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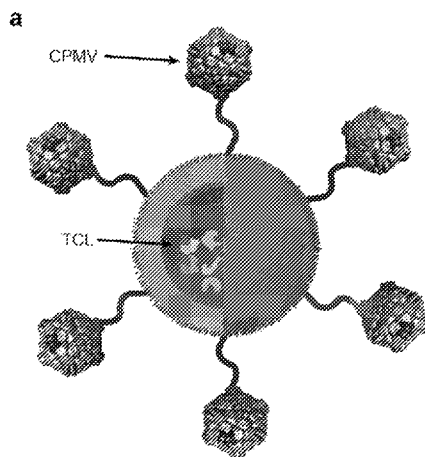
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(54) Title: ANTIGEN LOADED LIPOSOMES FOR COMBINATION WITH PLANT VIRUS NANOPARTICLE ADJUVANTS



(57) Abstract: Provided are compositions, methods and uses relating to conjugate comprising a liposome encapsulating one or cancer antigen(s) that is conjugated to a plant virus nanoparticle that is useful to treat cancer.



ANTIGEN LOADED LIPOSOMES FOR COMBINATION WITH PLANT VIRUS NANOPARTICLE ADJUVANTS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application No. 63/523751, filed on June 28, 2023, the entire contents of which are incorporated herein by reference.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with government support under Grant No. CA253615 awarded by the National Institutes of Health (NIH). The government has certain rights in the invention.

BACKGROUND

[0003] A serous ovarian carcinoma diagnosis carries a dismal prognosis. Due to the cancer's nonspecific clinical symptoms, the majority of patients are diagnosed with stage III or stage IV disease, among which the five-year survival rates are 42% and 26%, respectively⁷. Cancer immunotherapy has become the fourth pillar of cancer treatments since the approval of immune checkpoint blockade drugs targeting CLTA4, PD1 and PDL1¹⁻³. Unlike traditional cancer treatments, immunotherapy helps patients restore their natural immunity cycle and prevents recurrence by establishing antigen-specific anti-tumor immunity¹⁻³. However, this approach has yet to succeed with ovarian cancer^{4, 5} due to the complex immunosuppressive tumor microenvironment (TME)⁶. Advanced-stage ovarian cancer is one of the deadliest gynecological cancers in females^{7, 8}, with 19,710 new cases and 13,270 deaths predicted for 2023 in the USA⁹. Standard treatments involve surgical resection followed by chemotherapy to remove most of the malignant tissues, but after a short remission up to 70% of patients experience recurrence, which significantly reduces the survival rate and quality of life¹⁰. Following relapse, most patients succumb to their disease. Surgery also leads to postoperative trauma and the establishment of a new TME primed for recurrence and metastasis¹¹⁻¹³. Therefore, a personalized immunotherapy or cancer vaccine that reprograms the TME, restores the immunity cycle, and establishes tumor-specific anti-tumor immunity during remission would prevent recurrence and prolong survival. The

current standard of care includes surgical debulking, during which large quantities of tumor are removed from the peritoneal cavity. Accordingly, there is a significant need for therapies to treat ovarian and other cancers in general. This disclosure satisfies this need and provides related advantages as well.

SUMMARY OF THE DISCLOSURE

[0004] Ovarian cancer is a carcinoma that originates in the ovary or nearby structures. This is one of the most common malignancies in females and the majority of patients will experience at least one recurrence. Treatment options are currently limited to surgery followed by chemotherapy, with a severe effect on the quality of life. Here Applicant presents a personalized immunotherapy concept for post-surgical administration that aims to prevent or treat recurrent disease. Autologous tumor cell lysates (TCL) extracted from resected tumors are encapsulated in liposomes that are attached to cowpea mosaic virus (CPMV), a plant virus that acts as a potent adjuvant. The TCL-loaded liposomes are delivered to antigen-presenting cells, where the attached CPMV particles stimulate antigen processing and presentation. In a preclinical murine model of metastatic ovarian cancer, liposome-CPMV complexes carrying TCL from a murine cancer cell line elicited a targeted adaptive immune response in the intraperitoneal space and spleen, specifically involving tumor antigens. The resulting enhanced anti-tumor immunity resulted in the effective suppression of tumor initiation and growth. This disclosure provides a conjugate comprising a liposome that comprises, or consists essentially of, or yet further consists of one or more cancer antigen(s) conjugated to a plant virus nanoparticle. In one aspect the liposome comprising, consisting essentially of, or consisting of the one or more cancer antigen(s) is one or more of a micelle, a liposome or a lipid nanoparticle (LNP). In one aspect, the liposome is an LNP. As described herein, a cancer antigen includes a fragment thereof, such as for example, a fragment that induces or raises an immune response.

[0005] In one aspect, the plant virus nanoparticle is as described herein, e.g., one or more of a CPMV; a virus from the picornavirus family; a virus from the Comovirinae virus subfamily, such as CPMV, Broad bean wilt virus 1, and Tobacco ringspot virus; bean pod mottle virus (BPMV); rice tungro spherical virus; a Tobacco Mosaic Virus (TMV); and derivatives of

each thereof. In one aspect, the plant virus nanoparticle is a CMPV or a derivative thereof that is non-replicating and noninfectious toward mammals.

[0006] In one embodiment of the conjugates of this disclosure, the one or more cancer antigen(s) is selected from an antigen or neoantigen isolated from a cancer selected from a carcinoma, a sarcoma, or a hematological cancer. In a further aspect, the one or more cancer antigens is an antigen or neoantigen from a carcinoma, such as for example, an ovarian cancer cell. In a further aspect, the antigen or neoantigen is detectably labeled, examples of such are known in the art and described herein.

[0007] In a further aspect, a plurality of antigens or neoantigens are comprised in the conjugate, wherein the antigens or neoantigens target or induce an immune response against the same cancer cell type or different cancer cell types. Alternatively, at least two of the plurality of the antigens or neoantigens target or induce an immune response are different from each other but target the same cancer type. In a further aspect, the plurality of the antigens or neoantigens are identical to each other.

[0008] In another aspect, provided herein is a composition comprising, consisting essentially of, or consisting of the conjugate as provided herein, and at least one carrier, such as a pharmaceutically acceptable carrier or excipient. In another aspect, a plurality of the conjugates are provided herein, wherein the nanoparticles, antigens, and/or plant virus nanoparticles are the same or different from each other.

[0009] Also provided is a method to deliver a conjugate to a cell or tissue, such as a cancer cell comprising contacting the cell with a conjugate as described herein. In one aspect, the cell or tissue is a mammalian cell or tissue, such as a human cell or tissue. The contacting can be *in vitro* or *in vivo*.

[0010] Further provided is a method for one or more of in a subject in need thereof: delaying, slowing down, or preventing the relapse of a cancer; treating cancer; triggering or enhancing one or more of the following in the subject in need thereof: an anti-cancer immune response, an anti-cancer innate immune responses, a T cell-dependent anti-cancer immune response, T cell priming, a CD8⁺ T cell-dependent immune response, or an immunological memory, comprising, consisting essentially thereof, or consisting of administering an

effective amount of the conjugate, the plurality, or the composition as described herein to the subject. In one aspect, the one or more cancer antigen(s) are autologous to the subject. Alternatively, or in addition, the one or more cancer antigen(s) comprise ovarian cancer antigens and the subject in need thereof has been diagnosed with ovarian cancer. As used herein, the term “cancer antigen” can include in certain aspect, a cancer neoantigen or a fragment of the antigen or neoantigen.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIGURES 1A-1E. Conjugation of CPMV to TCL-loaded liposomes using click chemistry. (FIG. 1A) Structure of the TCL-Lip-CPMV complex. (FIG. 1B) The two-step conjugation process: CPMV is attached to the bifunctional linker (DBCO-PEG₄-NHS ester) via NHS chemistry to produce CPMV-DBCO, which is in turn reacted with azide groups on the DPPE-PEG2K-azide liposomes to produce TCL-Lip-CPMV. (FIG. 1C) NUPAGE of TCL-loaded lipids (TCL-Lip), native CPMV, CPMV-DBCO, the TCL-Lip + CPMV mixture, and the TCL-Lip-CPMV complex. The complex features two additional bands (L-PEG2K-DPPE and S-PEG2K-DPPE). (FIG. 1D) SEC analysis of TCL-Lip, CPMV, TCL-Lip + CPMV, and TCL-Lip-CPMV. The complex eluates as a single peak at the same column volume as the liposomes, whereas the TCL-Lip + CPMV mixture elutes as two peaks corresponding to the separate components. (FIG. 1E) Cryo-EM image of TCL-Lip-CPMV (blue arrows) showing CPMV closely associated with liposomes.

[0012] FIGURES 2A-2D. Treatment efficacy of CPMV-conjugated TCL-loaded liposomes. (FIG. 2A) Treatment and tumor injection schedule. The groups are HEPES, TCL-Lip, CPMV, TCL-Lip + CPMV, and TCL-Lip-CPMV (n = 8 per group). Each treatment consisted of 100 µg CPMV and/or 30 µg TCL-loaded liposomes. (FIG. 2B) Average body circumferences for all treatment groups. Data points are cut off when n < 5 for the treatment group. (FIG. 2C) Individual circumferences in each group. The number of tumor-free mice 100 days post-inoculation is shown in the top right corner. (FIG. 2D) Survival rates for all treatment groups. Statistical significance in (FIG. 2B) was calculated by two-way ANOVA (**p < 0.01, ****p < 0.0001). Statistical significance in (FIG. 2D) was calculated using the log-rank (Mantel-Cox) test (*p < 0.05, ****p < 0.0001). Treatments in FIG. 2B and 2D

indicated with arrows. Where there is substantial overlap, treatments are indicated together and separated by a semi-colon.

[0013] FIGURES 3A-3G. Co-delivery of labeled TCL-Lip-CPMV *in vitro* and *in vivo*.

(**FIG. 3A**) Analysis of OG488-TCL-Lip, CPMV-Cy5, OG488-TCL-Lip + CPMV-Cy5, and OG488-TCL-Lip-CPMV-Cy5 by agarose gel electrophoresis. (**FIG. 3B**) Analysis of the same constructs by NUPAGE. (**FIG. 3C**) Confocal images of BMDCs 24 h post-incubation with OG488-TCL-Lip, CPMV-Cy5, OG488-TCL-Lip + CPMV-Cy5, and OG488-TCL-Lip-CPMV-Cy5, showing the co-delivery of OG488-TCL-Lip and CPMV-Cy5. (**FIGS. 3D and 3E**) Flow cytometry data for double-negative BMDCs (**FIG. 3D**) and double-positive BMDCs (**FIG. 3E**) 24 h post-incubation with OG488-TCL-Lip, CPMV-Cy5, OG488-TCL-Lip + CPMV-Cy5, and OG488-TCL-Lip-CPMV-Cy5. (**FIGS. 3F and 3G**) Fluorescence intensity of OG488 and Cy5 in draining popliteal lymph nodes over time. Statistical significance was determined by ordinary one-way ANOVA (**** $p < 0.0001$). In **FIGS. 3F and 3G**, treatments are indicated with arrows.

[0014] FIGURES 4A-4G. Quantification of co-delivered TCL and CPMV in popliteal draining lymph nodes and activation of popliteal lymph nodes.

(**FIG. 4A**) Confocal images of harvested lymph node sections 4 h after footpad injections of TCL-Lip + CPMV and TCL-Lip-CPMV. DCs are stained with a PE-conjugated anti-CD11c antibody. Flow cytometry analysis of TCL⁺/CPMV⁺ (**FIG. 4B**), TCL⁺ (**FIG. 4C**), CPMV⁺ (**FIG. 4D**) CD11c⁺ DCs in harvested lymph nodes based on OG488 and Cy5 fluorescence. (**FIGS. 4E-4G**) Activation of DCs in popliteal draining lymph nodes 24 h after footpad injections. The activation status of CD11c⁺ MHC-II⁺ DCs in lymph nodes was determined by flow cytometry using expression levels of CD40 (**FIG. 4E**), CD80 (**FIG. 4F**), and CD86 (**FIG. 4G**) surface markers. Statistical significance was determined by ordinary one-way ANOVA (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). From left to right in each of **FIGS. 4B-4D**, at every timepoint, treatments are HEPES, TCL-Lip, CPMV, TCL-Lip + CPMV, TCL-Lip-CPMV.

[0015] FIGURES 5A-5J. CD4⁺ and CD8⁺ T cells within the i.p. space after treatment.

(**FIG. 5A**) Percentage of CD3⁺CD4⁺ T cells among CD45⁺ immune cells. (**FIG. 5B**) Percentage of CD44⁺ memory CD4⁺ T cells among CD45⁺ immune cells. (**FIG. 5C**)

Percentage of CD44⁺CD62L⁻ effector memory CD4⁺ T cells among CD45⁺ immune cells. (FIG. 5D) Percentage of CD44⁺CD62L⁺ central memory CD4⁺ T cells among CD45⁺ immune cells. (FIG. 5E) Percentage of CD69⁺ activated CD4⁺ T cells among CD45⁺ immune cells. (FIG. 5F) Percentage of CD3⁺CD8⁺ T cells among CD45⁺ immune cells. (FIG. 5G) Percentage of CD44⁺ memory CD8⁺ T cells among CD45⁺ immune cells. (FIG. 5H) Percentage of CD44⁺CD62L⁻ effector memory CD8⁺ T cells among CD45⁺ immune cells. (FIG. 5I) Percentage of CD44⁺CD62L⁺ central memory CD8⁺ T cells among CD45⁺ immune cells. (FIG. 5J) Percentage of CD69⁺ activated CD8⁺ T cells among CD45⁺ immune cells. Statistical significance was determined by ordinary one-way ANOVA (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

[0016] FIGURES 6A-6D. MHC-I specific CD8⁺ T cells. (FIG. 6A) The OVA-Lip-CPMV formulation. Figure was generated using Biorender and Adobe Illustrator. (FIG. 6B-6C) SIINFEKL⁺ CD8⁺ T cells within the i.p. space (FIG. 6B) and spleens (FIG. 6C) were quantified by flow cytometry using a PE-conjugated MHC-I SIINFEKL dextramer. (FIG. 6D) Representative flow cytometry plots of SIINFEKL⁺ CD8⁺ T cells within the i.p. space of different treatment groups. Statistical significance was determined by ordinary one-way ANOVA (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

[0017] FIGURES 7A-7F. Characterization of TCL and TCL-Lip. (FIG. 7A) NuPAGE of TCL isolated from ID8-Defb29/Vegf-a-Luc murine ovarian cancer cells. (FIG. 7B) DLS of 100 nm TCL-Lip. (FIG. 7C) NUPAGE analysis of flow-through fractions (FT1-5) from five cycles of TFF and purified TCL-Lip. (FIG. 7D) UV-Vis of FT fractions from TFF. (FIG. 7E) Quantification of the amount of loaded TCL per mg liposomes. (FIG. 7F) Cryo-EM imaging of TCL-Lip.

[0018] FIGURES 8A-8C. Characterization of CPMV and CPMV-DBCO. (FIG. 8A) UV-Vis of CPMV and CPMV-DBCO. (FIG. 8B) DLS of CPMV and CPMV-DBCO. (FIG. 8C) TEM of CPMV and CPMV-DBCO.

[0019] FIGURE 9. Cryo-EM of TCL-Lip + CPMV mixture. TCL-loaded liposomes (TCL-Lip) are indicated by red arrows and CPMV particles are indicated by black arrows.

[0020] FIGURE 10. Individual body weights of all treated mice.

[0021] FIGURES 11A-11C. Survival rates and individual circumferences of mice treaded with oxidized and irradiated TCL. (FIG. 11A) Survival rates for HEPES, OxTCL-Lip, CPMV, OxTCL-Lip + CPMV, and OxTCL-Lip-CPMV groups. **(FIG. 11B)** Survival rates for HEPES, IrTCL-Lip, CPMV, IrTCL-Lip + CPMV, and IrTCL-Lip-CPMV groups. **(FIG. 11C)** Individual circumferences for mice treated with OxTCL-Lip, OxTCL-Lip + CPMV, OxTCL-Lip-CPMV, IrTCL-Lip, IrTCL-Lip + CPMV, and IrTCL-Lip-CPMV. In **FIGS. 11A** and **11B** individual treatments are indicated with arrows.

[0022] FIGURES 12A-12F. Characterization of labeled formulations. (FIG. 12A) NuPAGE analysis of TCL and OG488-labelled TCL. Agarose gel electrophoresis **(FIG. 12B)** and NUPAGE **(FIG. 12C)** analysis of CPMV, CPMV-Cy5, and CPMV-DBCO-Cy5. **(FIG. 12D)** UV-Vis analysis of CPMV, CPMV-Cy5, and CPMV-DBCO-Cy5. **(FIG. 12E)** DLS analysis of CPMV, CPMV-Cy5, and CPMV-DBCO-Cy5. **(FIG. 12F)** TEM images of CPMV, CPMV-Cy5, and CPMV-DBCO-Cy5.

[0023] FIGURES 13A-13D. Co-delivery data. (FIG. 13A) Confocal micrograph images of BMDCs after incubation for 1 h with OG488-TCL-Lip, CPMV-Cy5, OG488-TCL-Lip + CPMV-Cy5, and OG488-TCL-Lip-CPMV-Cy5, showing the co-delivery of OG488-TCL-Lip and CPMV-Cy5. Flow cytometry analysis of double-negative BMDCs **(FIG. 13B)** and double-positive BMDCs **(FIG. 13C)** after incubation for 4 h with OG488-TCL-Lip, CPMV-Cy5, OG488-TCL-Lip + CPMV-Cy5, and OG488-TCL-Lip-CPMV-Cy5. **(FIG. 13D)** Flow cytometry analysis of CPMV-Cy5 positive BMDCs after incubation for 24 h with OG488-TCL-Lip, CPMV-Cy5, OG488-TCL-Lip + CPMV-Cy5, and OG488-TCL-Lip-CPMV-Cy5. Statistical significance was determined by ordinary one-way ANOVA (**** $p < 0.0001$).

[0024] FIGURES 14A-14C. Cy5 fluorescence imaging. (FIG. 14A) Representative IVIS images of footpads following the injection of labeled TCL-Lip + CPMV and TCL-Lip-CPMV. **(FIG. 14B)** Quantification of Cy5 fluorescence intensity (1 mg/mL of CPMV-Cy5 and CPMV-DBCO-Cy5). **(FIG. 14C)** IVIS images of harvested popliteal draining lymph nodes following footpad injections of OG488-TCL-Lip, CPMV-Cy5, OG488-TCL-Lip + CPMV-Cy5, and OG488-TCL-Lip-CPMV-Cy5. OG488-TCL was imaged using the GFP filter and CPMV-Cy5 was imaged using the Cy5.5 filter.

[0025] FIGURES 15A-15C. Flow cytometry analysis of F4/80⁺ macrophages within harvested lymph nodes. (FIG. 15A) double-positive for OG488-TCL and CPMV-Cy5, (FIG. 15B) positive for OG488-TCL, and (FIG. 15C) positive for CPMV-Cy5. Statistical significance was determined by ordinary one-way ANOVA (p < 0.01, ****p < 0.0001). From left to right in each of FIGS. 15A-15C, at every timepoint, treatments are HEPES, TCL-Lip, CPMV, TCL-Lip + CPMV, TCL-Lip-CPMV.**

[0026] FIGURES 16A-16C. Activation status of dendritic cells (DCs) within popliteal draining lymph nodes 4 h after footpad injections. The activation status of CD11c⁺ MHC-II⁺ DCs was measured by flow cytometry based on the expression levels of (FIG. 16A) CD40, (FIG. 16B) CD80, and (FIG. 16C) CD86 surface markers. Statistical significance was determined by ordinary one-way ANOVA (*p < 0.05).

[0027] FIGURES 17A-17B. Analysis of CD4⁺ and CD8⁺ T cells. (FIG. 17A) Schedules for treatments, tumor inoculations, i.p. wash and spleen harvest. (FIG. 17B) Gating strategies for CD4⁺ and CD8⁺ T cells and their subsets.

[0028] FIGURES 18A-18J. CD4⁺ and CD8⁺ T cells in spleens after treatment. (FIG. 18A) Percentage of CD3⁺CD4⁺ T cells among CD45⁺ immune cells. (FIG. 18B) Percentage of CD44⁺ memory CD4⁺ T cells among CD45⁺ immune cells. (FIG. 18C) Percentage of CD44⁺CD62L⁻ effector memory CD4⁺ T cells among CD45⁺ immune cells. (FIG. 18D) Percentage of CD44⁺CD62L⁺ central memory CD4⁺ T cells among CD45⁺ immune cells. (FIG. 18E) Percentage of CD69⁺ activated CD4⁺ T cells among CD45⁺ immune cells. (FIG. 18F) Percentage of CD3⁺CD8⁺ T cells among CD45⁺ immune cells. (FIG. 18G) Percentage of CD44⁺ memory CD8⁺ T cells among CD45⁺ immune cells. (FIG. 18H) Percentage of CD44⁺CD62L⁻ effector memory CD8⁺ T cells among CD45⁺ immune cells. (FIG. 18I) Percentage of CD44⁺CD62L⁺ central memory CD8⁺ T cells among CD45⁺ immune cells. (FIG. 18J) Percentage of CD69⁺ activated CD8⁺ T cells among CD45⁺ immune cells. Statistical significance was determined by ordinary one-way ANOVA.

[0029] FIGURES 19A-19B. Characterization of formulations containing ovalbumin (OVA). (FIG. 19A) DLS analysis of 100-nm OVA-Lip. (FIG. 19B) NuPAGE analysis of OVA-Lip-CPMV to confirm the conjugation of CPMV to OVA-Lip.

[0030] FIGURES 20A-20B. Analysis of antigen-specific immune response. (FIG. 20A) Schedule for OVA-Lip, CPMV, OVA-Lip + CPMV, and OVA-Lip-CPMV administration, i.p. wash and spleen collection. **(FIG. 20B)** Representative flow cytometry plots of SIINFEKL⁺ CD8⁺ T cells in the spleens of different treatment groups.

DETAILED DESCRIPTION

Definitions

[0031] As it would be understood, the section or subsection headings as used herein is for organizational purposes only and are not to be construed as limiting or separating or both limiting and separating the subject matter described.

[0032] Throughout this disclosure, various publications, patents and published patent specifications are referenced by an identifying citation. The disclosures of these publications, patents and published patent specifications are hereby incorporated by reference into the present disclosure in their entireties to more fully describe the state of the art to which this invention pertains.

