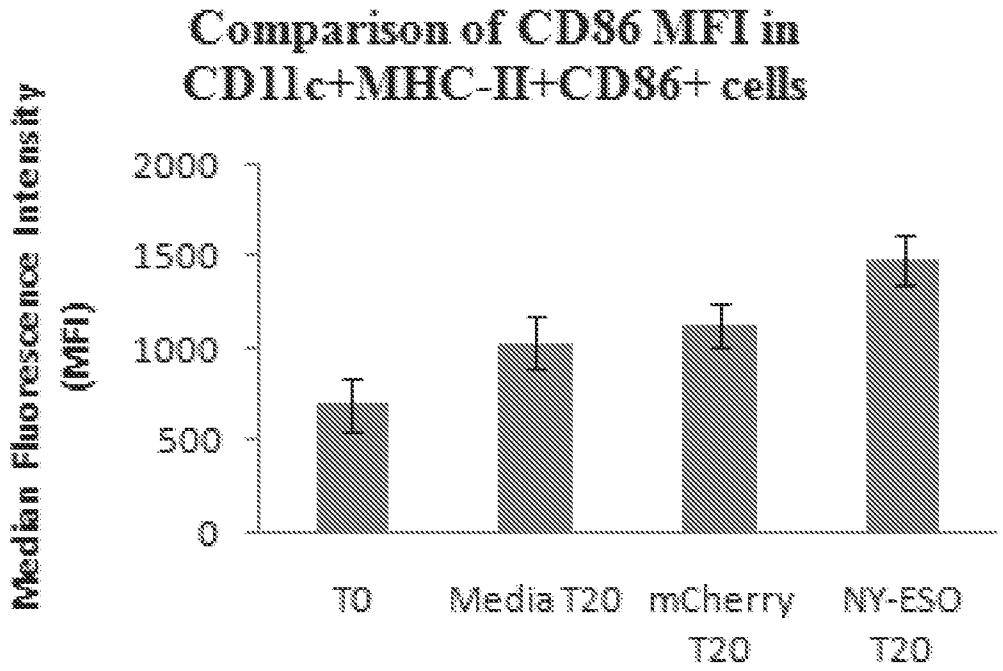
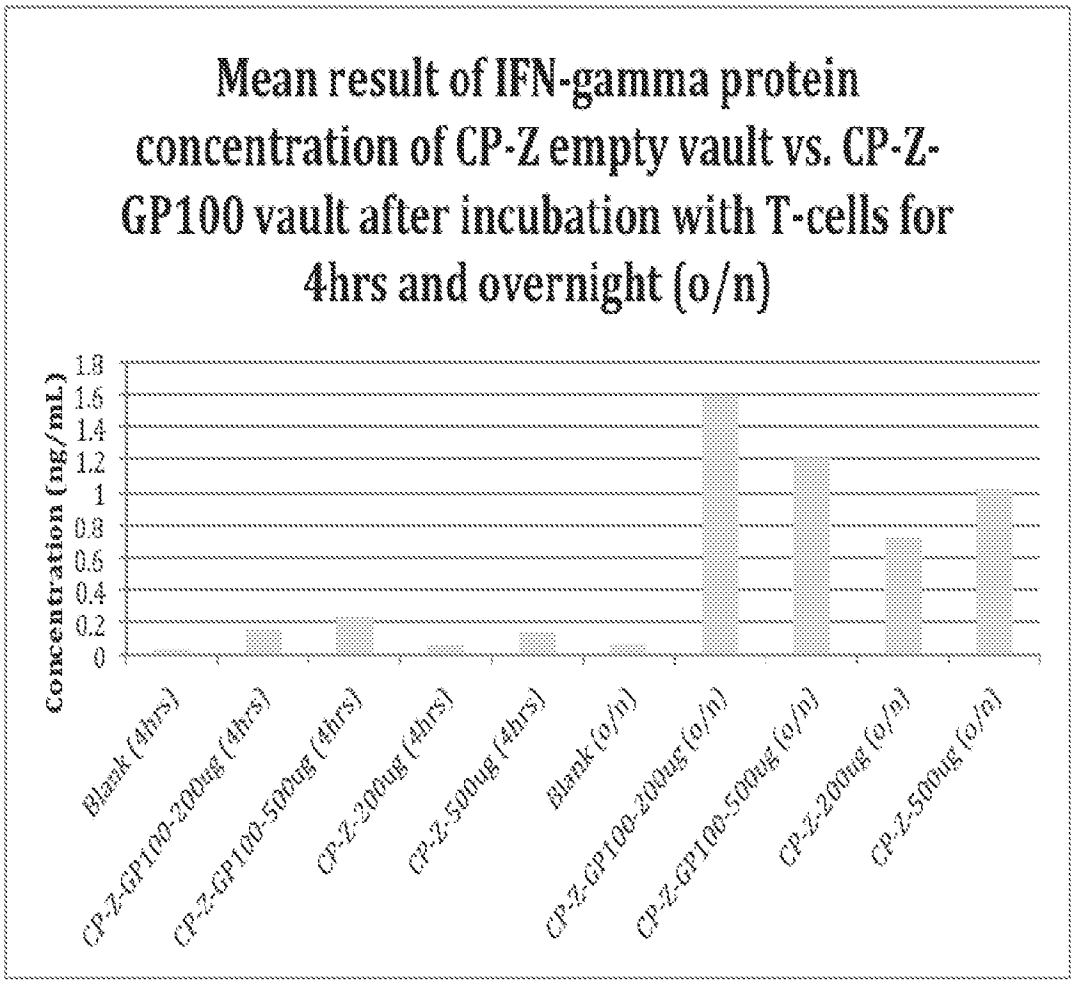