[0033] The practice of the present technology will employ, unless otherwise indicated, conventional techniques of organic chemistry, pharmacology, immunology, molecular biology, microbiology, cell biology and recombinant DNA, which are within the skill of the art. See, *e.g.*, Sambrook, Fritsch and Maniatis, *Molecular Cloning: A Laboratory Manual*, 2nd edition (1989); *Current Protocols In Molecular Biology* (F. M. Ausubel, et al. eds., (1987)); the series *Methods in Enzymology* (Academic Press, Inc.): *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) *Antibodies, a Laboratory Manual*, and *Animal Cell Culture* (R.I. Freshney, ed. (1987)).

[0034] As used in the specification and claims, the singular form “a,” “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a cell” includes a plurality of cells, including mixtures thereof.

[0035] As used herein, the term “comprising” is intended to mean that the compounds, compositions, and methods include the recited elements, but not exclude others. “Consisting essentially of” when used to define compounds, compositions, and methods, shall mean

excluding other elements of any essential significance to the combination. In one aspect, a composition consisting essentially of contains the active agent as the sole element of any essential significance to the combination. In another aspect, a composition consisting essentially of an active agent contains the active agent as the element of essential significance to the combination and can also contain additional agents such as an adjuvant. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants, e.g., from the isolation and purification method and pharmaceutically acceptable carriers, preservatives, flavoring agents, stabilizers, and the like. “Consisting of” shall mean excluding more than trace elements of other ingredients. Embodiments defined by each of these transition terms are within the scope of this technology.

[0036] “Optional” or “optionally” means that the subsequently described circumstance may or may not occur, so that the description includes instances where the circumstance occurs and instances where it does not.

[0037] As used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

[0038] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 1, 5, or 10%. It is to be understood, although not always explicitly stated that all numerical designations are preceded by the term “about.” It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[0039] As used herein, the term “about” is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value. The term “about” when used before a numerical designation, e.g., temperature, time, amount, and concentration, including range, indicates approximations which may vary by (+) or (-) 15%, 10%, 5%, 3%, 2%, or 1 %.

[0040] “Substantially” or “essentially” means nearly totally or completely, for instance, 95% or greater of some given quantity. In some embodiments, “substantially” or “essentially” means 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9%.

[0041] As used herein, the term “animal” refers to living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term “mammal” includes both human and non-human mammals.

[0042] The term “subject,” “host,” “individual,” and “patient” are as used interchangeably herein to refer to animals, typically mammalian animals. Any suitable mammal can be treated by a method described herein. Non-limiting examples of mammals include humans, non-human primates (e.g., apes, gibbons, chimpanzees, orangutans, monkeys, macaques, and the like), domestic animals (e.g., dogs and cats), farm animals (e.g., horses, cows, goats, sheep, pigs) and experimental animals (e.g., mouse, rat, rabbit, guinea pig). In some embodiments, a mammal is a human. A mammal can be any age or at any stage of development (e.g., an adult, teen, child, infant, or a mammal in utero). A mammal can be male or female. In some embodiments, a subject is a human.

[0043] A “composition” as used herein, refers to an active agent, such as a compound as disclosed herein and a carrier, inert or active. The carrier can be, without limitation, solid such as a bead or resin, or liquid, such as phosphate buffered saline.

[0044] Carriers also include pharmaceutical excipients and additives proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri, tetra-oligosaccharides, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/antibody components, which can also function in a buffering capacity, include alanine, arginine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like. Carbohydrate excipients are also intended within the scope of this technology, examples of which include but are not limited to monosaccharides such as fructose, maltose, galactose, glucose, D-

mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol) and myoinositol.

[0045] A “pharmaceutical composition” is intended to include the combination of an active agent with a carrier, inert or active, making the composition suitable for diagnostic or therapeutic use *in vitro*, *in vivo*, or *ex vivo*.

[0046] “Pharmaceutically acceptable carriers” refers to any diluents, excipients, or carriers that may be used in the compositions disclosed herein. Pharmaceutically acceptable carriers include ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances, such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, Mack Publishing Company, a standard reference text in this field. They may be selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrups and the like, and consistent with conventional pharmaceutical practices.

[0047] The compositions used in accordance with the disclosure can be packaged in dosage unit form for ease of administration and uniformity of dosage. The term “unit dose” or “dosage” refers to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of the composition calculated to produce the desired responses in association with its administration, i.e., the appropriate route and regimen. The quantity to be administered, both according to number of treatments and unit dose, depends on the result and/or protection desired. Precise amounts of the composition also depend on the judgment of the practitioner and are peculiar to each individual. Factors affecting dose include physical and clinical state of the subject, route of administration, intended goal of treatment

(alleviation of symptoms versus cure), and potency, stability, and toxicity of the particular composition. Upon formulation, solutions are administered in a manner compatible with the dosage formulation and in such amount as is therapeutically or prophylactically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described herein.

[0048] Administration or treatment in “combination” refers to administering two agents such that their pharmacological effects are manifest at the same time. Combination does not require administration at the same time or substantially the same time, although combination can include such administrations.

[0049] An “effective amount” is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages. Such delivery is dependent on a number of variables including the time period for which the individual dosage unit is to be used, the bioavailability of the therapeutic agent, the route of administration, etc. It is understood, however, that specific dose levels of the therapeutic agents disclosed herein for any particular subject depends upon a variety of factors including the activity of the specific compound employed, bioavailability of the compound, the route of administration, the age of the animal and its body weight, general health, sex, the diet of the animal, the time of administration, the rate of excretion, the drug combination, and the severity of the particular disorder being treated and form of administration. In general, one will desire to administer an amount of the compound that is effective to achieve a serum level commensurate with the concentrations found to be effective *in vivo*. These considerations, as well as effective formulations and administration procedures are well known in the art and are described in standard textbooks.

[0050] As used herein, “treating” or “treatment” of a disease in a subject refers to (1) preventing the symptoms or disease from occurring in a subject that is predisposed or does not yet display symptoms of the disease; (2) inhibiting the disease or arresting its development; or (3) ameliorating or causing regression of the disease or the symptoms of the disease. As understood in the art, “treatment” is an approach for obtaining beneficial or desired results, including clinical results. For the purposes of the present technology, beneficial or desired results can include one or more, but are not limited to, alleviation or

amelioration of one or more symptoms, diminishment of extent of a condition (including a disease), stabilized (*i.e.*, not worsening) state of a condition (including disease), delay or slowing of condition (including disease), progression, amelioration or palliation of the condition (including disease), states and remission (whether partial or total), whether detectable or undetectable. When the disease is cancer, the following clinical end points are non-limiting examples of treatment: reduction in tumor burden, slowing of tumor growth, longer overall survival, longer time to tumor progression, inhibition of metastasis or a reduction in metastasis of the tumor, or delay, slowing, or prevent of relapse. In one aspect, treatment excludes prophylaxis. In one aspect, treatment provides a longer progression free survival or a longer overall survival.

[0051] In one embodiment, the term “disease” or “disorder” as used herein refers to a cancer or a tumor (which are used interchangeably herein), a status of being diagnosed with such disease, a status of being suspect of having such disease, or a status of at high risk of having such disease.

[0052] “Cancer” or “malignancy” are used as synonymous terms and refer to any of a number of diseases that are characterized by uncontrolled, abnormal proliferation of cells, the ability of affected cells to spread locally or through the bloodstream and lymphatic system to other parts of the body (*i.e.*, metastasize) as well as any of a number of characteristic structural and/or molecular features. In some embodiments, the term “cancer” is used interchangeably with the term “tumor”. Non-limiting examples of cancers include carcinomas, sarcomas, and hematological cancers. In one aspect, the cancer is ovarian cancer such as ovarian serous carcinoma.

[0053] A “solid tumor” is an abnormal mass of tissue that usually does not contain cysts or liquid areas. Solid tumors can be benign or malignant. Different types of solid tumors are named for the type of cells that form them. Examples of solid tumors include sarcomas, carcinomas, and lymphomas. In some embodiments, a solid tumor comprises bladder cancer, bone cancer, brain cancer, breast cancer, colorectal cancer, esophageal cancer, eye cancer, head and neck cancer, kidney cancer, lung cancer, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, or stomach cancer.

[0054] As used herein, the term “hematologic cancer” refers to cancers with hematopoietic origin. In some instances, the hematologic malignancy is a B-cell malignancy. In some instances, the hematologic malignancy is a lymphoma, optionally a B-cell lymphoma. Exemplary hematologic malignancies include, but are not limited to, Diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), mantle cell lymphoma (MCL), marginal zone lymphomas, Burkitt lymphoma, Waldenström macroglobulinemia, hairy cell leukemia (HCL), primary central nervous system (CNS) lymphoma, or primary intraocular lymphoma.

[0055] The term “B-cell lymphoma or leukemia” refers to a type of cancer that forms in tissues of the lymphatic system or bone marrow and has undergone a malignant transformation that makes the cells within the cancer pathological to the host organism with the ability to invade or spread to other parts of the body.

[0056] As used herein, the term “antigen” refers to a compound, composition, or substance that may be specifically bound by the products of specific humoral or cellular immunity, such as an antibody molecule or T-cell receptor. Antigens can be any type of molecule including, for example, haptens, simple intermediary metabolites, sugars (e.g., oligosaccharides), lipids, and hormones as well as macromolecules such as complex carbohydrates (e.g., polysaccharides), phospholipids, and proteins. Common categories of antigens include, but are not limited to, viral antigens, bacterial antigens, fungal antigens, protozoa and other parasitic antigens, tumor antigens, antigens involved in autoimmune disease, allergy and graft rejection, toxins, and other miscellaneous antigens.

[0057] The term “tumor or cancer antigen” is an antigen that is expressed on a tumor cell that is expressed at a different level, or not at all on a counterpart normal cell. The term, as used herein, also includes tumor associated antigens and neoantigens as well as fragments thereof that in one aspect, raise an immune response against the cancer cell. Non-limiting examples of tumor antigens are CD19, mesothelin, ROR1, EGFRvIII, MAGE-D4B, PSMA, HER2, HER3, AFP, CEA CA-125, MUC-1, ETA, MUC-1, BAGE, GAGE-1, MAGE-A1, NY-ESO-1, Gp100, Melan-A/MART-1, Prostate-specific antigen, Mammoglobin-A, Alpha-fetoprotein, HER-2/neu, P53, K-ras, or TRP-2/INT2. Tumor associated antigens (TAAs) are antigens that are present on tumor cells and also normal cells. In some aspects, the TAA may be

overexpressed or underexpressed by the tumor cell relative to normal cells. Tumor specific antigens (TSAs) are antigens that may only be expressed by tumor cells and may not be expressed on any other cells. Tumor cell antigens of the instant disclosure include both known and yet to be identified tumor cell antigens. Additional examples are provided in **Table 1**, reproduced in part from Categories of Tumor Antigens, Cancer Medicine, Holland-Frei Cancer Medicine. 6th edition. Kufe DW, Pollock RE, Weichselbaum RR, et al., editors, Copyright 2003, BC Decker Inc.

[0058] Table 1. Exemplary Antigens.

Category	Example Antigen	Cancer Histology
Oncofetal	CEA	Colorectal carcinoma
	Immature laminin receptor	RCC
	TAG-72	Prostate carcinoma
Oncoviral	HPV E6, E7	Cervical carcinoma
Overexpressed/accumulated	BING-4	Melanoma
	Calcium-activated chloride channel 2	Lung carcinoma
	Cyclin-B ₁	Multi
	9D7	RCC
	Ep-CAM	Breast carcinoma
	EphA3	Multi
	Her2/neu	Multi
	Telomerase	Multi
	Mesothelin	Ductal pancreatic carcinoma
	SAP-1	Colorectal carcinoma
	Survivin	Multi
Cancer-Testis	BAGE family	Multi
	CAGE family	Multi
	GAGE family	Multi
	MAGE family	Multi

	SAGE family	Multi
	XAGE family	Multi
CT9, CT10		Multi
	NY-ESO-1/LAGE-1	Multi
	PRAME	Multi
	SSX-2	Melanoma, Multi
Lineage Restricted	Melan-A/MART-1	Melanoma
	Gp100/pmell7	Melanoma
	Tyrosinase	Melanoma
	TRP-1/-2	Melanoma
	P.plypeptide	Melanoma
	MC1R	Melanoma
	Prostate-pecific antigen	Prostate
Mutated	B-catenin	Melanoma, Prostate, HCC
	BRCA1/2	Breast, ovarian carcinoma
	CDK4	Multi
	CML66	CML
	Fibronectin	Multi
	MART-2	Melanoma
	p53	Multi
	Ras	Multi
	TGF- β RII	Colorectal carcinoma
Posttranslationally altered	MUC1	Ductal carcinoma, RCC
Idiotypic	IG, TCR	B, T leukemia, lymphoma, myeloma

BRCA = breast cancer antigen; CDK4 = cyclin-dependent kinase-4; CEA = carcinoembryonic antigen; CML66 = chronic myelogenous leukemia (antigen) 66; CT = cancer testis; HPV = human papilloma virus; Ep-CAM = epithelial cell adhesion molecule; Ig = immunoglobulin; MART-1/-2 = melanoma antigen recognized by T cells-1/-2; MC1R = melanocortin-1-receptor; SAP-1 = stomach cancer-associated protein tyrosine phosphatase-1; TAG-72 = tumor antigen-72; TCR = T cell receptor; TGF- β RII = transforming growth factor- β receptor II; TRP = tyrosinase-related protein.

[0059] As used herein, the term “cancer antigen” can include is certain aspect, a cancer neoantigen or a fragment thereof that in one aspect, raises an immune response against the cancer. A neoantigen is a newly formed antigen generated by tumor cells as a result of various tumor-specific alterations such as genomic mutation. See, e.g., Zhang et al. (2021) Neoantigen: a New Breakthrough in Tumor Immunotherapy” Front. Immunol. Vol. 12, <https://doi.org/10.3389/fimmu.2021.672356>, accessed on June 27, 2023.

[0060] As used herein, the term “antigen binding domain” refers to any protein or polypeptide domain that can specifically bind to an antigen target.

[0061] As used herein, the term “autologous,” in reference to cells refers to cells that are isolated and infused back into the same subject (recipient or host). “Allogeneic” refers to non-autologous cells.

[0062] As used herein, an ablative therapy is a treatment destroying or ablating cancer tumors. In one embodiment, the ablative therapy does not require invasive surgery. In other embodiments, the ablative therapy refers to removal of a tumor via surgery. In some embodiments, the step ablating the cancer includes immunotherapy of the cancer. Cancer immunotherapy is based on therapeutic interventions that aim to utilize the immune system to combat malignant diseases. It can be divided into unspecific approaches and specific approaches. Unspecific cancer immunotherapy aims at activating parts of the immune system generally, such as treatment with specific cytokines known to be effective in cancer immunotherapy (e.g. IL-2, interferon's, cytokine inducers).

[0063] The terms “oligonucleotide” or “polynucleotide” or “portion,” or “segment” thereof refer to a stretch of polynucleotide residues which is long enough to use in PCR or various hybridization procedures to identify or amplify identical or related parts of mRNA or DNA molecules. The polynucleotide compositions of this invention include RNA, cDNA, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those skilled in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.), charged linkages

(e.g., phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids, etc.). Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule.

[0064] The term “contacting” means direct or indirect binding or interaction between two or more. A particular example of direct interaction is binding. A particular example of an indirect interaction is where one entity acts upon an intermediary molecule, which in turn acts upon the second referenced entity. Contacting as used herein includes in solution, in solid phase, *in vitro*, *ex vivo*, in a cell and *in vivo*. Contacting *in vivo* can be referred to as administering, or administration.

[0065] The term “liposome” intends any of a small carrier molecule that has a diameter of about 1 and 1000 nanometers. Non-limiting examples include micelles, liposomes and lipid nanoparticles (LNPs). Example of such are known in the art and have most recently been used for the delivery of mRNA vaccines. These are commercially available and manufactured by, for example, Moderna, Alnylam, and ReCode Therapeutics. In one aspect of this disclosure, the liposome encapsulates the one or more antigens. Non-limiting examples of liposome nanoparticles are described in US 20200206362A1 and US Patent Nos. 10722508; 9546128B2; 9,737,528; and 10,8235,492B2. These can be lyophilized for ease of storage and use.

[0066] Modes for Carrying out the Disclosure

[0067] Plant Virus Nanoparticles

[0068] As used herein, the term “plant virus” includes viruses that infect plants or plant systems, e.g., leaves, root and/or stems. Plant viruses can be stably stored (and are stable without cold chain requirements). Plant viruses do not infect or replicate in mammalian cells, thus adding another layer of safety compared to oncolytic viral therapies. Non-limiting examples of plant viruses include Cowpea mosaic virus (CPMV), Broad bean wilt virus 1,

Tobacco ringspot virus, Bbean pod mottle virus (BPMV), Rice tungro spherical virus, and Potato Virus X.

[0069] The plant viruses can belong to the picornavirus family, such as a virus from the Comovirinae virus subfamily. In other aspects the plant virus is a different plant virus.

[0070] As used herein, the term “plant viral nanoparticle,” “plant virus nanoparticle,” or “VLP” refers to a non-replicating, viral shell, derived from one or more plant viruses (*e.g.*, one or more plant viruses described herein). VLPs are generally composed of one or more viral proteins, such as, but not limited to, those proteins referred to as capsid, coat, shell, surface and/or envelope proteins, or particle-forming polypeptides derived from these proteins. VLPs can form spontaneously upon recombinant expression of the protein in an appropriate expression system. VLPs can also be engineered, *e.g.*, comprising, or consisting essentially of, or yet further consisting of, one or more viral proteins that comprise, or consists essentially of, or yet further consists of, a modification. Methods for producing VLPs are known in the art. The presence of VLPs following recombinant expression of viral proteins can be detected using conventional techniques known in the art, such as by electron microscopy, biophysical characterization, and the like. Further, VLPs can be isolated by known techniques, *e.g.*, density gradient centrifugation and identified by characteristic density banding. See, for example, Baker et al. (1991) *Biophys. J.* 60:1445-1456; Hagensee et al. (1994) *J. Viral.* 68:4503-4505; Vincente et al. (2011) *J Invertebr Pathol.*, 107 Suppl:S42-8 and Schneider, Ohlum and Ross (2012) *Curr. Top. Microbial. Immunol.*, 354: 53073). As used herein, VLP intends naturally occurring (wild-type or native) VLP and engineered VLP, unless explicitly stated otherwise.

[0071] In some embodiments, the plant virus nanoparticle is a virus belonging to the order *Picornavirales* (i.e. a plant picornavirus). A plant picornavirus is a virus belonging to the family *Secoviridae*, which together with mammalian picornaviruses belong to the order of the *Picornavirales*. Plant picornaviruses are relatively small, nonenveloped, positive- stranded RNA viruses with an icosahedral capsid. In some embodiments, the virus particles are selected from the *Comovirinae* virus subfamily. The *Comovirinae* subfamily comprises over 60 species across at least 3 genera (*Comovirus*, *Fabavirus*, *Nepovirus*). Some *Picornavirales* have a monopartite genome, while others have a bipartite genome.

[0072] In one embodiment, the plant virus nanoparticle is a Cowpea mosaic virus (CPMV). CPMV is a plant-infecting member of the order *Picornavirales*, which has a relatively simple, non-enveloped capsid that has been extensively studied and a positive-sense, single-stranded RNA genome. For CPMV, the genome is bipartite, with RNA-1 (6 kb) and RNA-2 (3.5 kb) being separately encapsidated. CPMV has an icosahedral capsid structure, which is ~30 nm in diameter and is formed from 60 copies each of a Large (L) and Small (S) coat protein. These two coat proteins are processed from a single RNA-2-encoded precursor polyprotein (VP60) by the action of the 24 K viral proteinase which is encoded by RNA-1. As used herein, the terms “coat protein” and “capsid protein” are used interchangeably. Thus capsid assembly, as well as viral infection, is dependent on the presence of both genomic segments in an infected plant cell.

[0073] In some cases, the large capsid protein is a wild-type large capsid protein, optionally expressed by SB or Vu strain. In other instances, the large capsid protein is a modified large capsid protein, e.g., comprising, or consisting essentially of, or yet further consisting of, one or more substitutions, insertions, and/or deletions. In some cases, the large capsid protein comprises, or consists essentially of, or yet further consists of, the sequence as set forth in the UniProtKB ID P03599 (residues 460-833):

MEQNLFALSLDDTSSVRGSLDTKFAQTRVLLSKAMAGGDVLLDEYLYDVVNGQD
FRATVAFLRTHVITGKIKVTATTNISDNSGCCCLMLAINSGVRGKYSTDVYTICSQDSM
TWNPGCKKNFSFTFNPNPCGDSWSAEMISRSRVRMTVICVSGWTLSPTTDVIAKLDW
SIVNEKCEPTIYHLADCQNWLPLNRWMGKLTFPQGVTSVRRMPLSIGGGAGATQA
FLANMPNSWISMWRYFRGELHFEVTKMSSPYIKATVTFLIAFGNLSDAFGFYESFPHR
IVQFAEVEEKCTLVFSQQEFVTAWSTQVNPRTTLEADGCPYLYAIIHDSTTGTISGDF
NLGVKLVGIKDFCGIGSNPGIDGSRLGAIQA (SEQ ID NO: 1), or an equivalent thereof.

[0074] In some cases, the mature small capsid protein is a wild-type mature small capsid protein, optionally expressed by SB or Vu strain. In other instances, the mature small capsid protein is a modified mature small capsid protein, e.g., comprising, or consisting essentially of, or yet further consisting of, one or more substitutions, insertions, and/or deletions. In some cases, the mature small capsid protein comprises, or consists essentially of, or yet further consists of, the sequence as set forth in the UniProtKB ID P03599 (residues 834-1022):

GPVCAEASDVYSPCMIASTPPAPFSDVTA VTFDLINGKITPVGDDNWNTHIYNPPIMN
 VLRTAAWKSGTIHVQLNVRGAGVKRADWDGQVFVYLRQSMNPESYDARTFVISQP
 GSAMLNFSFDIIGPNSGFEFAESPWANQTTWYLECVATNPRQIQQFEVNMRFDPNFR
 VAGNILMPPFPLSTETPPL (SEQ ID NO: 2), or an equivalent thereof.

[0075] The terms “CPMV” “CPMV virus” or “CPMV particles” are used interchangeably, referring to a CPMV comprising, or alternatively consisting essentially of, or yet consisting of a capsid and an RNA genome (which is also referred to herein as a viral genome) encapsidated in the capsid. In some embodiments, the CPMV particles have been treated, prepared and/or inactivated by a method as disclosed herein. In some embodiments, the CPMV particle further comprises a heterologous RNA, which is heterologous to (i.e., not naturally presented in) a native CPMV free of any human intervention.

[0076] In some embodiments, the CPMV can be substituted by another plant virus, for example another plant picornavirus, optionally from the *Comovirinae* subfamily. However, a bacteriophage or mammalian virus can be used in some embodiments of the invention. Exemplary plant viruses from the *Comovirinae* subfamily include, but are not limited to Broad bean wilt virus 1, Tobacco ringspot virus, Cowpea severe mosaic virus, Turnip ringspot virus, Broad bean wilt virus 2, Bean pod mottle virus (BPMV), Potato virus B, Tomato ringspot virus, and Rice tungro spherical virus. Additional information about plant picornaviruses can be found in Zell et al (2017) Journal of General Virology, 98: 2421–2422.

[0077] In some embodiments, the plant virus is Potato Virus X (PVX). PVX belongs to the *Potexvirus* genus of the family *Flexivirida*. PVX virions are described as flexible, thread-like bodies measuring approximately 500-515 nanometers in length and 13-15 nanometers in diameter. Each viral particle comprises around 1300-1350 helically folded identical CP subunits enclosing a 6.4 knt viral RNA, with each turn of the primary helix consisting of 8.9 CP subunits. In some cases, the coat protein comprises, or consists essentially of, or yet further consists of, the sequence as set forth in the UniProt ID P17782:

MSAPASTTQATGSTTSTTTKTAGATPATASGLFTIPDGDFFSTARAIVASNAVATNED
 LSKIEAIWKDMKVPTDTMAQAAWDLVRHCADVGSQAQTEMIDTGPYSNGISRARLA
 AAIKEVCTLRQFCMKYAPVVWNWMLTNNSPPANWQAQGFKPEHKFAAFDFFNGVT
 NPAAIMPKEGLIRPPSEAEMNAAQTAAAFVKITKARAQSNDFASLDAAVTRGRITGTT
 TAEAVVTLPPP (SEQ ID NO: 3)

[0078] The virus can be obtained according to various methods known to those skilled in the art. In embodiments where plant virus particles are used, the virus particles can be obtained from the extract of a plant infected by the plant virus or using the method disclosed herein. For example, cowpea mosaic virus can be grown in black eyed pea plants, which can be infected within 10 days of sowing seeds. Plants can be infected by, for example, coating the leaves with a liquid containing the virus, and then rubbing the leaves, preferably in the presence of an abrasive powder which wounds the leaf surface to allow penetration of the leaf and infection of the plant. Within a week or two after infection, leaves are harvested and viral nanoparticles are extracted. In the case of cowpea mosaic virus, 100 mg of virus can be obtained from as few as 50 plants. Procedures for obtaining plant picornavirus particles using extraction of an infected plant are known to those skilled in the art. See Wellink J., Meth Mol Biol, 8, 205- 209 (1998). Procedures are also available for obtaining virus-like particles. Saunders et al., Virology, 393(2):329-37 (2009). The disclosures of both of these references are incorporated herein by reference.

[0079] In some embodiments, the plant virus nanoparticle, VLP, or CPMV or CMPV particle has a diameter of from about 15 nm to about 60 nm, or from about 15 nm to about 50 nm, or from about 10 nm to about 40 nm, or from about 20 or 25 nm to about 50 nm, or from about 20 or 25 nm to about 40 nm, or from about 15 nm to about 35 nm, or from about 15 nm to about 40 nm, or from about 15 nm to about 45 nm, or alternatively about 15 nm, or about 20 nm, or about 25 nm, or about 30 nm, or about 35 nm, or about 40 nm, or about 45 nm, or about 50 nm, or about 55 nm, or about 60 nm.

[0080] In some embodiments, the plant virus nanoparticle may include at least one Toll-like Receptor (TLR) agonist.

[0081] The plant virus nanoparticle is a derivative and can comprise modified capsid polypeptides, for example, non-conservative and conservative substitutions of the capsid amino acid sequences.

[0082] As used herein, the term “conservative substitution” denotes the replacement of an amino acid residue by another, chemically or biologically similar residue. Biologically similar means that the substitution does not destroy a biological activity or function, *e.g.*, assembly of a viral capsid. Structurally similar means that the amino acids have side chains

with similar length, such as alanine, glycine and serine, or a similar size. Chemical similarity means that the residues have the same charge or are both hydrophilic and hydrophobic. Particular examples of conservative substitutions include the substitution of a hydrophobic residue such as isoleucine, valine, leucine or methionine for another, the substitution of a polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. The term "conservative substitution" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid. Such proteins that include amino acid substitutions can be encoded by a nucleic acid. Consequently, nucleic acid sequences encoding proteins that include amino acid substitutions are also provided.

[0083] Modified proteins also include one or more D-amino acids substituted for L-amino acids (and mixtures thereof), structural and functional analogues, for example, peptidomimetics having synthetic or non-natural amino acids or amino acid analogues and derivatized forms. Modifications include cyclic structures such as an end-to-end amide bond between the amino and carboxy-terminus of the molecule or intra- or inter-molecular disulfide bond.

[0084] Modified forms further include "chemical derivatives," in which one or more amino acids has a side chain chemically altered or derivatized. Such derivatized polypeptides include, for example, amino acids in which free amino groups form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups; the free carboxy groups form salts, methyl and ethyl esters; free hydroxyl groups that form O-acyl or O-alkyl derivatives as well as naturally occurring amino acid derivatives, for example, 4-hydroxyproline, for proline, 5-hydroxylysine for lysine, homoserine for serine, ornithine for lysine etc. Also included are amino acid derivatives that can alter covalent bonding, for example, the disulfide linkage that forms between two cysteine residues that produces a cyclized polypeptide.

[0085] In some instances, a plant virus nanoparticle described herein further comprise, or consists essentially of, or yet further consists of, a label or a tag, e.g., such as a detectable label. A detectable label can be attached to, e.g., to the surface of a virus or plant virus nanoparticle.

[0086] Non-limiting exemplary detectable labels also include a radioactive material, such as a radioisotope, a metal or a metal oxide. Radioisotopes include radionuclides emitting alpha, beta or gamma radiation. In particular embodiments, a radioisotope can be one or more of: ^3H , ^{10}B , ^{18}F , ^{11}C , ^{14}C , ^{13}N , ^{18}O , ^{15}O , ^{32}P , P^{33} , ^{35}S , ^{35}Cl , ^{45}Ti , ^{46}Sc , ^{47}Sc , ^{51}Cr , ^{52}Fe , ^{59}Fe , ^{57}Co , ^{60}Cu , ^{61}Cu , ^{62}Cu , ^{64}Cu , ^{67}Cu , ^{67}Ga , ^{68}Ga , ^{72}As , ^{76}Br , ^{77}Br , $^{81\text{m}}\text{Kr}$, ^{82}Rb , ^{85}Sr , ^{89}Sr , ^{86}Y , ^{90}Y , ^{95}Nb , $^{94\text{m}}\text{Tc}$, $^{99\text{m}}\text{Tc}$, ^{97}Ru , ^{103}Ru , ^{105}Rh , ^{109}Cd , ^{111}In , ^{113}Sn , $^{113\text{m}}\text{In}$, ^{114}In , I^{125} , I^{131} , ^{140}La , ^{141}Ce , ^{149}Pm , ^{153}Gd , ^{157}Gd , ^{153}Sm , ^{161}Tb , ^{166}Dy , ^{166}Ho , ^{169}Er , ^{169}Y , ^{175}Yb , ^{177}Lu , ^{186}Re , ^{188}Re , ^{201}Tl , ^{203}Pb , ^{211}At , ^{212}Bi or ^{225}Ac .

[0087] Additional non-limiting exemplary detectable labels include a metal or a metal oxide. In particular embodiments, a metal or metal oxide is one or more of: gold, silver, copper, boron, manganese, gadolinium, iron, chromium, barium, europium, erbium, praseodymium, indium, or technetium. In additional embodiments, a metal oxide includes one or more of: Gd(III), Mn(II), Mn(III), Cr(II), Cr(III), Cu(II), Fe(III), Pr(III), Nd(III), Sm(III), Tb(III), Yb(III), Dy(III), Ho(III), Eu(II), Eu(III), or Er(III).

[0088] Further non-limiting exemplary detectable labels include contrast agents (e.g., gadolinium; manganese; barium sulfate; an iodinated or noniodinated agent; an ionic agent or nonionic agent); magnetic and paramagnetic agents (e.g., iron-oxide chelate); nanoparticles; an enzyme (horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase); a prosthetic group (e.g., streptavidin/biotin and avidin/biotin); a fluorescent material (e.g., umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin); a luminescent material (e.g., luminol); or a bioluminescent material (e.g., luciferase, luciferin, aequorin).

[0089] Additional non-limiting examples of tags and/or detectable labels include enzymes (horseradish peroxidase, urease, catalase, alkaline phosphatase, beta-galactosidase, chloramphenicol transferase); enzyme substrates; ligands (e.g., biotin); receptors (avidin); GST-, T7-, His-, myc-, HA- and FLAG®-tags; electron-dense reagents; energy transfer molecules; paramagnetic labels; fluorophores (fluorescein, fluorscamine, rhodamine, phycoerythrin, phycocyanin, allophycocyanin); chromophores; chemi-luminescent (imidazole, luciferase, acridinium, oxalate); and bio-luminescent agents.

[0090] As set forth herein, a detectable label or tag can be linked or conjugated (e.g., covalently) to the virus or plant virus nanoparticle or nanoparticle. In various embodiments a detectable label, such as a radionuclide or metal or metal oxide can be bound or conjugated to the agent, either directly or indirectly. A linker or an intermediary functional group can be used to link the molecule to a detectable label or tag. Linkers include amino acid or peptidomimetic sequences inserted between the molecule and a label or tag so that the two entities maintain, at least in part, a distinct function or activity. Linkers may have one or more properties that include a flexible conformation, an inability to form an ordered secondary structure or a hydrophobic or charged character which could promote or interact with either domain. Amino acids typically found in flexible protein regions include Gly, Asn and Ser. The length of the linker sequence may vary without significantly affecting a function or activity.

[0091] Linkers further include chemical moieties, conjugating agents, and intermediary functional groups. Examples include moieties that react with free or semi-free amines, oxygen, sulfur, hydroxy or carboxy groups. Such functional groups therefore include mono and bifunctional crosslinkers, such as sulfo-succinimidyl derivatives (sulfo-SMCC, sulfo-SMPB), in particular, disuccinimidyl suberate (DSS), BS3 (Sulfo-DSS), disuccinimidyl glutarate (DSG) and disuccinimidyl tartrate (DST). Non-limiting examples include diethylenetriaminepentaacetic acid (DTPA) and ethylene diaminetetracetic acid.

[0092] Conjugates

[0093] This disclosure provides a conjugate comprising a liposome comprising one or more cancer antigen(s) conjugated to at least one plant virus nanoparticle. In one aspect the liposome comprising the one or more cancer antigen(s) is one or more of a micelle, a liposome or a lipid nanoparticle (LNP). In one aspect, the liposome is an LNP or an 80 DOPC and DPPE-PEG2K liposome. **FIG. 1A** shows an exemplary conjugate, wherein the liposome is represented by the circle, surrounded by plant virus nanoparticles.

[0094] In one aspect, the plant virus nanoparticle is as described herein, e.g., one or more of a CPMV; a virus from the picornavirus family; a virus from the *Comovirinae* virus subfamily, such as CPMV, Broad bean wilt virus 1, Tobacco ringspot virus, Bean pod mottle virus

(BPMV); Rice tungro spherical virus. In one aspect, the plant virus nanoparticle is a CPMV or a derivative thereof that is non-replicating and noninfectious toward mammals.

[0095] In some embodiments, the plant virus nanoparticle or CPMV or CMPV particle has a diameter of from about 15 nm to about 60 nm, or from about 15 nm to about 50 nm, or from about 10 nm to about 40 nm, or from about 20 or 25 nm to about 50 nm, or from about 20 or 25 nm to about 40 nm, or from about 15 nm to about 35 nm, or from about 15 nm to about 40 nm, or from about 15 nm to about 45 nm, or alternatively about 15 nm, or about 20 nm, or about 25 nm, or about 30 nm, or about 35 nm, or about 40 nm, or about 45 nm, or about 50 nm, or about 55 nm, or about 60 nm. According to one embodiment, the plant virus nanoparticle or the CPMV particle has a diameter of about 30 nm. In one aspect, the CPMV particle conjugated to a DBCO group is about 30 nm in diameter (**FIG. 8B**).

[0096] In some embodiments, the liposome or LNP has a diameter of from about 15 to about 1000 nm, or from about 30 nm to about 800 nm, or from about 45 nm to about 600 nm, or from about 60 nm to about 400 nm, or from about 75 nm to about 200 nm, or from about 90 nm to about 150 nm, or from about 90 nm to about 130 nm, or from about 95 nm to about 125 nm, or from about 100 nm to about 120 nm. According to one embodiment, the liposome or LNP has a diameter of about 50 nm to about 200 nm. According to one embodiment, the liposome or LNP has a diameter of about 100 nm.

[0097] In some embodiments, the conjugate has a diameter from about 30 nm to about 1100 nm, or from about 80 nm to about 800 nm, or from about 120 nm to about 500 nm, or from about 150 nm to about 200 nm. According to one embodiment, the conjugate has a diameter of about 160 nm. In a further aspect, the nanoparticle and/or the liposome is detectably labeled, examples of such are known in the art and described herein.

[0098] In one embodiment of the conjugates of this disclosure, the one or more cancer antigen(s) is selected from an antigen or neoantigen isolated from a cancer selected from a carcinoma, a sarcoma, or a hematological cancer. In a further aspect, the one or more cancer antigens is an antigen or neoantigen from a carcinoma, such as for example, an ovarian cancer cell. In a further aspect, the antigen or neoantigen is detectably labeled, examples of such are known in the art and described herein, see **Table 1**.

[0099] Further provided is a conjugate comprising a plant virus nanoparticle conjugated to a liposome and comprising one or more cancer antigens and/or neoantigens. In one aspect, the liposome comprises one or more cancer antigens and/or neoantigens that are the same or different from each other.

[0100] In a further aspect, a plurality of antigens or neoantigens are comprised in the conjugate, wherein the antigens or neoantigens target or induce an immune response against the same cancer cell type or different cancer cell types. Alternatively, at least two of the plurality of the antigens or neoantigens target or induce an immune response are different from each other but target the same cancer type. In a further aspect, the plurality of the antigens or neoantigens are identical to each other.

[0101] In one aspect, the one or more cancer antigens are derived from tumor cell lysate (TCL, see e.g. **FIG. 1A**). In one embodiment, the tumor cell lysate is derived from a known tumor cell line, for example ID8-Defb29/Vegf-a-Luc. In other embodiments the tumor cell lysate is derived from another known tumor cell line. In some embodiments the tumor cell lysate is derived from the tumor of a subject with cancer. In one aspect, the antigen is ovalbumin.

[0102] In one embodiment, the tumor cell lysate is oxidized or irradiated. In another aspect, the tumor cell lysate is freshly isolated prior to forming the conjugate.

[0103] Further provided is a conjugate comprising a plant virus nanoparticle, e.g., selected from a CMPV or derivative thereof conjugated to a liposome and comprising one or more cancer antigens and/or neoantigens. In a further aspect, one or more of the nanoparticles, the liposome or the antigen or neoantigen is detectably labeled, examples of such are known in the art and described herein. In a further aspect, a plurality of antigens or neoantigens are comprised in the conjugate, wherein the antigens or neoantigens target or induce an immune response against the same cancer cell type or different cancer cell types. Alternatively, at least two of the plurality of the antigens or neoantigens target or induce an immune response are different from each other but target the same cancer type. In a further aspect, the plurality of the antigens or neoantigens are identical to each other.

[0104] Further provided is a conjugate comprising a plant virus nanoparticle selected from a CMPV or derivative thereof conjugated to a liposome and comprising one or more ovarian cancer antigens and/or neoantigens. In a further aspect, one or more of the nanoparticles, the liposome or the antigen or neoantigen is detectably labeled, examples of such are known in the art and described herein. Alternatively, the conjugate comprises a plurality of ovarian cancer antigens or neoantigens that are the same or different from each other.

[0105] Further provided is a conjugate comprising a CMPV plant virus nanoparticle conjugated to a liposome and comprising one or more ovarian cancer antigens and/or neoantigens. In a further aspect, one or more of the plant virus nanoparticle, the liposome, or the antigen or neoantigen is detectably labeled, examples of such are known in the art and described herein.

[0106] Alternatively, the conjugate comprises a plurality of ovarian cancer antigens or neoantigens that are the same or different from each other.

[0107] In each of the above embodiments, a further aspect of the conjugate is that the plant virus nanoparticle is conjugated to the liposome via a DBCO group, for example wherein the DBCO group is chemically conjugated to the plant virus nanoparticle via terminal NH₂ group (see e.g. FIG. 1).

[0108] Also provided is a plurality of conjugates, wherein one or more of the plant virus nanoparticles, the liposome and/or the cancer antigens or neoantigens are the same or different from each other. One or more of the conjugates in the plurality can be detectably labeled.

[0109] Compositions

[0110] In another aspect, provided herein is a composition comprising, consisting essentially of, or consisting of the conjugate as provided herein, and at least one carrier, such as a pharmaceutically acceptable carrier or excipient. In another aspect, a plurality of the conjugates are provided herein, wherein the nanoparticles, antigens, and/or plant virus nanoparticles are the same or different from each other.

[0111] In one aspect, the composition further comprises a preservative or stabilizer, that can be in one aspect, exogenously added or non-naturally occurring.

[0112] In one embodiment, this technology relates to a composition comprising a combination of conjugates as described herein and a carrier.

[0113] In another embodiment, this technology relates to a pharmaceutical composition comprising a combination of conjugates as described herein and a pharmaceutically acceptable carrier.

[0114] In another embodiment, this technology relates to a pharmaceutical composition comprising an effective amount or a therapeutically effective amount of a conjugate or plurality of such as described herein and a pharmaceutically acceptable carrier.

[0115] Compositions, including pharmaceutical compositions comprising, consisting essentially of, or consisting of the multivalent nanoparticle-peptide conjugate formulation alone or in combination of other therapeutic agents can be manufactured by means of conventional mixing, dissolving, granulating, dragee-making levigating, emulsifying, encapsulating, entrapping, or lyophilization processes. These can be formulated in conventional manner using one or more physiologically acceptable carriers, diluents, excipients, or auxiliaries which facilitate processing of the combinations of compounds provided herein into preparations which can be used pharmaceutically.

[0116] In some embodiments, the pharmaceutical formulations described herein are administered to a subject by multiple administration routes, including but not limited to, parenteral, oral, buccal, rectal, sublingual, or transdermal administration routes. In some cases, parenteral administration comprises, or consists essentially of, or yet further consists of, intravenous, subcutaneous, intramuscular, intracerebral, intranasal, intra-arterial, intra-articular, intradermal, intravitreal, intraosseous infusion, intraperitoneal, or intrathecal administration. In some instances, the pharmaceutical composition is formulated for local administration. In other instances, the pharmaceutical composition is formulated for systemic administration.

[0117] In some embodiments, the pharmaceutical formulations include, but are not limited to, lyophilized formulations, aqueous liquid dispersions, self-emulsifying dispersions, solid

solutions, liposomal dispersions, aerosols, solid dosage forms, powders, immediate release formulations, controlled release formulations, fast melt formulations, tablets, capsules, pills, delayed release formulations, extended release formulations, pulsatile release formulations, multiparticulate formulations (e.g., nanoparticle formulations), and mixed immediate and controlled release formulations.

[0118] In some embodiments, the pharmaceutical formulations include a carrier or carrier materials selected on the basis of compatibility with the composition disclosed herein, and the release profile properties of the desired dosage form. Exemplary carrier materials include, e.g., binders, suspending agents, disintegration agents, filling agents, surfactants, solubilizers, stabilizers, lubricants, wetting agents, diluents, and the like. Pharmaceutically compatible carrier materials include, but are not limited to, acacia, gelatin, colloidal silicon dioxide, calcium glycerophosphate, calcium lactate, maltodextrin, glycerine, magnesium silicate, polyvinylpyrrolidone (PVP), cholesterol, cholesterol esters, sodium caseinate, soy lecithin, taurocholic acid, phosphatidylcholine, sodium chloride, tricalcium phosphate, dipotassium phosphate, cellulose and cellulose conjugates, sugars sodium stearyl lactylate, carrageenan, monoglyceride, diglyceride, pregelatinized starch, and the like. See, e.g., Remington: The Science and Practice of Pharmacy, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995), Hoover, John E., Remington 's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania 1975, Liberman, H.A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y., 1980, and Pharmaceutical Dosage Forms and Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins, 1999).

[0119] In some instances, the pharmaceutical formulations further include pH adjusting agents or buffering agents which include acids such as acetic, boric, citric, lactic, phosphoric and hydrochloric acids, bases such as sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, sodium lactate and tris-hydroxymethylaminomethane, and buffers such as citrate/dextrose, sodium bicarbonate and ammonium chloride. Such acids, bases and buffers are included in an amount required to maintain pH of the composition in an acceptable range.

[0120] In some instances, the pharmaceutical formulation includes one or more salts in an amount required to bring osmolality of the composition into an acceptable range. Such salts

include those having sodium, potassium or ammonium cations and chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate or bisulfite anions, suitable salts include sodium chloride, potassium chloride, sodium thiosulfate, sodium bisulfite and ammonium sulfate.

[0121] In some embodiments, the pharmaceutical formulations include, but are not limited to, sugars like trehalose, sucrose, mannitol, maltose, glucose, or salts like potassium phosphate, sodium citrate, ammonium sulfate and/or other agents such as heparin to increase the solubility and in vivo stability of polypeptides.