Comparison of CD86 MFI in CD11c+MHC-II+CD86+ cells
Median Fluorescence Intensity (MFI)
2000
1500
1000
500
0
T0
Media T20
mCherry T20
NY-ESO T20

Figure 15


Mean result of IFN-gamma protein concentration of CP-Z empty vault vs. CP-Z-GP100 vault after incubation with T-cells for 4hrs and overnight (o/n)
Concentration (ng/mL)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Blank (4hrs)
CP-Z-GP100-200ug (4hrs)
CP-Z-GP100-500ug (4hrs)
CP-Z-200ug (4hrs)
CP-Z-500ug (4hrs)
Blank (o/n)
CP-Z-GP100-200ug (o/n)
CP-Z-GP100-500ug (o/n)
CP-Z-200ug (o/n)
CP-Z-500ug (o/n)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/049816

A. CLASSIFICATION OF SUBJECT MATTER

A61K 38/17(2006.01)i, A61K 38/16(2006.01)i, A61K 47/48(2006.01)i, A61P 37/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 38/17; A61K 38/16; A61K 47/48; A61P 37/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: vault, tumor antigen, INT, MVP, VPARP

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHAMPION et al., `A vault nanoparticle vaccine induces protective mucosal immunity` PLoS One, Vol.4, No.4, Article No.e5409 (pages 1-12) (2009) See abstract; pages 1-6, 9-10.	16-27
A	KAR et al., `Novel CCL21-vault nanocapsule intratumoral delivery inhibits lung cancer growth` PloS One, Vol.6, No.5, Article No.e18758 (pages 1-8) (2011) See the whole document.	16-27
A	FARRIS et al., `CD4+ T cells and antibody are required for optimal major outer membrane protein vaccine-induced immunity to Chlamydia muridarum genital infection` Infection and Immunity, Vol.78, No.10, pp.4374-4383 (2010) See the whole document.	16-27
A	HAN et al., `Targeted vault nanoparticles engineered with an endosomolytic peptide deliver biomolecules to the cytoplasm` ACS Nano, Vol.5, No.8, pp.6128-6137 (2011) See the whole document.	16-27

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

☒ See patent family annex.

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

Date of the actual completion of the international search	Date of mailing of the international search report
15 October 2013 (15.10.2013)	**15 October 2013 (15.10.2013)**

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701, Republic of Korea
Facsimile No. +82-42-472-7140

Authorized officer
KIM Seung Beom
Telephone No. +82-42-481-3371

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/049816

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

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

1. ☒ Claims Nos.: 1-15, 28-41
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-15 and 28-41 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 42
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/049816

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CASANAS et al., `Vault particles: a new generation of delivery nanodevices` Current Opinion in Biotechnology, Vol.23, No.6, pp.972-977 (Epub 06 June 2012) See the whole document.	16-27
PX	KAR et al., `Vault nanocapsules as adjuvants favor cell-mediated over antibody-mediated immune responses following immunization of mice` PloS One, Vol.7, No.7, Article No.e38553 (pages 1-13) (11 July 2012) See abstract; pages 3-4, 6-11.	16-27

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2013/049816

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
None			