[0122] In some instances, the pharmaceutical formulations further include diluent which are used to stabilize compounds because they can provide a more stable environment. Salts dissolved in buffered solutions (which also can provide pH control or maintenance) are utilized as diluents in the art, including, but not limited to a phosphate buffered saline solution. In certain instances, diluents increase bulk of the composition to facilitate compression or create sufficient bulk for homogenous blend for capsule filling. Such compounds can include e.g., lactose, starch, mannitol, sorbitol, dextrose, microcrystalline cellulose such as AVICEL[®], dibasic calcium phosphate, dicalcium phosphate dihydrate, tricalcium phosphate, calcium phosphate, anhydrous lactose, spray-dried lactose, pregelatinized starch, compressible sugar, such as Di- PAC[®] (Amstar), mannitol, hydroxypropylmethylcellulose, hydroxypropylmethylcellulose acetate stearate, sucrose-based diluents, confectioner's sugar, monobasic calcium sulfate monohydrate, calcium sulfate dihydrate, calcium lactate trihydrate, dextrans, hydrolyzed cereal solids, amylose, powdered cellulose, calcium carbonate, glycine, kaolin, mannitol, sodium chloride, inositol, bentonite, and the like.

[0123] In some cases, the pharmaceutical formulations include disintegration agents or disintegrants to facilitate the breakup or disintegration of a substance. The term “disintegrate” include both the dissolution and dispersion of the dosage form when contacted with gastrointestinal fluid. Examples of disintegration agents include a starch, e.g., a natural starch such as corn starch or potato starch, a pregelatinized starch such as National 1551 or AMIJEL[®], or sodium starch glycolate such as PROMOGEL[®] or EXPLOTAB[®], a cellulose such as a wood product, methylcrystalline cellulose, e.g., AVICEL[®], AVICEL[®] PH101,

AVICEL[®] PH102, AVICEL[®] PH105, ELCEMA[®] P100, EMCOCEL[®], VIVACEL[®], MING TIA[®], and SOLKA-FLOC[®], methylcellulose, croscarmellose, or a cross-linked cellulose, such as cross-linked sodium carboxymethylcellulose (AC-DI-SOL[®]), cross-linked carboxymethylcellulose, or cross-linked croscarmellose, a cross-linked starch such as sodium starch glycolate, a cross-linked polymer such as crospovidone, a cross-linked polyvinylpyrrolidone, alginate such as alginic acid or a salt of alginic acid such as sodium alginate, a clay such as VEEGUM[®] HV (magnesium aluminum silicate), a gum such as agar, guar, locust bean, Karaya, pectin, or tragacanth, sodium starch glycolate, bentonite, a natural sponge, a surfactant, a resin such as a cation-exchange resin, citrus pulp, sodium lauryl sulfate, sodium lauryl sulfate in combination starch, and the like.

[0124] In some instances, the pharmaceutical formulations include filling agents such as lactose, calcium carbonate, calcium phosphate, dibasic calcium phosphate, calcium sulfate, microcrystalline cellulose, cellulose powder, dextrose, dextrates, dextran, starches, pregelatinized starch, sucrose, xylitol, lactitol, mannitol, sorbitol, sodium chloride, polyethylene glycol, and the like.

[0125] Lubricants and glidants are also optionally included in the pharmaceutical formulations described herein for preventing, reducing or inhibiting adhesion or friction of materials.

[0126] Exemplary lubricants include, e.g., stearic acid, calcium hydroxide, talc, sodium stearyl fumarate, a hydrocarbon such as mineral oil, or hydrogenated vegetable oil such as hydrogenated soybean oil (STEROTEX[®]), higher fatty acids and their alkali-metal and alkaline earth metal salts, such as aluminum, calcium, magnesium, zinc, stearic acid, sodium stearates, glycerol, talc, waxes, STEAROWET[®], boric acid, sodium benzoate, sodium acetate, sodium chloride, leucine, a polyethylene glycol (e.g., PEG-4000) or a methoxypolyethylene glycol such as CARBOWAX[™], sodium oleate, sodium benzoate, glyceryl behenate, polyethylene glycol, magnesium or sodium lauryl sulfate, colloidal silica such as SYLOID[™], CAB-O-SIL[®], a starch such as corn starch, silicone oil, a surfactant, and the like.

[0127] Plasticizers include compounds used to soften the microencapsulation material or film coatings to make them less brittle. Suitable plasticizers include, e.g., polyethylene glycols

such as PEG 300, PEG 400, PEG 600, PEG 1450, PEG 3350, and PEG 800, stearic acid, propylene glycol, oleic acid, triethyl cellulose and triacetin. Plasticizers can also function as dispersing agents or wetting agents.

[0128] Solubilizers include compounds such as triacetin, triethyl citrate, ethyl oleate, ethyl caprylate, sodium lauryl sulfate, sodium doccusate, vitamin E TPGS, dimethylacetamide, N-methylpyrrolidone, N-hydroxyethylpyrrolidone, polyvinylpyrrolidone, hydroxypropylmethyl cellulose, hydroxypropyl cyclodextrins, ethanol, n-butanol, isopropyl alcohol, cholesterol, bile salts, polyethylene glycol 200-600, glycofurol, transcitol, propylene glycol, and dimethyl isosorbide and the like.

[0129] Stabilizers include compounds such as any antioxidation agents, buffers, acids, preservatives and the like. Exemplary stabilizers include L-arginine hydrochloride, tromethamine, albumin (human), citric acid, benzyl alcohol, phenol, disodium biphosphate dehydrate, propylene glycol, metacresol or m-cresol, zinc acetate, poly sorb ate-20 or TWEEN® 20, or trometamol.

[0130] Suspending agents include compounds such as polyvinylpyrrolidone, e.g., polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, polyvinylpyrrolidone K25, or polyvinylpyrrolidone K30, vinyl pyrrolidone/vinyl acetate copolymer (S630), polyethylene glycol, e.g., the polyethylene glycol can have a molecular weight of about 300 to about 6000, or about 3350 to about 4000, or about 7000 to about 5400, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, hydroxymethylcellulose acetate stearate, polysorbate-80, hydroxyethylcellulose, sodium alginate, gums, such as, e.g., gum tragacanth and gum acacia, guar gum, xanthans, including xanthan gum, sugars, cellulosics, such as, e.g., sodium carboxymethylcellulose, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose, polysorbate-80, sodium alginate, polyethoxylated sorbitan monolaurate, polyethoxylated sorbitan monolaurate, povidone and the like.

[0131] Surfactants include compounds such as sodium lauryl sulfate, sodium docusate, Tween 60 or 80, triacetin, vitamin E TPGS, sorbitan monooleate, polyoxyethylene sorbitan monooleate, polysorbates, polaxomers, bile salts, glyceryl monostearate, copolymers of ethylene oxide and propylene oxide, e.g., PLURONIC® (BASF), and the like. Additional

surfactants include polyoxyethylene fatty acid glycerides and vegetable oils, e.g., polyoxyethylene (60) hydrogenated castor oil, and polyoxyethylene alkyl ethers and alkylphenyl ethers, e.g., octoxynol 10, octoxynol 40. Sometimes, surfactants is included to enhance physical stability or for other purposes.

[0132] Viscosity enhancing agents include, e.g., methyl cellulose, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, hydroxypropylmethyl cellulose acetate stearate, hydroxypropylmethyl cellulose phthalate, carbomer, polyvinyl alcohol, alginates, acacia, chitosans and combinations thereof.

[0133] Wetting agents include compounds such as oleic acid, glyceryl monostearate, sorbitan monooleate, sorbitan monolaurate, triethanolamine oleate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monolaurate, sodium docusate, sodium oleate, sodium lauryl sulfate, sodium docusate, triacetin, Tween 80, vitamin E TPGS, ammonium salts and the like.

[0134] The pharmaceutical compositions for the administration of the combinations of compounds can be conveniently presented in dosage unit form and can be prepared by any of the methods well known in the art of pharmacy. The pharmaceutical compositions can be, for example, prepared by uniformly and intimately bringing the compounds provided herein into association with a liquid carrier, a finely divided solid carrier or both, and then, if necessary, shaping the product into the desired formulation. In the pharmaceutical composition, each compound of the combination provided herein is included in an amount sufficient to produce the desired therapeutic effect. For example, pharmaceutical compositions of the present technology may take a form suitable for virtually any mode of administration, including, for example, topical, ocular, oral, buccal, systemic, nasal, injection, infusion, transdermal, rectal, and vaginal, or a form suitable for administration by inhalation or insufflation.

[0135] For topical administration, the combination of compounds can be formulated as solutions, gels, ointments, creams, suspensions, etc., as is well-known in the art.

[0136] Systemic formulations include those designed for administration by injection (*e.g.*, subcutaneous, intravenous, infusion, intramuscular, intrathecal, or intraperitoneal injection) as well as those designed for transdermal, transmucosal, oral, or pulmonary administration.

[0137] Useful injectable preparations include sterile suspensions, solutions, or emulsions of the compounds provided herein in aqueous or oily vehicles. The compositions may also contain formulating agents, such as suspending, stabilizing, and/or dispersing agents. The formulations for injection can be presented in unit dosage form, *e.g.*, in ampules or in multidose containers, and may contain added preservatives.

[0138] Alternatively, the injectable formulation can be provided in powder form for reconstitution with a suitable vehicle, including but not limited to sterile pyrogen free water, buffer, and dextrose solution, before use. To this end, the combination of compounds provided herein can be dried by any art-known technique, such as lyophilization, and reconstituted prior to use.

[0139] For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are known in the art.

[0140] For oral administration, the pharmaceutical compositions may take the form of, for example, lozenges, tablets, or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose, or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc, or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulfate). The tablets can be coated by methods well known in the art with, for example, sugars, films, or enteric coatings.

[0141] Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions, and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents, and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the combination of compounds provided herein in admixture with non-toxic pharmaceutically acceptable excipients which are suitable

for the manufacture of tablets. These excipients can be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents (e.g., corn starch or alginic acid); binding agents (e.g. starch, gelatin, or acacia); and lubricating agents (e.g., magnesium stearate, stearic acid, or talc). The tablets can be left uncoated or they can be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate can be employed. They may also be coated by the techniques well known to the skilled artisan. The pharmaceutical compositions of the present technology may also be in the form of oil-in-water emulsions.

[0142] Liquid preparations for oral administration may take the form of, for example, elixirs, solutions, syrups, or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives, or hydrogenated edible fats); emulsifying agents (e.g., lecithin, or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol, cremophoreTM, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, preservatives, flavoring, coloring, and sweetening agents as appropriate.

[0143] In some embodiments, one or more compositions disclosed herein are contained in a kit. Accordingly, in some embodiments, provided herein is a kit comprising, consisting essentially of, or consisting of one or more the conjugates and/or compositions disclosed herein and instructions for their use.

[0144] Therapeutic Methods

[0145] Also provided is a method to deliver a conjugate to a cell or tissue, such as a cancer cell comprising, or consisting essentially of, or yet further consisting of, contacting the cell with a conjugate as described herein. In one aspect, the cell or tissue is a mammalian cell or tissue, such as a human cell or tissue. The contacting can be *in vitro* or *in vivo*.

[0146] In one aspect, the conjugate contains one or more cancer antigen(s), that in one aspect, are derived from tumor cell lysate. In some embodiments, the tumor cell lysate may be oxidized, irradiated, or freshly prepared from the tumor cell. Preferably, the tumor cell lysate is freshly prepared from the tumor cell. Treatment with a conjugate comprising tumor cell lysate packaged in a liposome, and CPMV led to increased survival than treatment with only tumor cell lysate packaged in a liposome, CPMV, or a combination of tumor cell lysate packaged in a liposome+CPMV (**FIG. 2**). In one aspect the antigen is ovalbumin. In one aspect, the plant virus nanoparticle is conjugated to the LNP via a DBCO groups, optionally wherein the DBCO are chemically conjugated to the plant virus nanoparticle via terminal NH₂ groups.

[0147] In some embodiments, the cancer cell may be a primary cancer or a metastatic cancer. The cell can be from a cell line or a commercially available cell line. According to one embodiment, the cell is an ovarian cancer and the antigen is an ovarian antigen that optionally has been isolated from a tumor cell lysate. In other embodiments, the cancer may be a blood cancer, a glioma, a carcinoma, a sarcoma, a lung cancer, an intraperitoneal cancer, a colorectal cancer, a colon cancer, an ovarian cancer, a melanoma, or a breast cancer.

[0148] When performed *in vitro*, the method can be used to assay for personalized therapies, new therapies or to test combination therapies. As is known to those of skill in the art, it is preferable to also test one or more positive and negative control cell lines and agents for efficacy. When practiced *in vivo*, the method can be practiced in a mammal such as an animal model to assay for personalized therapies, new therapies or to test combination therapies. As is known to those of skill in the art, it is preferable to also test one or more positive and negative control animal models and agents for efficacy. In addition, the method is practiced *in vivo* in a subject in need thereof, such as a human patient.

[0149] Also provided is a method to deliver an antigen or fragment thereof to a cell or tissue, such as a cancer cell comprising, or consisting essentially of, or yet further consisting of, contacting the cell with a conjugate as described herein. In one aspect, the cell or tissue is a mammalian cell or tissue, such as a human cell or tissue. The contacting can be *in vitro* or *in vivo*.

[0150] In one aspect, the conjugate contains one or more cancer antigen(s), that in one aspect, are derived from tumor cell lysate. In some embodiments, the tumor cell lysate may be oxidized, irradiated, or freshly prepared from the tumor cell (**FIGS. 2 and 11**) . Preferably, the tumor cell lysate is freshly prepared from the tumor cell. Treatment with a conjugate comprising tumor cell lysate packaged in a liposome, and CPMV led to increased survival than treatment with only tumor cell lysate packaged in a liposome, CPMV, or a combination of tumor cell lysate packaged in a liposome+CPMV (**FIG. 2**). In one aspect the antigen is ovalbumin. In one aspect, the plant virus nanoparticle is conjugated to the LNP via a DBCO groups, optionally wherein the DBCO are chemically conjugated to the plant virus nanoparticle via terminal NH₂ groups.

[0151] Further provided is a method for one or more of in a subject in need thereof: delaying, slowing down, or preventing the relapse of a cancer; treating cancer; triggering or enhancing one or more of the following in the subject in need thereof: an anti-cancer immune response, an anti-cancer innate immune responses, a T cell-dependent anti-cancer immune response, T cell priming, a CD8⁺ T cell-dependent immune response, or an immunological memory, comprising, consisting essentially thereof, or consisting of administering an effective amount of the conjugate, the plurality, or the composition as described herein to the subject. For example, as indicated in **FIG. 2**, the conjugates can decrease tumor circumference and increase the survival rate as compared to the negative controls and individual components of the conjugates added separately or in combination. Further, as indicated in **FIG. 4**, the conjugates can improve delivery of both liposomes and plant virus nanoparticles to antigen producing cells. Further still, the conjugates can augment T cell infiltration and activation in the intraperitoneal space (**FIGS. 5E, 5J, and 6**).

[0152] “Administration” can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. Suitable dosage formulations and methods of administering the agents are known in the art. Route of administration can also be determined and method of determining the most effective route of administration are

known to those of skill in the art and will vary with the composition used for treatment, the purpose of the treatment, the health condition or disease stage of the subject being treated, and target cell or tissue. Non-limiting examples of route of administration include systemic or localized administration, e.g., oral administration, intratumorally, or localized at the site of the cancer or tumor, nasal administration, injection, and topical application. Additional non-limiting routes of administration include transdermal, intranasal, vaginal, rectal, subcutaneous intravenous, intravenous, intraarterial, intramuscular, intraosseous, intraperitoneal, intraocular, subconjunctival, sub-Tenon's, intravitreal, retrobulbar, intracameral, intratumoral, epidural and intrathecal. In one aspect, administration is systemic or intraperitoneal.

[0153] In one aspect, the one or more cancer antigen(s) used in the methods of treatment are derived from tumor cell lysate. In some embodiments, the tumor cell lysate may be oxidized, irradiated, or freshly prepared from the tumor cell (**FIGs. 2 and 11**). Preferably, the tumor cell lysate is freshly prepared from the tumor cell. Treatment with a conjugate comprising tumor cell lysate packaged in a liposome, and CPMV led to increased survival than treatment with only tumor cell lysate packaged in a liposome, CPMV, or a combination of tumor cell lysate packaged in a liposome+CPMV (**FIG. 2**).

[0154] In some embodiments, the cancer may be a primary cancer or a metastatic cancer. According to one embodiment, the cancer is ovarian cancer. In other embodiments, the cancer may be a blood cancer, a glioma, a carcinoma, a sarcoma, a lung cancer, an intraperitoneal cancer, a colorectal cancer, a colon cancer, an ovarian cancer, a melanoma, or a breast cancer.

[0155] In one aspect, the one or more cancer antigen(s) are autologous to the subject, for example in tumor cell lysate derived from the tumor of the subject. Alternatively, or in addition, the one or more cancer antigen(s) comprise ovarian cancer antigens and the subject in need thereof has been diagnosed with ovarian cancer. In one aspect the antigen is ovalbumin.

[0156] In a further aspect, the subject is an animal or mammal, such as a human patient.

[0157] In another aspect, the method further comprises isolating one or more cancer antigens from the subject and using the one or more cancer antigens to prepare preparing the conjugate. Alternatively, the conjugate administered to the subject comprises one or more cancer antigens isolated from the subject or are derived from a cancer antigen isolated from the subject.

[0158] In a further aspect of this disclosure, the administration(s) of the conjugate or composition is repeated for at least once, or at least twice, or more, and/or wherein two administrations are about 1 day to about 1 year apart, or about 1 week apart, or about 2 weeks apart, or about 3 weeks apart, or about 4 weeks apart, or about 1 month apart, or about 2 months apart, or about 3 months apart, or about 6 months apart, and/or wherein the administration is subsequent to tumor resection, and/or wherein the subject is a cancer patient who has been treated by one or more of: an ablative therapy, a chemotherapy, a radiation therapy, an immune checkpoint blockade therapy, or another anti-cancer therapy.

[0159] In one embodiment, the method further comprises treating the subject with one or more of: an ablative therapy, a chemotherapy, a radiation therapy, an immune checkpoint blockade therapy, or another anti-cancer therapy, and/or wherein the conjugate or composition is administered as a first line therapy, a second line therapy, a third line therapy or a fourth line therapy, and/or wherein the plant viral nanoparticle has a diameter of from about 15 nm to about 60 nm.

[0160] According to one embodiment is method for treating cancer in a subject in need thereof, comprising administering an effective amount of a conjugate comprised of a liposome and a plant virus nanoparticle, wherein the liposome comprises at least one cancer-associated antigen, and wherein the plant virus nanoparticle is CPMV, optionally wherein the cancer is ovarian cancer, optionally wherein the cancer-associated antigen is derived from tumor cell lysate, and optionally wherein the tumor cell lysate is oxidized or irradiated.

[0161] As is apparent to the skilled artisan, in one aspect, an effective amount is administered which is determined by the treating physician or veterinarian.

[0162] When administered to an animal, the methods provide an animal model to test combination therapy or personalized therapies. For example, the animal can be inoculated

with tumor cells from a human patient. Alternatively, when the subject is a non-human animal, the methods provide veterinarian therapies. When the subject is a human patient, the methods provide methods to achieve one or more of the disclosed therapeutic benefits.

[0163] Further provided is a kit comprising the conjugate, and/or the plurality, and/or the composition as described herein, and an optional instruction for use.

[0164] Dosages and Dosing Regimens

[0165] The appropriate amount and dosing regimen of the component or the combination, when present to be administered to the subject according to any of the methods disclosed herein, may be determined by one of ordinary skill in the art.

[0166] In some embodiments, the component or the combination as disclosed herein, may be administered to a subject in need thereof, either alone or as part of a pharmaceutically acceptable formulation, once a week, once a day, twice a day, three times a day, or four times a day, or even more frequently.

[0167] Administration of the component or the combination as disclosed herein may be effected by any method that enables delivery of the component or the combination to the site of action. These methods include oral routes, intraduodenal routes, parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion), topical, and rectal administration. Bolus doses can be used, or infusions over a period of 1, 2, 3, 4, 5, 10, 15, 20, 30, 60, 90, 120 or more minutes, or any intermediate time period can also be used, as can infusions lasting 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 20, 24 or more hours or lasting for 1-7 days or more. Infusions can be administered by drip, continuous infusion, infusion pump, metering pump, depot formulation, or any other suitable means.

[0168] Dosage regimens may be adjusted to provide the optimum desired response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form, as used herein, refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit containing a predetermined quantity of active

compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on (a) the unique characteristics of the chemotherapeutic agent and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0169] Thus, the skilled artisan would appreciate, based upon the disclosure provided herein, that the dose and dosing regimen is adjusted in accordance with methods well-known in the therapeutic arts. That is, the maximum tolerable dose can be readily established, and the effective amount providing a detectable therapeutic benefit to a patient may also be determined, as can the temporal requirements for administering each agent to provide a detectable therapeutic benefit to the patient. Accordingly, while certain dose and administration regimens are exemplified herein, these examples in no way limit the dose and administration regimen that may be provided to a patient in practicing the present disclosure.

[0170] It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated, and may include single or multiple doses. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. For example, doses may be adjusted based on pharmacokinetic or pharmacodynamic parameters, which may include clinical effects such as toxic effects and/or laboratory values. Thus, the present disclosure encompasses intra-patient dose-escalation as determined by the skilled artisan. Determining appropriate dosages and regimens for administration of the chemotherapeutic agent are well-known in the relevant art and would be understood to be encompassed by the skilled artisan once provided the teachings disclosed herein.

[0171] **Experimental Methods**

[0172] In this study, Applicant conjugated tumor cell lysates (TCL)-laden liposomes to CPMV particles to develop an antigen–adjuvant combination immunotherapy for ovarian

cancer. As proof of concept, Applicant introduced murine metastatic ovarian cancer cells (ID8-Defb29/Vegf-a-Luc) into C57BL/6J mice⁵⁴ and loaded liposomes with the TCL isolated from cultured cells of the same line, mimicking the use of autologous TCL. The efficacy of the TCL-Lip-CPMV complex was evaluated in mice with ID8-Defb29/Vegf-a-Luc ovarian tumors. Applicant also examined the co-delivery of TCL and CPMV to antigen-presenting cells (APCs) and the immunomodulatory and anti-tumor mechanisms.

[0173] Results and Discussion

[0174] *Production of TCL and TCL-laden liposomes*

[0175] TCL were derived from the syngeneic murine ovarian cancer cell line ID8-Defb29/Vegf-a-Luc, which closely resembles human high-grade serous carcinoma (HGSC)⁵⁴. Confluent cells were harvested, washed, and exposed to five freeze–thaw cycles, and the supernatant (TCL) was recovered by centrifugation²² (**FIG. 7A**). The TCL were then loaded into 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC; 90% molar) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-azido(polyethylene glycol)-2000 (DPPE-PEG2K-azide; 10% molar) liposomes by thin-film rehydration^{22, 55}. The heterogeneous liposome population was processed by extrusion to generate more uniform 100-nm liposomes, as confirmed by dynamic light scattering (DLS) (**FIG. 7B**). Unpackaged TCL was removed by tangential flow filtration (TFF) until no proteins were detected in the flow-through (FT) fraction by NUPAGE and UV-Vis spectrophotometry (**FIGS. 7C, 7D**). The average TCL loading capacity was 103.4 ± 13.4 μg TCL/mg lipids based on six independent preparations (**FIG. 7E**). The formulation was validated by cryo-electron microscopy (cryo-EM) (**FIG. 7F**).

[0176] *Conjugation of TCL-laden liposomes to CPMV*

[0177] Applicant previously showed that CPMV can be conjugated to irradiated ovarian cancer cells, forming an effective vaccine that protects mice from ovarian cancer⁵⁶. The co-delivery of a tumor antigen and an adjuvant to the same APCs therefore appears to promote antigen processing and presentation, triggering an antigen-specific anti-tumor immune response⁵⁷. Here, Applicant conjugated CPMV to the TCL loaded liposomes (TCL-Lip) to form complexes (hereafter TCL-Lip-CPMV, **FIG. 1A**) using azide and DBCO click chemistry (**FIG. 1B**). Azide groups were introduced during liposome formulation using

appropriate lipids (DPPE-PEG2K-azide). The surface-exposed lysine residues of CPMV were then functionalized with an alkyne handle using NHS chemistry^{33, 58, 59}. CPMV was therefore conjugated to DBCO-PEG₄-NHS ester, a bifunctional linker, to produce CPMV-DBCO⁶⁰. Purified CPMV-DBCO particles were similar to native CPMV in terms of absorbance (A_{260/280} ~1.7) indicating the presence of intact particles containing RNA⁶¹ (**FIG. 8A**). DLS revealed the particles were ~30 nm in diameter (**FIG. 8B**) and transmission electron microscopy (TEM) confirmed that CPMV and CPMV-DBCO were intact and monodisperse (**FIG. 8C**).

[0178] The CPMV-DBCO particles were then mixed with the TCL-loaded liposomes to facilitate the reaction between DBCO and DPPE-PEG2K-azide. The success of the reaction was confirmed by NUPAGE (**FIG. 1C**). The two CPMV coat proteins (L and S) have molecular weights of ~42 and ~24 kDa⁶², which can be seen in the lanes containing the individual reaction components. Only the TCL-Lip-CPMV lane contained heavier bands corresponding to L-PEG2K-DPPE (~44.7 kDa) and S-PEG2K-DPPE (~26.7 kDa) in addition to the unconjugated L and S proteins (**FIG. 1C**); the difference in molecular weight corresponds to the molecular weight of the lipids. Successful conjugation of the TCL-Lip to CPMV was further confirmed by size exclusion chromatography (SEC), showing that TCL-Lip-CPMV eluted as a single peak rather than separate peaks for the liposomes and CPMV particles (**FIG. 1D**). No free proteins were observed at end of the column volume, indicating that conjugation to CPMV did not break the liposomes. Cryo-EM images showed that the liposomes were closely associated with CPMV particles (**FIG. 1E**) whereas these components were more widely separated in the unconjugated mixture (**FIG. 9**). The presence of free CPMV-DBCO particles and TCL-loaded liposomes indicated that conjugation was not 100% efficient.

[0179] *TCL-Lip-CPMV improves ovarian cancer treatment efficacy*

[0180] The efficacy of TCL-Lip-CPMV was assessed in C57BL/6J mice with ovarian tumors induced by intraperitoneal (i.p.) challenge using ID8-Defb29/Vegf-a-Luc cells. Applicant started the treatment with TCL-Lip-CPMV 3 days before inducing the tumors to test its ability to prevent recurrence. Accordingly, Applicant administered the first i.p. dose of TCL-Lip-CPMV on day -3 and then injected 5 x 10⁶ ID8-Defb29/Vegf-a-Luc cells i.p. on day 0 to

induce tumors. Starting from day 4, each mouse received five further i.p. doses of TCL-Lip-CPMV at weekly intervals. Each treatment comprised 30 µg TCL within liposomes and 100 µg CPMV. Applicant used unconjugated CPMV and TCL-loaded liposomes (individually or mixed) as controls, as well as a HEPES buffer control, with n = 8 mice per treatment group (**FIG. 2A**). Tumor progression was monitored by measuring body weight gain (**FIG. 10**) and increases in body circumference (**FIGS. 2B, 2C**) resulting from the tumor burden and ascites. Mice that were tumor free on day 100 were treated as survivors. Mice were euthanized if their body weight exceeded 35 g or their circumference exceeded 9 cm³³.

[0181] Tumor growth was significantly inhibited by the TCL-Lip-CPMV treatment, with 5/8 mice remaining tumor free by the end of the study (**FIGS. 2B, 2C**). TCL-loaded liposomes alone had no significant effect, similar to the HEPES control. However, CPMV alone or mixed with the liposomes delayed the induction and growth of ovarian tumors, albeit at lower efficacy compared to the TCL-Lip-CPMV treatment (**FIG. 2D**). The TCL-Lip-CPMV group differed significantly from the HEPES control ($p < 0.0001$) and the TCL-Lip group ($p < 0.05$). In terms of overall survival, the TCL-Lip-CPMV group fared best (5/8) followed by the CPMV group (2/8, $p < 0.247$) and TCL-Lip + CPMV mixture group (1/8, $p < 0.064$) (**FIGS. 2B-2C**). The efficacy of TCL-Lip-CPMV was confirmed by its undefined median survival, whereas all other groups had a defined median survival: 45 days for the HEPES group, 42 days for the TCL-Lip group, 64 days for the TCL-Lip + CPMV group, and 69 days for the CPMV group. This demonstrates that CPMV and TCL-Lip + CPMV improve the median survival for treated mice compared to HEPES and TCL-Lip but the co-delivery of CPMV and TCL using the TCL-Lip-CPMV formulation increases the efficacy and survival benefit even further. Applicant also prepared oxidized TCL (OxTCL) and irradiated TCL (IrTCL) and loaded them into liposomes (i.e. OxTCL-Lip and IrTCL-Lip) to determine whether chemical oxidation or X-ray exposure of TCL would improve the efficacy even more (**FIGS. 10 and 11**). The OxTCL showed a marginal improvement over CPMV alone (**FIGS. 11A and 11C**). Although IrTCL were more efficacious than the CPMV treatment (**FIGS. 11B and 11C**), the original TCL-Lip-CPMV formulation still outperformed all other groups. Therefore, Applicant focused on the utilization of freshly isolated TCL.

[0182] *Co-delivery of TCL and CPMV in vitro and in vivo*

[0183] To determine whether TCL-Lip-CPMV improves the co-delivery of TCL and CPMV particles to APCs, Applicant labeled TCL with Oregon Green 488 (OG488) and CPMV with Cy5. OG488-TCL was produced by incubating purified TCL with OG488 maleimide and OG488 carboxylic acid succinimidyl ester to label the free thiol and amine groups, as confirmed by NUPAGE (**FIG. 12A**). Applicant produced CPMV-DBCO-Cy5 and CPMV-Cy5 particles by incubating CPMV with sulfo-Cy5-NHS ester with or without DBCO-PEG₄-NHS ester. NUPAGE and agarose gel electrophoresis confirmed the conjugation of Cy5 (**FIGS. 12B and 12C**). The UV-Vis spectra of CPMV, CPMV-Cy5 and CPMV-DBCO-Cy5 particles were similar, indicating the presence of ~55 Cy5 molecules per CPMV-Cy5 and ~44 per CPMV-DBCO-Cy5 (**FIG 12D**). DLS confirmed that CPMV-Cy5 and CPMV-DBCO-Cy5 were ~30 nm in diameter, the same as native CPMV (**FIG. 12F**) and both particle types were found to be intact and monodisperse by TEM (**FIG. 12F**). After unconjugated free dye molecules were removed, OG488-TCL were loaded into 100-nm liposomes as described above. Applicant then prepared OG488-TCL-Lip + CPMV-Cy5 mixtures and conjugated the two components by mixing OG488-TCL-Lip and CPMV-DBCO-Cy5. Agarose gel electrophoresis showed separate signals for CPMV and TCL-Lip in all lanes except OG488-TCL-Lip-CPMV-Cy5, where the OG488 and Cy5 signals were colocalized due to conjugation (**FIG. 3A**). NUPAGE showed two additional bands in the OG488-TCL-Lip-CPMV-Cy5 lane, confirming the presence of CPMV coat proteins with linkers (**FIG. 3B**).

[0184] Having formulated the different fluorescent constructs, Applicant first compared their uptake and co-delivery *in vitro* using bone marrow derived dendritic cells (BMDCs) isolated and prepared from C57BL/6J mice⁵³. OG488-TCL-Lip-CPMV-Cy5 and formulations of the individual and mixed components were incubated with BMDCs for 1 and 24 h. The BMDCs were then analyzed by confocal microscopy and flow cytometry. At both time points, BMDCs were able to take up the TCL and/or CPMV in all groups (**FIGS 3C and 13A**), but differences between OG488-TCL-Lip-CPMV-Cy5 and the other formulations were observed by flow cytometry. After 1 h, nearly 40% of BMDCs were double-negative (no uptake of TCL-Lip or CPMV) in the TCL-Lip, TCL-Lip + CPMV, and TCL-Lip-CPMV groups and >70% were double-negative in the CPMV group, probably because BMDCs prefer to take up larger particles (**FIG. 12B**). However, after 24 h, few of the BMDCs were double-negative in the OG488-TCL-Lip-CPMV-Cy5 group whereas >20% of BMDCs remained double-negative without either or both components (**Fig. 3d**). There were significantly more double-

positive BMDCs in the TCL-Lip + CPMV group (26%) than the TCL-Lip-CPMV group (16%) after 1 h (**FIG. 13C**), but after 24 h the difference was marginal, with 64% double-positive for TCL-Lip-CPMV and 70% for TCL-Lip + CPMV (**FIG. 3E**). One explanation for this result is that TCL-Lip and CPMV can interact with BMDCs independently when they are presented as a mixture but not when they are conjugated, thus resulting in a slower uptake, especially in an *in vitro* environment. Even so, the conjugation of CPMV to TCL-Lip resulted in almost 100% of the BMDCs becoming CPMV positive after 24 h (**FIG. 13D**), which is necessary to facilitate antigen processing.

[0185] Next, Applicant determined whether the TCL-Lip-CPMV formulation could improve the co-delivery of TCL and CPMV *in vivo* for antigen processing. The labeled TCL-Lip-CPMV and other formulations were injected subcutaneously (s.c.) into the footpads of C57BL/6J mice (**FIG. 14A**). Popliteal draining lymph nodes (PDLNs) were harvested 4, 8, 24, 72 and 168 h post injection for IVIS imaging and confocal microscopy. IVIS imaging revealed the presence of TCL and CPMV in the PDLNs (**FIG. 14B**). OG488 fluorescence from TCL-Lip was the most intense after 4 and 8 h followed by rapid intensity decay within 72 h, which indicated that TCL was quickly processed within 3 days inside the PDLNs and transported to other lymphatic organs (**FIG. 3F**). In contrast, Cy5 fluorescence from CPMV remained relatively stable over the time course of the experiment, indicating that CPMV was slowly processed in PDLNs due to its exceptional stability (**FIG. 3G**). Indeed, Applicant previously observed that CPMV circulated for >7 days in the lymphatic system regardless of the injection route due to its stable structure^{53, 63}. Cy5 fluorescence in the TCL-Lip-CPMV group was stronger than the other CPMV-Cy5 groups, indicating more CPMV was taken up by lymphocytes. However, Applicant cannot rule out the possibility that the intrinsic fluorescence intensity of CPMV-DBCO-Cy5 was higher even though it had ~44 Cy5 per CPMV vs ~55 Cy5 for CPMV-Cy5 (**FIGS. 12D and 14C**). Confocal images of lymph node sections also indicated the colocalization of OG488 and Cy5 with DCs, macrophages, B cells and T cells in the TCL-Lip + CPMV and TCL-Lip-CPMV groups after 4 and 24 h (**FIGS. 4A**, additional data available but not shown). More importantly, even though most of the TCL-Lip and CPMV signals were colocalized in the TCL-Lip + CPMV group, the CPMV signal had penetrated deeper into the lymph nodes, whereas almost all the TCL-Lip and CPMV were colocalized in the TCL-Lip-CPMV group. These results indicate that conjugation led to the trafficking of TCL-Lip and CPMV together, whereas the components

were trafficked independently when presented as a mixture, resulting in the deeper penetration of CPMV.

[0186] To examine the uptake of the formulations by CD11c⁺ DCs and F4/80⁺ macrophages in the lymph nodes, single-cell suspensions were prepared from the PDLNs and analyzed by flow cytometry. Significantly more TCL and CPMV particles were co-delivered to DCs and macrophages in the TCL-Lip-CPMV group vs the mixture after 4 h, but the difference became negligible after 72 h (**FIGS. 4B and 15A**). Similarly, the number of DCs and macrophages positive only for TCL at the 4 h time point was higher in the TCL-Lip-CPMV group, but this was not the case for CPMV delivery (**FIGS. 4C and 15B**). Although conjugating CPMV to liposomes did not improve the delivery of CPMV to DCs and macrophages at the early time point, it dramatically changed the residence time as demonstrated by the faster drop in the number of CPMV⁺ DCs and macrophages over time, possibly due to the faster digestion of CPMV or trafficking of CPMV to other lymphatic organs (**FIGS. 4D and 15c**). Additionally, throughout the time course of the experiment, the TCL-Lip + CPMV and CPMV groups showed similar percentages of CPMV⁺ DCs and macrophages, confirming that CPMV was trafficked freely and independently when mixed with liposomes. Overall, these data show that conjugating CPMV to TCL-Lip not only improved the co-delivery of both components to APCs but also increased the processing of CPMV to stimulate the immune system.

[0187] *Activation of DCs within draining lymph nodes*

[0188] To determine whether TCL-Lip-CPMV complexes can activate APCs, Applicant administered the unlabeled formulations by s.c. injection into the footpads and characterized the activation status of CD11c⁺ MHC-II⁺ DCs after 4 and 24 h by measuring the levels of surface markers CD40, CD80 and CD86. After 4 h, CD40 and CD80 remained at basal levels in all groups and CD86 remained at basal levels in the TCL-Lip group and PBS control but was elevated ~1.3 fold in all three groups containing CPMV (**FIG. 16**). After 24 h, CD40, CD80 and CD86 were elevated ~1.6 fold (CPMV), ~2 fold (TCL-Lip + CPMV), and ~2.5 fold (TCL-Lip-CPMV, respectively, relative to the TCL-Lip group and PBS control (**FIGS. 4E-4G**). There was no significant difference between the TCL-Lip group and PBS control, ruling out immune stimulation by DOPC, DPPE-PEG2K-azide lipids or the TCL.

However, there was also no significant difference between the CPMV, TCL-Lip + CPMV, and TCL-Lip-CPMV groups, suggesting that CPMV could stimulate the DCs effectively when delivered alone or combined with liposomes⁵³. Improving the delivery of TCL into activated DCs by conjugating CPMV to TCL-Lip thus promotes tumor antigen processing and enhances anti-tumor immunity.

[0189] *Modulation of the adaptive immune system within the i.p. space and spleen*

[0190] To investigate how the different treatments modulate the immune cell population within the i.p. space and spleen, mice (n = 5 per group) were sacrificed 7 days after the third treatment (day 18 post-inoculation, **FIG. 17A**), and cells isolated from the i.p. washes and spleens were dissociated into splenocyte suspensions for analysis by flow cytometry (**FIG. 17B**). More CD4⁺ and CD8⁺ T cells infiltrated into the i.p. space for all the treatment groups involving CPMV compared to the TCL-Lip group and HEPES control (**FIGS. 5A, 5G**), this can be explained by the immunomodulatory nature of CPMV⁴⁷. With regard to the distribution of CD4⁺ T cells, the CPMV, CPMV + TCL-Lip and TCL-Lip-CPMV groups showed similar percentages of memory (CD44⁺) T cells (**FIG. 5B**) including effector memory (CD44⁺CD62L⁻) T cells, higher than the TCL-Lip group and HEPES control (**FIG. 5C**). In contrast, the CPMV, CPMV + TCL-Lip and TCL-Lip-CPMV groups featured significantly less central memory (CD44⁺CD62L⁺) T cells than the other groups (**FIG. 5D**). CPMV can reside within the i.p. space for over 7 days⁶⁴. Therefore, this result may be attributed to the prolonged CPMV residing within the i.p. space, therefore increasing the duration of antigen exposure, which favors CD62L^{Lo} memory CD4 T cells⁶⁵. Interestingly, the TCL-Lip-CPMV group featured significantly more activated (CD69⁺) T cells than all other groups. With regard to the distribution of CD8⁺ T cells, there was no significant difference between the TCL-Lip-CPMV and TCL-Lip + CPMV groups, but there was an increasing trend in the overall number of CD8⁺ T cells and the proportions of CD44⁺ memory T cells, CD44⁺CD62L⁻ effector memory T cells, CD44⁺CD62L⁺ central memory T cells, and CD69⁺ activated T cells in the following sequence: HEPES → TCL-Lip → CPMV → TCL-Lip + CPMV → TCL-Lip-CPMV (**FIGS. 5F-5J**). The analysis of splenocytes by flow cytometry showed no differences among the T cell populations in all five groups (**FIG. 18**). In summary, all treatments containing CPMV improved the infiltration of CD4⁺ and CD8⁺ T cells into the i.p. space, whereas the addition of TCL-Lip, especially when conjugated to

CPMV, further augmented CD8⁺ T cell infiltration and activation in the i.p. space. This may explain why TCL-Lip-CPMV showed better efficacy in slowing down the onset of tumor growth because it encouraged more inflammation within the i.p. space, creating an environment hostile to tumors. When approaching the onset of tumor growth, T cells within the i.p. space could attack the cancer cells, establishing adaptive immunity within the spleen to improve survival benefits.

[0191] Finally, to test for TCL-specific adaptive immunity, Applicant evaluated the antigen-specific CD8⁺ T cells within the i.p. space and spleen. TCL contains many different tumor proteins, so Applicant replaced them with a single protein – ovalbumin (OVA) – as a model antigen and evaluated CD8⁺ T cells that recognize the identified MHC-I peptide SIINFEKL. First, Applicant generated 100-nm OVA-laden liposomes (OVA-Lip) (**FIG. 19A**) and used the click chemistry approach described above to produce OVA-Lip-CPMV complexes (**FIGS. 6A and 19B**) analogous to TCL-Lip. Next, Applicant administered two i.p. doses of OVA-Lip, CPMV, OVA-Lip + CPMV, and OVA-Lip-CPMV into C57BL/6J mice (n = 5) at weekly intervals in a prime–boost regimen. Seven days after the second dose, i.p. washes and spleens were harvested and processed to identify SIINFEKL⁺ CD8⁺ cells in an MHC-I dextramer assay (**FIG. 20A**). Applicant observed significantly more SIINFEKL⁺ CD8⁺ T cells in the OVA-Lip-CPMV group compared to all other groups in both the i.p. washes and splenocytes (**FIGS. 6B, 6C, and 20**). In the i.p. space, the proportion of SIINFEKL⁺ cells among all CD8⁺ cells were ~5.4% for the OVA-Lip-CPMV group compared to ~4.2% for OVA-Lip + CPMV, ~2.8% for CPMV, and ~2% for OVA-Lip (**FIG. 6B**). In the spleen, the proportion of SIINFEKL⁺ CD8⁺ cells remained below 1.5% in all groups but was highest in the OVA-Lip-CPMV group (~1.3%) and below 1% in the others (**FIG. 6C**). Applicant observed a greater overall number of CD8⁺ T cells in the i.p. space following the liposome-CPMV treatment. Boosting the number of antigen-specific CD8 T cells would enhance the anti-tumor adaptive immune response, leading to greater efficacy and higher survival benefits.

[0192] Conclusion

[0193] Applicant has designed a novel immunotherapeutic modality (TCL-Lip-CPMV) that combines a plant virus with TCL-loaded liposomes to prevent the recurrence of ovarian

cancer. Autologous TCL provides a personalized source of tumor antigens, which could be used to treat cancer patients after the removal of tumors. As proof of principle, Applicant loaded liposomes with TCL isolated from murine ovarian cancer cells and combined them with CPMV as an immunogenic adjuvant, triggering both innate and adaptive immunity. The TCL-Lip-CPMV complex achieves the simultaneous delivery of CPMV and TCL to DCs and macrophages in draining lymph nodes, enhancing antigen presentation. Moreover, the same complex establishes adaptive immunity within the intraperitoneal (i.p.) space and spleen, promoting T cell infiltration and the generation of antigen-specific CD8⁺ T cells. Given that ovarian cancer often metastasizes within the i.p. space, this approach targets the TME specific to ovarian cancer and prevents tumor development. Furthermore, the TCL-Lip-CPMV complex offers a promising new approach for cancer vaccine development and immunotherapy. This versatile platform allows the loading of various agents such as drugs, antigens and nucleic acids into liposomes, followed by conjugation with CPMV as an adjuvant. This will facilitate the development of cancer vaccines and combination therapies with enhanced efficacy.

[0194] Materials and Methods

[0195] *Production of CPMV and CPMV conjugates* – CPMV was propagated in black eyed pea no. 5 plants and purified as previously reported^{56, 66}. CPMV-DBCO, CPMV-Cy5 and CPMV-DBCO-Cy5 derivatives were synthesized by attaching DBCO-PEG4-NHS ester (649.7 g/mol, BroadPharm) and/or sulfo-Cy5 NHS ester (777.95 g/mol, Lumiprobe) linkers to solvent-exposed lysine residues on the CPMV surface. CPMV-DBCO was prepared by mixing a 1200-molar excess of DBCO-PEG4-NHS ester with 2 mg/mL (final concentration) of CPMV (molecular weight = 5.6×10^6 g/mol) in PBS. CPMV-Cy5 was prepared by mixing a 1500-molar excess of sulfo-Cy5 NHS ester with CPMV as above. CPMV-DBCO-Cy5 was prepared by mixing a 1200-molar excess of DBCO-PEG₄-NHS ester and a 1500-molar excess of sulfo-Cy5 NHS ester with CPMV as above. All reactions were incubated for 2 h at room temperature. To remove unconjugated DBCO-PEG₄-NHS ester and sulfo-Cy5 NHS ester, the reaction mixtures were loaded onto Amicon Ultra-0.5 mL centrifugal filters with a 100-kDa cutoff (Millipore Sigma) and washed four times with 50 mM HEPES (pH 7.4). Purified CPMV-DBCO, CPMV-Cy5, and CPMV-DBCO-Cy5 particles were stored at 4 °C.

[0196] *Cell culture and production of TCL* – ID8-Defb29/Vegf-a-Luc cells were cultured in RPMI 1640 medium with L-glutamine (Corning) supplemented with 10% (v/v) fetal bovine serum (FBS) (VWR), 1% (v/v) penicillin/streptomycin (Pen/Strep) (Cytiva), 1 mM sodium pyruvate (Thermo Fisher Scientific), and 0.05 mM β -mercaptoethanol (Thermo Fisher Scientific) in an incubator at 37 °C with a 5% CO₂ atmosphere. The cells were harvested in trypsin-EDTA (Corning), washed three times with PBS (Corning) and resuspended in 50 mM HEPES (pH 7.4) at 1×10^7 cells/mL. After five freeze–thaw cycles using liquid nitrogen and a 37 °C water bath, cell debris was removed by two rounds of centrifugation ($14,000 \times g$, 10 min, room temperature). TCL was recovered and stored at –80 °C. The concentration of proteins was determined using a bicinchoninic acid (BCA) assay kit (Thermo Fisher Scientific) on a Tecan plate reader.

[0197] To track TCL *in vitro* and *in vivo*, they were labeled with OG488 by incubating 5 mg TCL and 1 mg OG488 carboxylic acid, succinimidyl ester, 5-isomer (Thermo Fisher Scientific) and 1 mg OG488 maleimide (Thermo Fisher Scientific) in 5 mL 50 mM HEPES (pH 7.4) overnight at 4 °C. OG488-labeled TCL (OG488-TCL) were then purified twice on PD-10 desalting columns (Cytiva) using 50 mM HEPES (pH 7.4).

[0198] *Production of TCL-Lip and OVA-Lip* – TCL-Lip and OVA-Lip were produced using the thin-film rehydration method⁶⁷. First, DOPC (25 mg/mL) and DPPE-PEG2K-azide (20 mg/mL) lipids (Avanti Polar Lipids) in chloroform were mixed together to give a 10% molar ratio of azide groups. After removing the chloroform using a rotary evaporator at room temperature for 30 min, 0.5 mg/mL TCL or OVA (Sigma-Aldrich) in 50 mM HEPES (pH 7.4) was added to rehydrate the lipid layer using the rotary evaporator at room temperature for 30 min, giving 1 mg TCL or OVA per 5 mg lipids. After rehydration and five freeze–thaw cycles as above, TCL-Lip and OVA-Lip were extruded 10 times using a GJE-10-mL jacketed liposome extruder (Genizer) and Whatman Nuclepore track-etched membranes (Millipore Sigma) with pore sizes of 200 and 100 nm. After the final extrusion, unpackaged TCL and OVA were removed by TFF using a MicroKros hollow-fiber filter (Repligen) with a 500-kDa cutoff and five washes in 50 mM HEPES (pH 7.4) until no protein was detected in the FT fraction using a BCA assay and UV-Vis. After the final TFF cycle, the volume of TCL-Lip and OVA-Lip was reduced to ~1 mL. The lipid concentration was determined using a phosphatidylcholine assay kit (Sigma-Aldrich) and the concentration of packaged

TCL and OVA was determined using a BCA assay (after mixing the liposomes with 1% SDS to break them). OG488-TCL-Lip was prepared using the same method.

[0199] *Conjugation of CPMV to TCL-Lip and OVA-Lip* – Applicant mixed 1 mg/mL CPMV-DBCO and 0.3 mg/mL TCL for 8 h at room temperature to form the conjugated TCL-Lip-CPMV complex. The unconjugated mixture (TCL-Lip + CPMV) was prepared by mixing 1 mg/mL CPMV with 0.3 mg/mL TCL. OVA-Lip-CPMV and OVA-Lip + CPMV were prepared in the same manner. OG488-TCL-Lip-CPMV-Cy5 was prepared by mixing 1 mg/mL CPMV-DBCO-Cy5 and 0.3 mg/mL OG488-TCL. The OG488-TCL-Lip + CPMV-Cy5 mixture was prepared by mixing 1 mg/mL CPMV-Cy5 and 0.3 mg/mL OG488-TCL-Lip.

[0200] *Characterization of CPMV, CPMV conjugates, TCL, and liposomes*

[0201] *NuPAGE* – Samples were mixed with 4× lithium dodecylsulfate buffer (Thermo Fisher Scientific) and 10× reducing agent (Invitrogen) and heated to 95 °C for 8 min before loading 20 µL per lane onto a 4–12% NuPAGE gel (Thermo Fisher Scientific). Gels were run at 200 V, 120 mA, and 25 W for 35 min in 1x MOPS buffer (Thermo Fisher Scientific). For the labeled samples, Applicant first imaged OG488 (MultiColor green filter) and Cy5 (MultiColor red filter) then stained the gels with Coomassie Brilliant Blue for protein imaging. All images were recorded using a ProteinSimple FluorChem R imager.

[0202] *UV-Vis spectrophotometry* – The concentrations of CPMV and its derivatives were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) and the extinction coefficient (ϵ) of CPMV at 260 nm = 8.1 mL / (mg x cm). The number of conjugated sulfo-Cy5 units per CPMV-Cy5 or CPMV-DBCO-Cy5 particle was determined using the molar extinction coefficient (ϵ) for sulfo-Cy5 (271000 L / (mol x cm)) at 647 nm. The FT fractions produced by TFF were also examined by spectrophotometry to ensure the complete removal of unpackaged TCL from TCL-Lip.

[0203] *Cy5 fluorescence intensity measurement* – Applicant compared the intensity of CPMV-Cy5 and CPMV-DBCO-Cy5 fluorescence by measuring the emission of 1 mg/mL CPMV-Cy5 and CPMV-DBCO-Cy5 on a Tecan plate reader (excitation wavelength 633 nm, emission wavelength of 665 nm).

[0204] *Agarose gel electrophoresis* – Samples comprising 10 µg CPMV and/or 30 µg TCL were mixed with 6× Gel Loading Purple dye (Biolabs) and loaded onto a 1.2% (w/v) agarose gel stained with GelRed nucleic acid gel stain (Gold Biotechnologies) and run at 100 V and 400 mA for 35 min in 1× Tris acetate EDTA (TAE) buffer (Thermo Fisher Scientific). RNA (UV light), OG488, and Cy5 signals were imaged before staining with Coomassie Brilliant Blue to detect proteins. Gels were imaged using a ProteinSimple FluorChem R imager.

[0205] *Dynamic light scattering (DLS)* – All CPMVs and liposomes were analyzed by DLS using a Zetasizer Nano ZSP/Zen5600 instrument (Malvern Panalytical). Specifically, Applicant loaded 100-µL aliquots of 1 mg/mL CPMV or liposomes containing 0.3 mg/mL TCL into a cuvette and measured them three times at room temperature.

[0206] *Transmission electron microscopy (TEM)* – CPMV particles were diluted to 0.5 mg/mL in 50 mM HEPES (pH 7.4), and 4 µL of each sample was applied to a glow-discharged carbon film with a 300-mesh Cu grid for 30 s. After blotting on filter paper, washing with Milli-Q water for 30 s, and blotting again, the samples were stained with 4 µL 1% (w/v) uranyl acetate (Electron Microscopy Sciences) for 30 s, followed by blotting with filter paper and air drying. The samples were imaged using a Talos TEM (Thermo Fisher Scientific) at a nominal magnification of 120,000×.

[0207] *Cryogenic electron microscopy (Cryo-EM)* – Before loading the samples, 200-mesh copper TEM grids with lacey carbon film were glow discharged using a Quorum Emiteck K100x device. Applicant then applied 4 µL of each sample onto the grids using an FEI Vitrobot Mark III (Thermo Fisher Scientific). The samples were visualized using a Talos Arctica microscope (Thermo Fisher Scientific) at a nominal magnification of 92,000×.

[0208] *Size exclusion chromatography (SEC)* – Samples were analyzed by SEC using a Superose 6 Increase 10/300 GL column mounted on an AKTA purifier system (GE Healthcare). The column was injected with 100-µL aliquots of 1 mg/mL CPMVs and/or liposomes with 0.3 mg/mL TCL at a flow rate of 0.5 mL/min, and the absorbance was monitored at 280 nm.

[0209] *Mouse models*

[0210] *Ethical statement* – All studies involving mice were carried out in accordance with the

guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University of California, San Diego (UCSD) and were approved by the Animal Ethics Committee of UCSD. Applicant obtained 7-week-old female C57BL/6J mice from Jackson Laboratories.

[0211] *Efficacy studies*

[0212] Five treatment groups (n = 8) were established: HEPES, CPMV, TCL-Lip, TCL-Lip + CPMV, and TCL-Lip-CPMV. All mice received six i.p. doses at weekly intervals on days – 3, 4, 11, 18, 25 and 32. Each dose comprised 100 µg CPMV and/or 30 µg TCL in 200 µL 50 mM HEPES (pH 7.4). On Day 0, Applicant injected 2×10^6 ID8-Defb29/Vegf-a-Luc cells in 200 µL PBS i.p. into each mouse. Mice were monitored every 2 days and the tumor burden was recorded as the increase in body weight and circumference. After 100 days, surviving mice were re-challenged by i.p. injection of 2×10^6 ID8-Defb29/Vegf-a-Luc cells in 200 µL PBS. Mice were euthanized when their body weight reached 35 g or their circumference reached 9 cm.

[0213] *Analysis of bone marrow-derived dendritic cells (BMDCs)*

[0214] BMDCs were prepared from a single-cell suspension of whole bone marrow cells isolated from the femurs and tibias of five female C57BL/6J mice. The cells were washed once with PBS, and the red blood cells were lysed for 5 min at room temperature using RBC lysis buffer (Invitrogen). The cells were washed twice with PBS, then resuspended in T-cell medium (RPMI 1640 medium + L-glutamine supplemented with 10% (v/v) FBS, 1% (v/v) Pen/Strep, 1 mM sodium pyruvate and 0.05 mM β-mercaptoethanol). To differentiate BMDCs, 3×10^6 cells per well were seeded into Costar TC treated 6-well plates (Corning). The medium was supplemented with 10 ng/mL mouse IL-4 and 15 ng/mL mouse GM-CSF (both from Biolegend) and cultured for 6 days at 37 °C in a 5% CO₂ atmosphere. On day 4, fresh medium was added containing 10 ng/mL mouse IL-4 and 15 ng/mL mouse GM-CSF. After 6 days, BMDCs were harvested and adjusted to 1×10^6 cells/mL in fresh medium and seeded into 12-well plates. Treatments containing 1 mg/mL CPMV-Cy5 and/or 0.3 mg/mL OG488-TCL and were added to the BMDCs and incubated as above for 1 or 24 h. The cells were then harvested for flow cytometry and confocal microscopy. For flow cytometry, BMDCs were blocked using anti-CD16/32 Fc block (Biolegend) on ice for 30 min, then stained using a PE-conjugated anti-CD11c antibody on ice for 1 h and fixed using the

Stabilizing Fixative 3x Concentrate (BD Biosciences). After washing, BMDCs were resuspended in BD Pharmingen Stain Buffer (BD Biosciences) and analyzed using a BD Accuri C6 Plus flow cytometer (BD Biosciences). Data were analyzed using Flowjo_v10.7. For confocal microscopy, leftover BMDCs from flow cytometry experiments were centrifuged onto Superfrost Plus Microscope Slides (Thermo Fisher Scientific) using a Statspin Cytofuge Cytocentrifuge (Beckman Coulter). All slides were then stained and mounted using Fluoroshield with DAPI (Millipore Sigma). Fluorescence images were obtained using a Nikon A1R confocal microscope with an Apo TIRF 100×/1.49 oil objective (Nikon). Collected images were analyzed using NIS-Elements AR Analysis v5.30 (Nikon).

[0215] *Lymphocytes – uptake experiments*

[0216] To study the co-delivery of TCL and CPMV into draining lymph nodes, treatments containing 100 µg CPMV-Cy5 and/or 30 µg OG488-TCL in 50 µL 50 mM HEPES (pH 7.4) were injected s.c. into the footpad of female C57BL/6J mice. After 4, 8, 24, 72 and 168 h, the mice were sacrificed to collect the PDLNs for fluorescence imaging using a Xenogen IVIS 200 imaging system to quantify OG488 and Cy5 fluorescence. The collected PDLNs were then processed to prepare slides for immunofluorescence imaging. In detail, PDLNs were flash frozen in Tissue-Tek OCT medium (Sakura) under liquid nitrogen, then 10-µm sections were prepared using a Leica CM1860 Cryostat. Sections were fixed in 4% paraformaldehyde (Electron Microscopy Sciences) at room temperature for 10 min, then washed with PBS and blocked with 1% (w/v) bovine serum albumin (BSA) (Thermo Fisher Scientific) for 1 h at room temperature. DCs, macrophages, B cells and T cells were stained with the following primary antibodies in PBS containing 1% BSA at 4°C for overnight: Armenian hamster anti-mouse CD11c monoclonal antibody (Biolegend, 1:100 dilution), rat anti-mouse F4/80 monoclonal antibody (Biolegend, 1:100 dilution), rat anti-mouse B220 monoclonal antibody (Biolegend, 1:100 dilution), and rat anti-mouse CD3 monoclonal antibody (Biolegend, 1:100 dilution). The next day, DCs were stained using a goat anti-Armenian hamster TRITC polyclonal antibody (abcam, 1:500 dilution) and the other cells were stained using a goat anti-rat Alexa Fluor 555 polyclonal antibody (Invitrogen, 1:500 dilution) at room temperature for 1 h. After washing with PBS and drying, the sections were mounted with Fluoroshield containing DAPI and imaged using a Nikon A1R confocal microscope with a filter set (DAPI, TRITC, FITC and APC) and a 20× objective lens. All

images were then processed using NIS-Elements AR Analysis v5.30. Single lymphocytes were prepared from the harvested PDLNs using the Spleen Dissociation Kit (Miltenyi Biotec) and a gentleMACS Octo Dissociator with heaters (Miltenyi Biotec). Lymphocytes were blocked, stained and fixed in preparation for flow cytometry as described above.

[0217] *Lymphocytes – activation experiments*

[0218] To study the activation of DCs in PDLNs, treatments containing 100 µg CPMV and/or 30 µg TCL were injected in the left and right footpad of female C57BL/6J mice and the PDLNs were processed as described above. Lymphocytes were then stained using LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific) on ice for 30 min to identify live cells. After washing, lymphocytes were blocked as above then stained with Pacific Blue anti-CD45 antibody (Biolegend), Super Bright 780 anti-CD11c antibody (Thermo Fisher Scientific), APC MHC-II antibody (Thermo Fisher Scientific), FITC anti-CD40 antibody (Thermo Fisher Scientific), Brilliant Violet 605 anti-CD80 antibody (Biolegend), and PerCP-Cy5.5 anti-CD86 antibody (Biolegend) on ice for 1 h. UltraComp eBeads (Thermo Fisher Scientific) were used to prepare single color staining for compensation. Lymphocytes were then fixed, washed and resuspended for flow cytometry as described above.

[0219] *Assessment of CD4⁺ and CD8⁺ T cells*

[0220] To evaluate T cell modulation within the i.p. space and spleen, treatments were administered as described above for the efficacy study. Seven days after the third injection, mice were sacrificed and i.p. washes and spleens were collected from five mice per group. Spleens were processed into single cell splenocytes using the Spleen Dissociation Kit and a gentleMACS Octo Dissociator with heaters. The red blood cells in the samples were lysed using RBC lysis buffer and the remaining cells were prepared using the LIVE/DEAD Fixable Aqua Dead Cell Stain Kit as described above. After washing and blocking as described above, cells were stained with Pacific Blue anti-CD45 antibody (Biolegend), APC-Cy7 anti-CD3 antibody (Biolegend), FITC anti-CD4 antibody (Biolegend), Brilliant Violet 785 anti-CD8 antibody (Biolegend), Brilliant Violet 605 anti-CD44 antibody (Biolegend), APC anti-CD62L antibody (Biolegend), PerCp-Cy5.5 anti-CD69 antibody (Biolegend) on ice for 1 h. UltraComp eBeads (ThermoFisher Scientific) were used to prepare single color staining for

compensation. The cells were then fixed, washed and resuspended for flow cytometry as described above.

[0221] *Assessment of antigen-specific CD8⁺ T cells*

[0222] TCL were replaced with the model antigen ovalbumin (OVA) (ThermoFisher Scientific) and the corresponding treatments containing 100 µg CPMV and/or 30 µg OVA were injected i.p. into C57BL/6J mice (n = 5) in a prime–boost regimen on days 0 and 7. On day 14, the mice were sacrificed and i.p. washes and spleens were harvested and processed as described above. The cells were further prepared according to the MHC I Dextramer Staining Protocol. Briefly, the cells were stained with the PE-conjugated H-2 Kb SIINFEKL Dextramer (Immudex) for 10 min on ice, then with Pacific Blue anti-CD45 antibody (Biolegend), APC-Cy7 anti-CD3 antibody (Biolegend), FITC anti-CD4 antibody (Biolegend) and Brilliant Violet 785 anti-CD8 antibody (Biolegend) for an additional 20 min. After five washes, the cells were resuspended for analysis by flow cytometry as described above.

[0223] *Production of Oxidized tumor cell lysates and irradiated tumor cell lysates*

[0224] ID8-Defb29/Vegf-a-Luc cells were cultured in RPMI 1640 medium with l-glutamine supplemented with 10% (v/v) FBS, 1% (v/v) Pen/Strep, 1 mM sodium pyruvate, and 0.05 mM β-mercaptoethanol in an incubator at 37 °C with a 5% CO₂ atmosphere.

[0225] *Oxidized tumor cell lysates* – ID8-Defb29/Vegf-a-Luc cells were harvested in trypsin-EDTA, washed, resuspended in growth media containing 60 µM HOCl (ThermoFisher Scientific) for 1 h at 37°C⁶⁸. Cells were then washed 3 times using PBS and adjusted to 1×10^7 cells/mL in 50 mM HEPES (pH 7.4). After five freeze–thaw cycles using liquid nitrogen and a 37 °C water bath, cell debris was removed by two rounds of centrifugation (14,000 × g, 10 min, room temperature). TCL was recovered and stored at –80 °C.

[0226] *Irradiated tumor cell lysates*

[0227] ID8-Defb29/Vegf-a-Luc cells were harvested in trypsin-EDTA, washed, resuspended in growth media, and irradiated at 70 Gray using an x-ray source. Cells were further cultured for 12 hours. After cells were harvested then washed 3 times using PBS and adjusted to 1×10^7 cells/mL in 50 mM HEPES (pH 7.4). After five freeze–thaw cycles using liquid nitrogen

and a 37 °C water bath, cell debris was removed by two rounds of centrifugation ($14,000 \times g$, 10 min, room temperature). TCL was recovered and stored at $-80\text{ }^{\circ}\text{C}$.

[0228] *Software for figure generation*

[0229] CPMV structure is generated using UCSF Chimera 1.16 using PDB: 1NY7.

Chemical structures were generated using ChemDraw 20.0.0. TCLs-Lip-CPMV and OVA-Lip-CPMV were generated using Biorender. Treatment and injection schedule figures were generated using Biorender and Adobe Illustrator. Other software includes GraphPad Prism 8 and FlowJo 10.7.1.

[0230] Embodiments

[0231] Additional embodiments include the following numbered embodiments:

[0232] 1. A conjugate comprising a liposome comprising one or more cancer antigen(s) conjugated to a plant virus nanoparticle.

[0233] 2. The conjugate of embodiment 1, wherein the liposome comprising the one or more cancer antigen(s) is a lipid nanoparticle (LNP).

[0234] 3. The conjugate of embodiment 1 or 2, wherein the plant virus nanoparticle comprises a cowpea mosaic virus (CPMV) particle.

[0235] 4. The conjugate of any of embodiments 1-3, wherein the CPMV particle is non-replicating and noninfectious toward mammals.

[0236] 5. The conjugate of any of embodiments 1-4, wherein the one or more cancer antigen(s) is selected from an antigen isolated from a cancer selected from a carcinoma, a sarcoma, or a hematological cancer.

[0237] 6. The conjugate of any of embodiments 1-5, wherein the one or more cancer antigen(s) comprise ovarian cancer cell antigens.

[0238] 7. The conjugate of any of embodiments 1-6, wherein the one or more cancer antigen(s) is derived from tumor cell lysate, optionally wherein the tumor cell lysate is oxidized or irradiated.

[0239] 8. The conjugate of any of embodiments 1-6, wherein the liposome comprises a plurality of the one or more cancer antigens that are the same or different from each other.

[0240] 9. The conjugate of embodiment 8, wherein the liposome comprises a LNP and the plant virus nanoparticle comprises a CPMV particle.

[0241] 10. The conjugate of any of embodiments 1-9, wherein the plant virus nanoparticle is conjugated to the liposome via a DBCO groups.

[0242] 11. The conjugate of embodiment 10, wherein the DBCO are chemically

conjugated to the plant virus nanoparticle via terminal NH₂ groups.

[0243] 12. A plurality of the conjugate of any of embodiments 1-11, wherein any one or more of the liposome, the one or more cancer antigen(s), or the plant virus nanoparticle are the same or different from each other.

[0244] 13. A composition comprising a carrier and the conjugate of any of embodiments 1-11 or the plurality of embodiment 12.

[0245] 14. The composition of embodiment 13, wherein the carrier is a pharmaceutically acceptable carrier.

[0246] 15. A method for one or more of in a subject in need thereof: delaying, slowing down, or preventing the relapse of a cancer; treating cancer; triggering or enhancing one or more of the following in the subject in need thereof: an anti-cancer immune response, an anti-cancer innate immune responses, a T cell-dependent anti-cancer immune response, T cell priming, a CD8⁺ T cell-dependent immune response, a CD4⁺ T cell-dependent immune response, or an immunological memory, comprising administering an effective amount of the conjugate of any of embodiments 1-11, the plurality of embodiment 12, or the composition of embodiment 13 or 14, to the subject.

[0247] 16. The method of embodiment 15, wherein the one or more cancer antigen(s) are derived from tumor cell lysate, optionally wherein the tumor cell lysate is oxidized or irradiated.

[0248] 17. The method of embodiment 15 or 16, wherein the one or more cancer antigen(s) are autologous to the subject.

[0249] 18. The method of any of embodiments 15-17, wherein the one or more cancer antigen(s) comprise ovarian cancer antigens and the subject in need thereof has been diagnosed with ovarian cancer.

[0250] 19. The method of any of embodiments 15-18, further comprising isolating one or more cancer antigens from the subject.

[0251] 20. The method of embodiment 19, further comprising preparing the conjugate of

any of embodiment 1-11 with the one or more cancer antigen(s) isolated from the subject.

[0252] 21. The method of any of embodiments 15-20, further comprising isolating a cancer sample from the subject.

[0253] 22. The method of any one of embodiments 15-21, wherein the conjugate of any of embodiments 1-11, the plurality of embodiment 12, or the composition of embodiment 13 or 14 is administered to the patient by oral, intratumoral, nasal, topical, transdermal, intranasal, vaginal, rectal, subcutaneous intravenous, intravenous, intraarterial, intramuscular, intraosseous, intraperitoneal, intraocular, subconjunctival, sub-Tenon's, intravitreal, retrobulbar, intracameral, epidural or intrathecal administration.

[0254] 23. The method of any of embodiments 15-21, wherein the conjugate of any of embodiments 1-11, the plurality of embodiment 12, or the composition of embodiment 13 or 14 administered to the patient by intraperitoneal administration.

[0255] 24. The method of any of embodiments 15-23, wherein the cancer is a primary cancer or a metastatic cancer.

[0256] 25. The method of any of embodiments 15-23, wherein the cancer is selected from a blood cancer, a carcinoma, a sarcoma, a lung cancer, an intraperitoneal cancer, a colorectal cancer, a colon cancer, an ovarian cancer, a melanoma, or a breast cancer.

[0257] 26. The method of any one of embodiments 15-22, wherein the administration(s) of the conjugate or composition is repeated for at least once, or at least twice, or more.

[0258] 27. The method of embodiment 23, wherein two administrations are about 1 day to about 1 year apart, or about 1 week apart, or about 2 weeks apart, or about 3 weeks apart, or about 4 weeks apart, or about 1 month apart, or about 2 months apart, or about 3 months apart, or about 6 months apart.

[0259] 28. The method of any one of embodiments 15-24, wherein the administration is subsequent to tumor resection.

[0260] 29. The method of any one of embodiments 15-25, wherein the subject is a cancer patient who has been treated by one or more of: an ablative therapy, a chemotherapy, a

radiation therapy, an immune checkpoint blockade therapy, or another anti-cancer therapy.

[0261] 30. The method of any one of embodiments 15-26, further comprising treating the subject with one or more of: an ablative therapy, a chemotherapy, a radiation therapy, an immune checkpoint blockade therapy, or another anti-cancer therapy. 31. The method of any one of embodiments 15-27, wherein the conjugate or composition is administered as a first line therapy, a second line therapy, a third line therapy or a fourth line therapy.

[0262] 32. The conjugate of any of embodiments 1-11, the plurality of claim 12, the composition of claim 13 or 14, or the method of any one of claims 15-31, wherein the plant virus nanoparticle has a diameter of from about 15 nm to about 60 nm.

[0263] 33. A kit comprising the conjugate of any of embodiments 1-11, the plurality of embodiment 12, or the composition of embodiment 13 or 14; and an optional instruction for use.

[0264] 34. A method for treating cancer in a subject in need thereof, comprising administering an effective amount of a conjugate comprised of a liposome and a plant virus nanoparticle, wherein the liposome comprises at least one cancer-associated antigen, and wherein the plant virus nanoparticle is CPMV, optionally wherein the subject in need has been diagnosed with ovarian cancer, optionally wherein the cancer-associated antigen is derived from tumor cell lysate, and optionally wherein the tumor cell lysate is oxidized or irradiated.

[0265] 35. The method of embodiment 34, wherein the cancer is an ovarian cancer.

[0266] 36. The method of embodiment 34, wherein the plant viral nanoparticle is administered intraperitoneally.

[0267] **Equivalents**

[0268] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs.

[0269] The present technology illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising,” “including,” “containing,” etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the present technology claimed.

[0270] Thus, it should be understood that the materials, methods, and examples provided here are representative of preferred aspects, are exemplary, and are not intended as limitations on the scope of the present technology.

[0271] It should be understood that although the present invention has been specifically disclosed by certain aspects, embodiments, and optional features, modification, improvement and variation of such aspects, embodiments, and optional features can be resorted to by those skilled in the art, and that such modifications, improvements and variations are considered to be within the scope of this disclosure.

[0272] The present technology has been described broadly and generically herein. Each of the narrower species and sub-generic groupings falling within the generic disclosure also form part of the present technology. This includes the generic description of the present technology with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0273] In addition, where features or aspects of the present technology are described in terms of Markush groups, those skilled in the art will recognize that the present technology is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0274] All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entireties, to the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control.

[0275] Other aspects are set forth within the following claims.

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WHAT IS CLAIMED IS:

1. A conjugate comprising a liposome comprising one or more cancer antigen(s) conjugated to a plant virus nanoparticle, optionally wherein the liposome comprising the one or more cancer antigen(s) is a lipid nanoparticle (LNP), optionally wherein the plant virus nanoparticle comprises a plant picornavirus nanoparticle.
2. The conjugate of claim 1, wherein the plant virus nanoparticle comprises a cowpea mosaic virus (CPMV) particle, wherein the CPMV particle is non-replicating and noninfectious toward mammals.
3. The conjugate of claim 1 or 2, wherein the one or more cancer antigen(s) is selected from an antigen isolated from a cancer selected from a carcinoma, a sarcoma, or a hematological cancer.
4. The conjugate of any of claims 1-3, wherein the one or more cancer antigen(s) comprise ovarian cancer cell antigens.
5. The conjugate of any of claims 1-4, wherein the one or more cancer antigen(s) is derived from tumor cell lysate, optionally wherein the tumor cell lysate is oxidized or irradiated.
6. The conjugate of any of claims 1-5, wherein the liposome comprises a plurality of the one or more cancer antigens that are the same or different from each other.
7. The conjugate of claim 1, wherein the liposome comprises a LNP and the plant virus nanoparticle comprises a CPMV particle.
8. The conjugate of any of claims 1-7, wherein the plant virus nanoparticle is conjugated to the liposome via a DBCO groups, optionally wherein the DBCO are chemically conjugated to the plant virus nanoparticle via terminal NH₂ groups.
9. A plurality of the conjugate of any of claims 1-8, wherein any one or more of the liposome, the one or more cancer antigen(s), or the plant virus nanoparticle are the same or different from each other.
10. A composition comprising a carrier and the conjugate of any of claims 1-8 or the plurality of claim 9, optionally wherein the carrier is a pharmaceutically acceptable carrier.
11. A method for one or more of in a subject in need thereof:

delaying, slowing down, or preventing the relapse of a cancer;

treating cancer;

triggering or enhancing one or more of the following in the subject in need thereof: an anti-cancer immune response, an anti-cancer innate immune responses, a T cell-dependent anti-cancer immune response, T cell priming, a CD8⁺ T cell-dependent immune response, a CD4⁺ T cell-dependent immune response, or an immunological memory,

comprising administering an effective amount of the conjugate of any of claims 1-11, the plurality of claim 12, or the composition of claim 13 or 14, to the subject, optionally wherein the administration is intraperitoneal, and optionally wherein the cancer is ovarian,

optionally further comprising isolating one or more cancer antigens from the subject, further optionally further comprising preparing the conjugate of any of claims 1-11 with the one or more cancer antigen(s) isolated from the subject, and further optionally isolating a cancer sample from the subject.

12. The method of claim 11, wherein the one or more cancer antigen(s) are derived from tumor cell lysate, optionally wherein the tumor cell lysate is oxidized or irradiated.

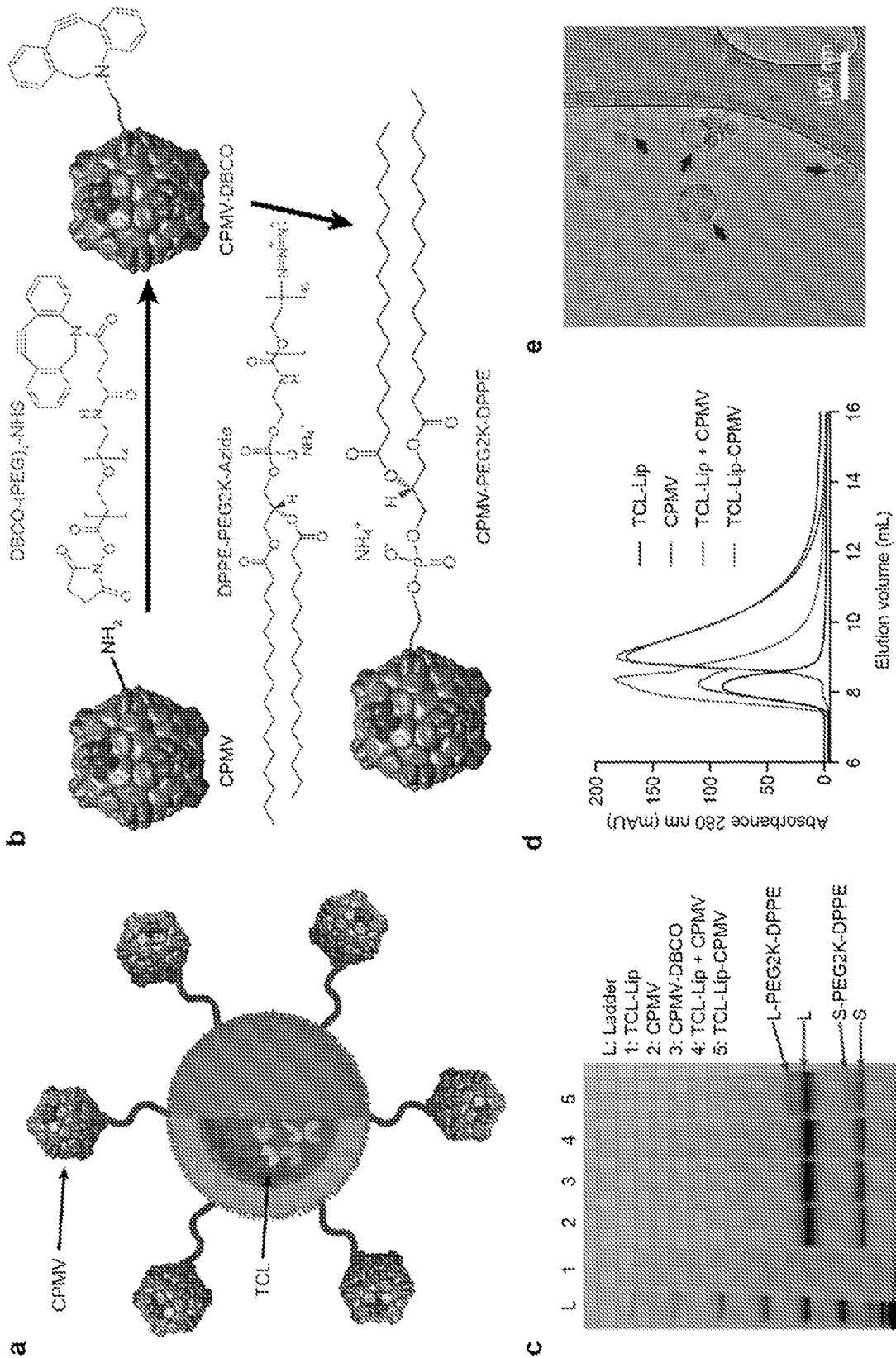
13. The method of claim 11, wherein the one or more cancer antigen(s) are autologous to the subject.

14. The method of any of claims 11-13, wherein the one or more cancer antigen(s) comprise ovarian cancer antigens and the subject in need thereof has been diagnosed with ovarian cancer.

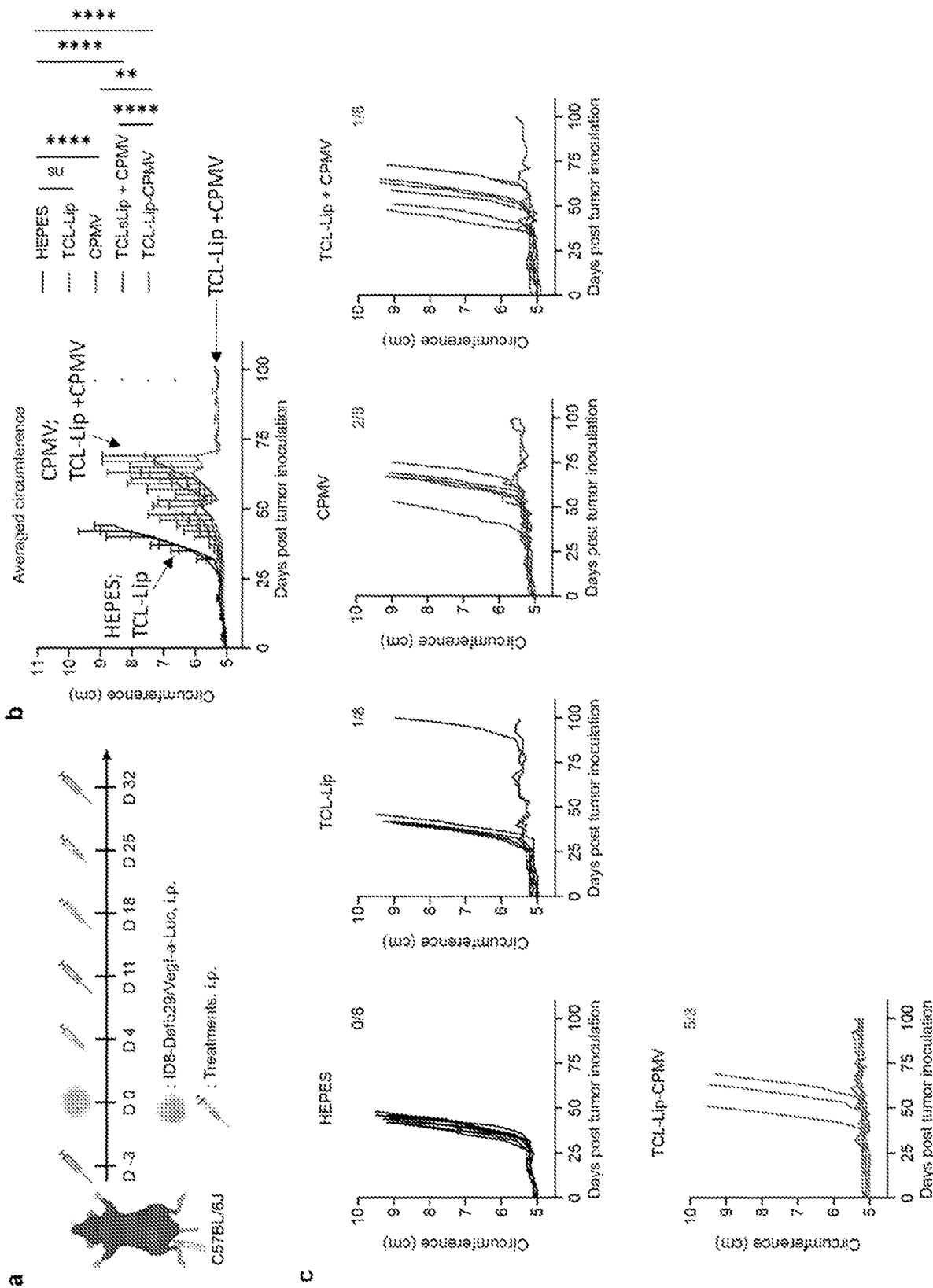
15. The method of any one of claims 11-14, wherein the administration(s) of the conjugate or composition is repeated for at least once, or at least twice, or more, wherein administrations are about 1 day to about 1 year apart, or about 1 week apart, or about 2 weeks apart, or about 3 weeks apart, or about 4 weeks apart, or about 1 month apart, or about 2 months apart, or about 3 months apart, or about 6 months apart.

16. The method of any one of claims 11-15, wherein the subject is a cancer patient who has been treated by one or more of an ablative therapy, a chemotherapy, a radiation therapy, an immune checkpoint blockade therapy, or another anti-cancer therapy, or the subject has had a tumor resection.

17. The method of any one of claims 11-16, further comprising treating the subject with one or more of: an ablative therapy, a chemotherapy, a radiation therapy, an immune checkpoint blockade therapy, or another anti-cancer therapy, wherein the conjugate, plurality or composition is administered as a first line therapy, a second line therapy, a third line therapy or a fourth line therapy.
18. The conjugate of any of claims 1-8, the plurality of claim 9, the composition of claim 10 or the method of any one of claims 11-17, wherein the plant virus nanoparticle has a diameter of from about 15 nm to about 60 nm.
19. A kit comprising the conjugate of any of claims 1-8, the plurality of claim 9, or the composition of claim 10; and an optional instruction for use.
20. A method for treating cancer in a subject in need thereof, comprising administering an effective amount of a conjugate comprised of a liposome and a plant virus nanoparticle, wherein the liposome comprises at least one cancer-associated antigen, and wherein the plant virus nanoparticle is CPMV, optionally wherein the cancer is ovarian cancer optionally wherein the cancer-associated antigen is derived from tumor cell lysate, and optionally wherein the tumor cell lysate is oxidized or irradiated.



FIGURES 1A-1E



FIGURES 2A-2C

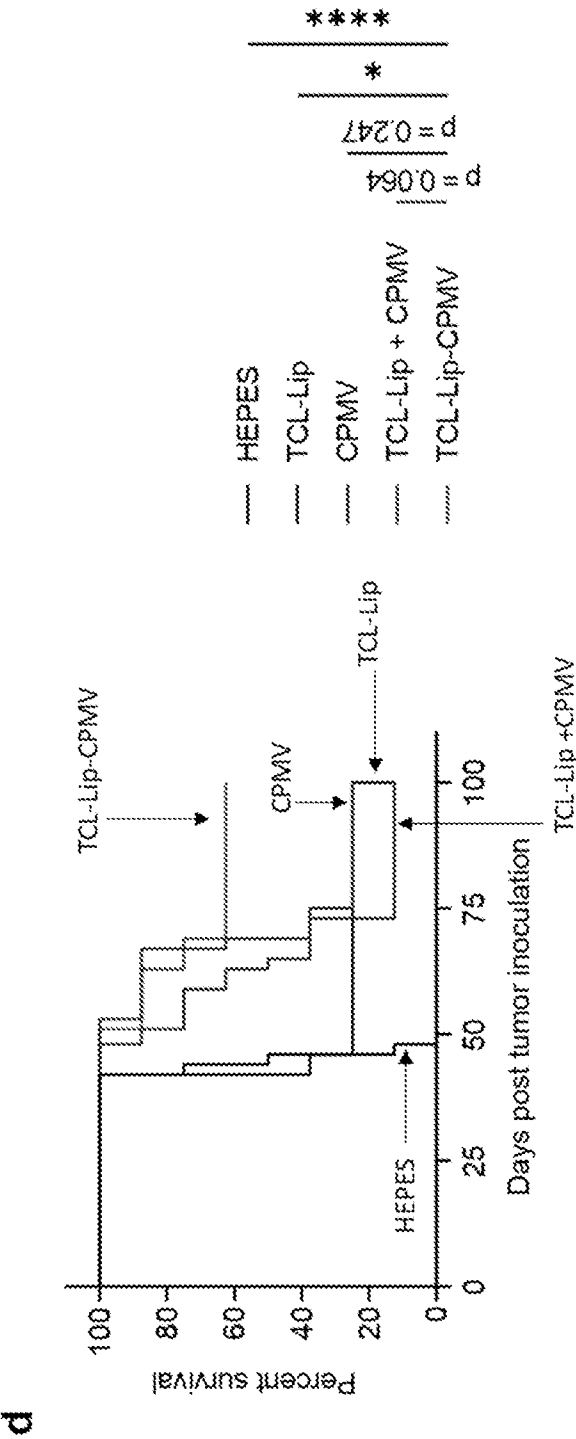
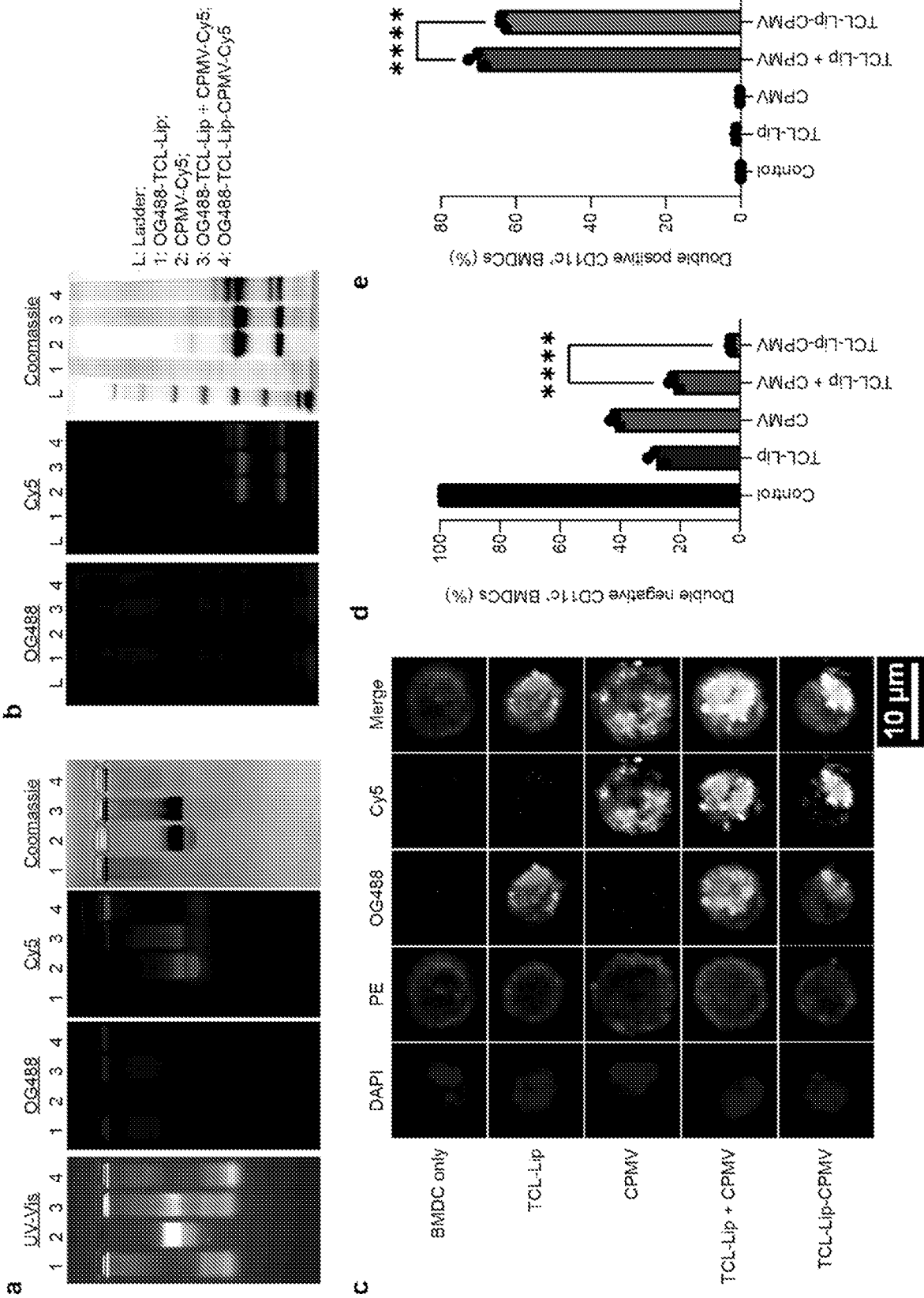
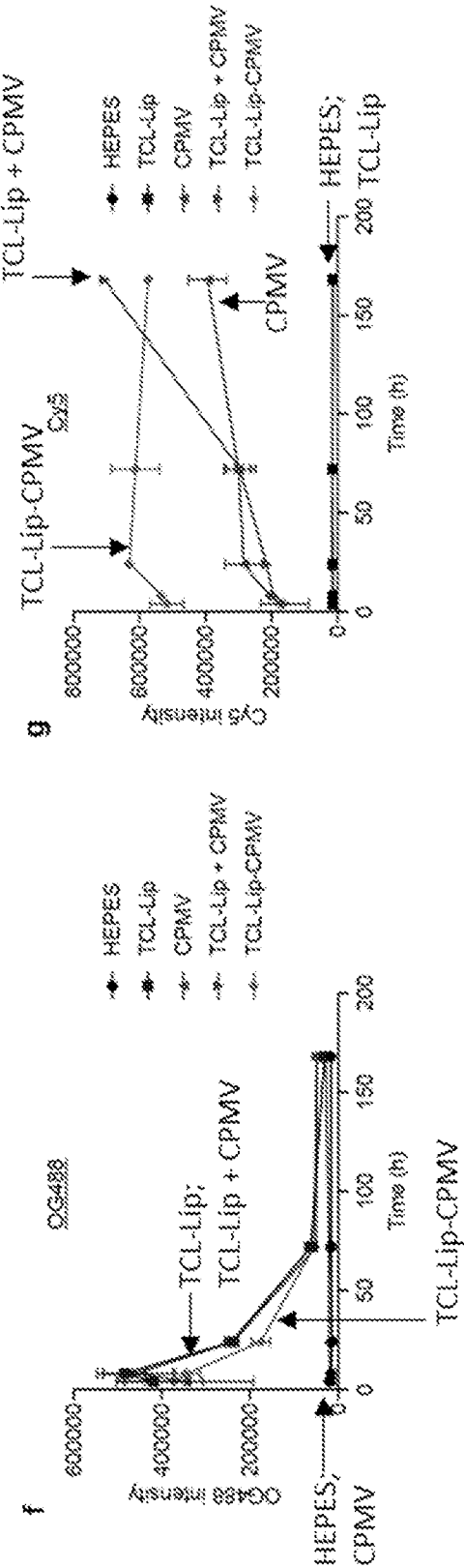


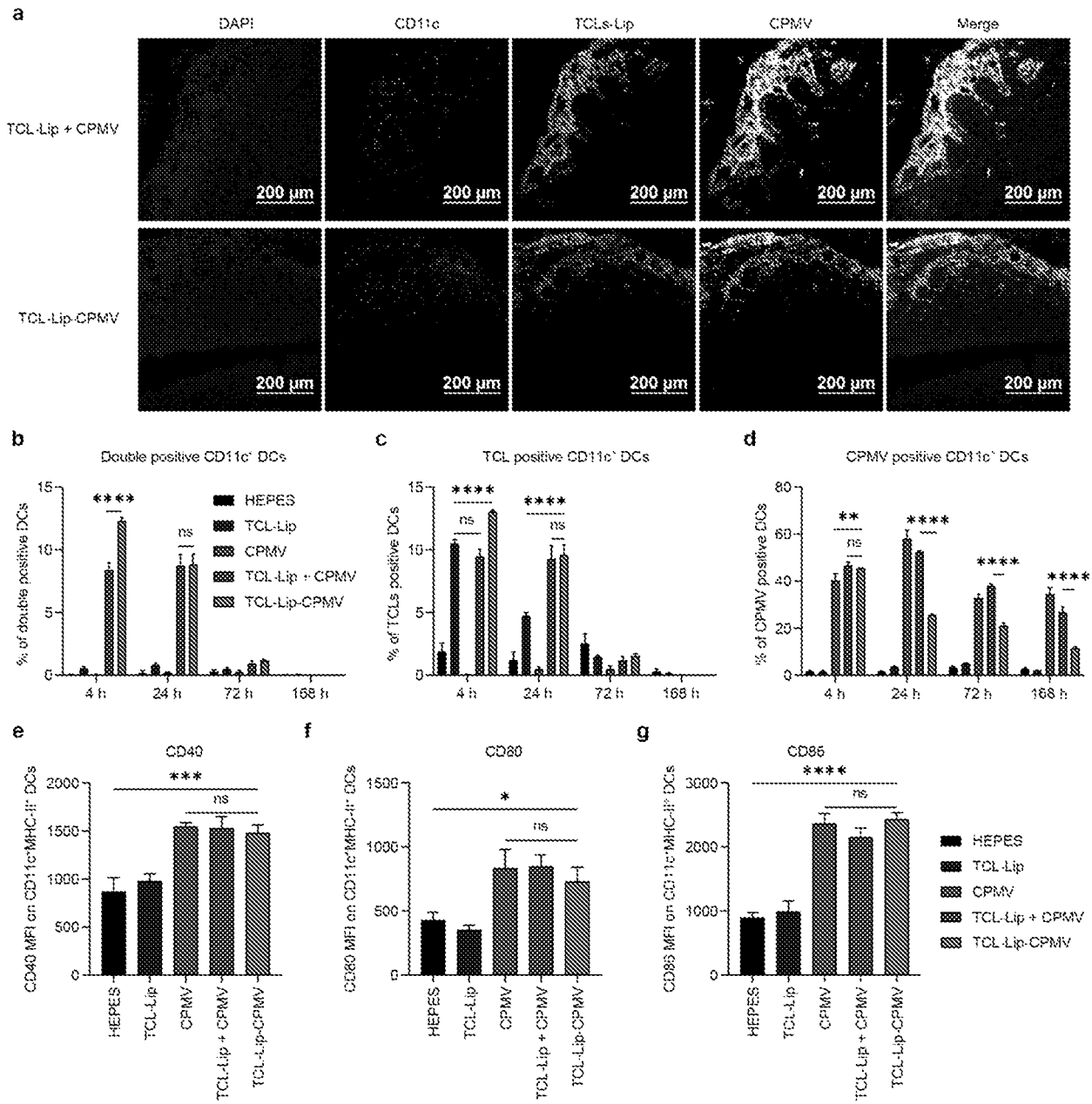
FIGURE 2D



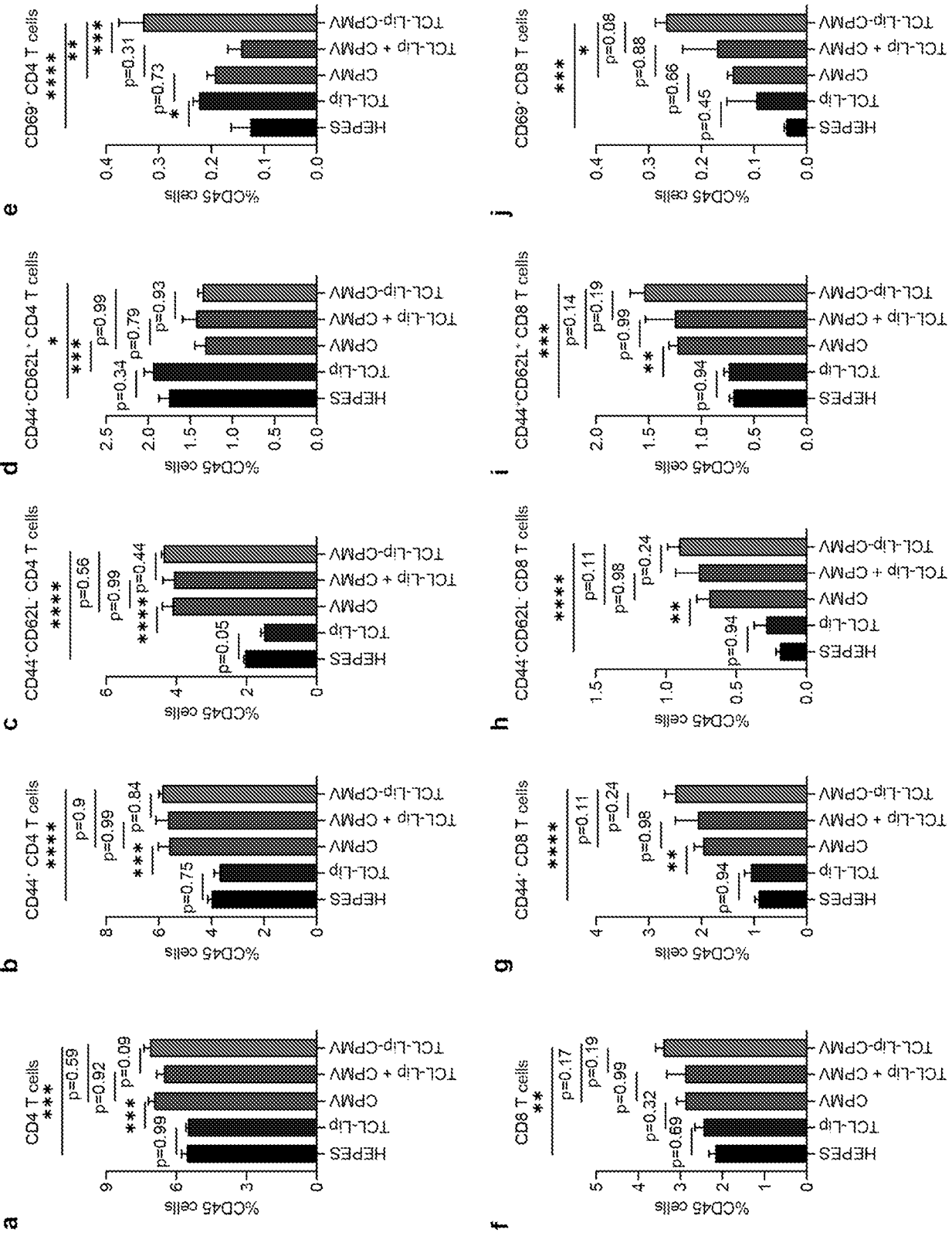
FIGURES 3A-3E



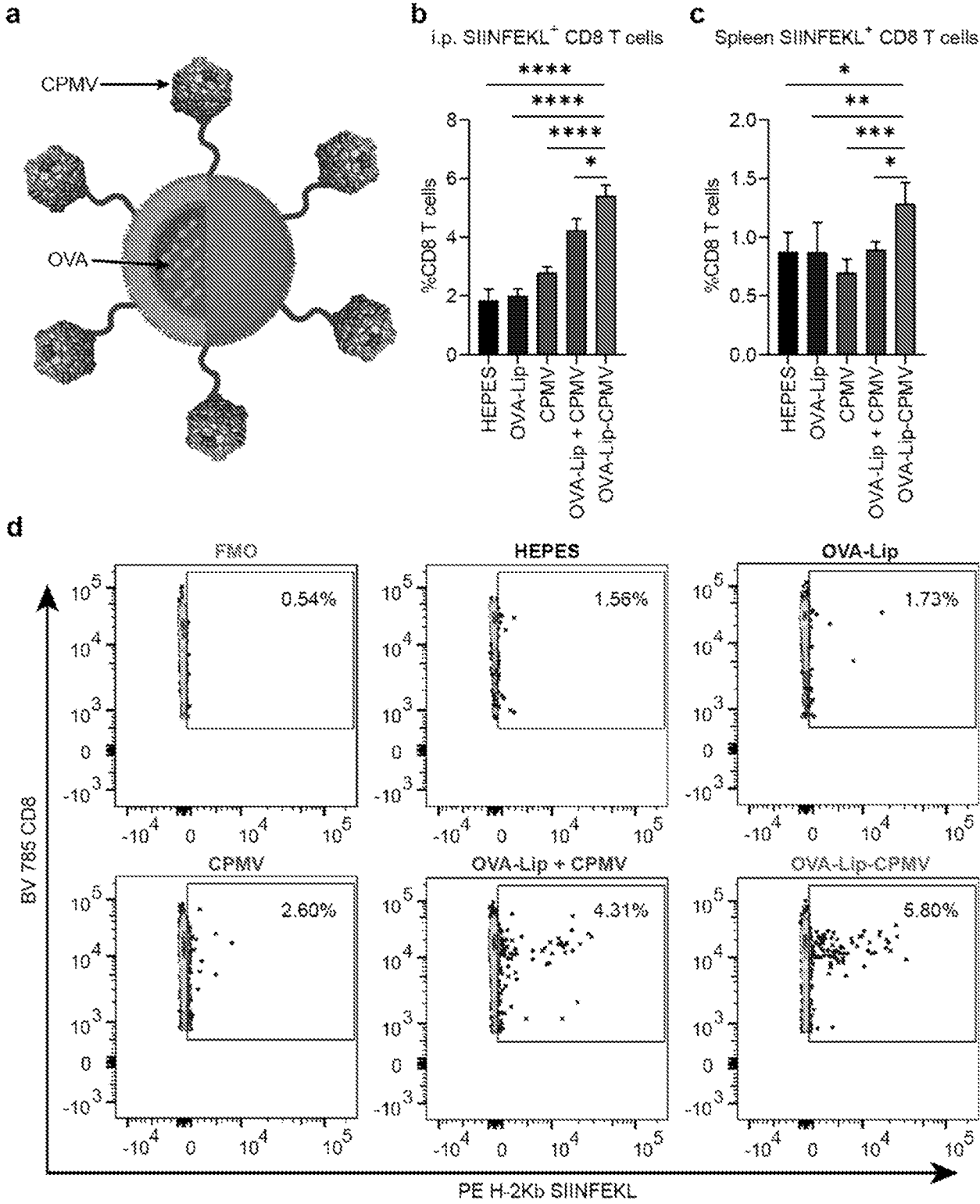
FIGURES 3F-3G



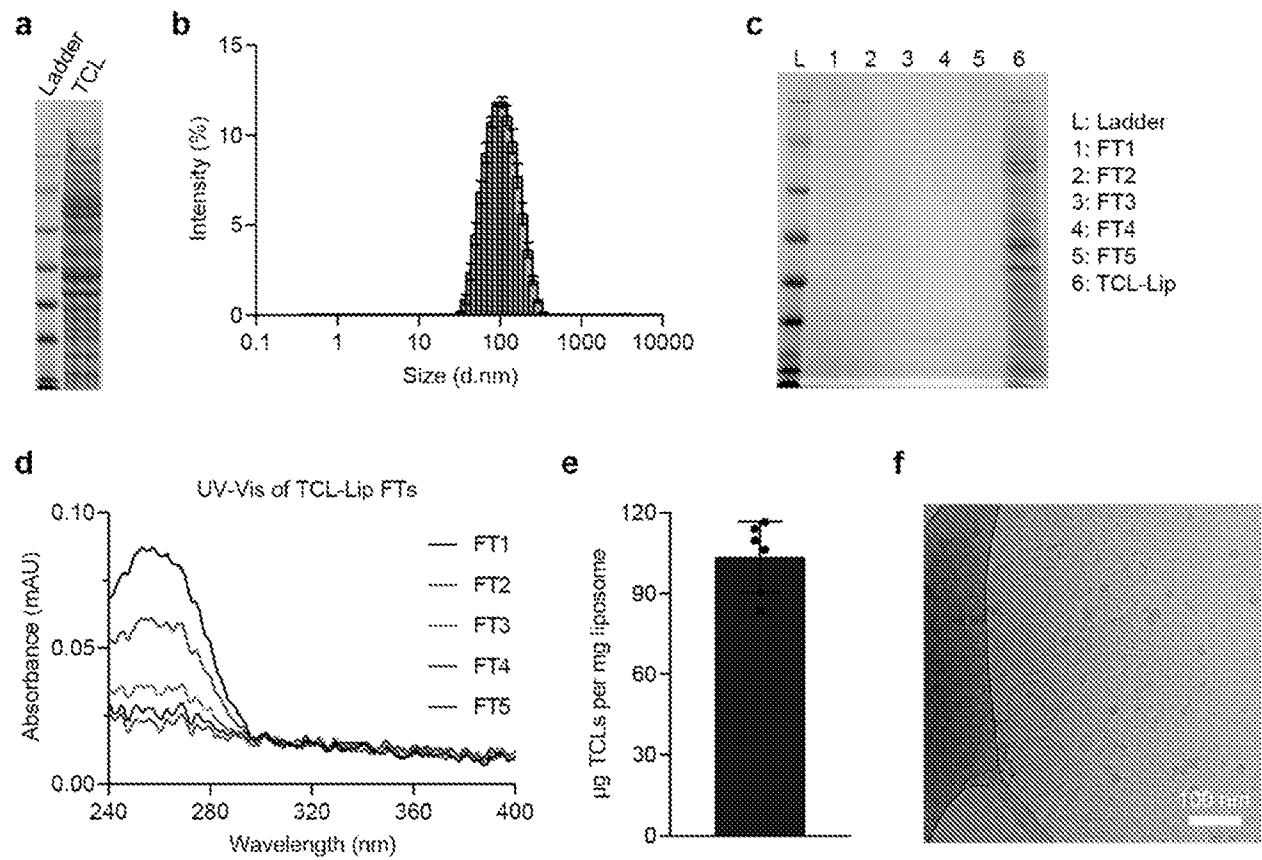
FIGURES 4A-4G



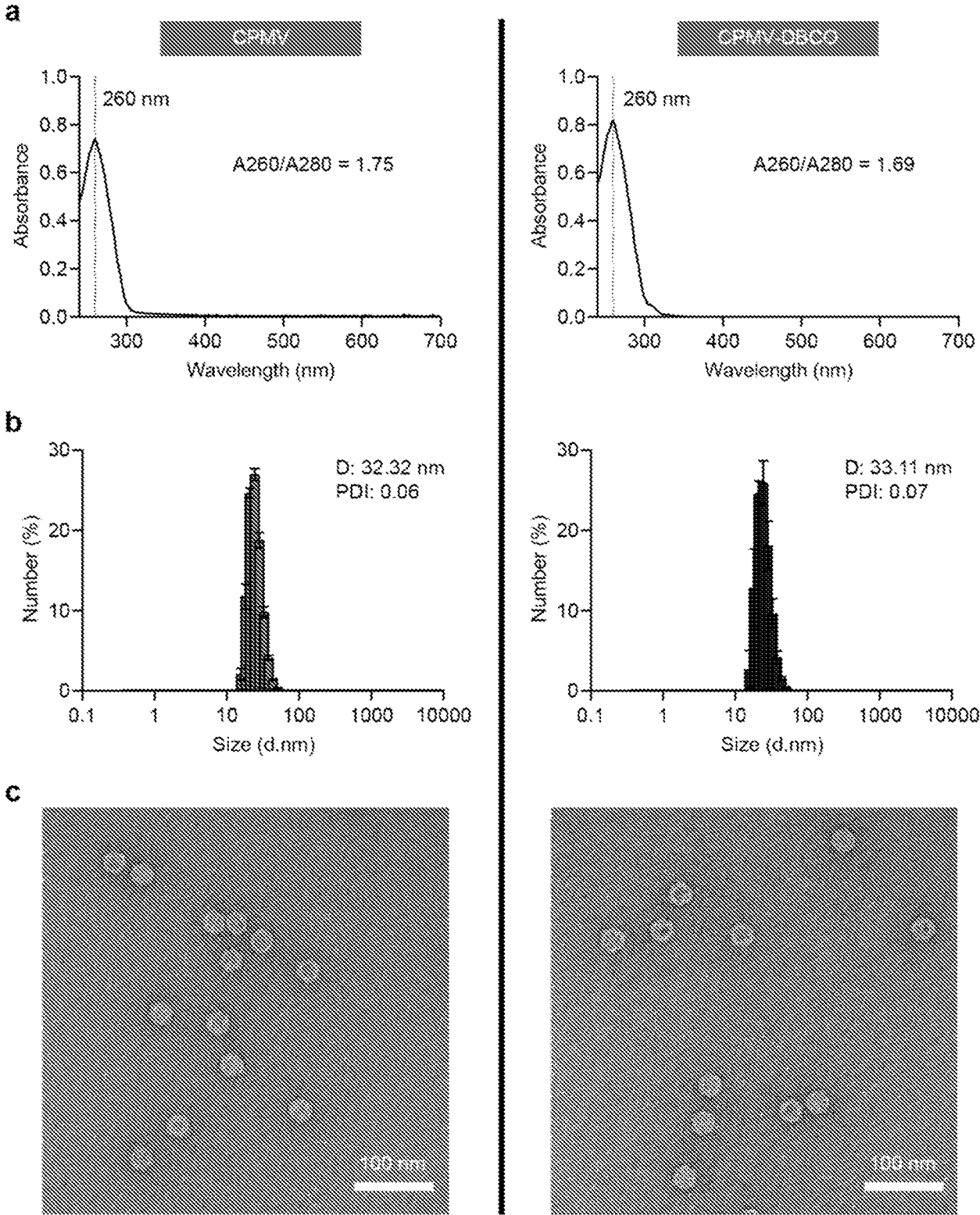
FIGURES 5A-5J



FIGURES 6A-6D



FIGURES 7A-7F



FIGURES 8A-8C

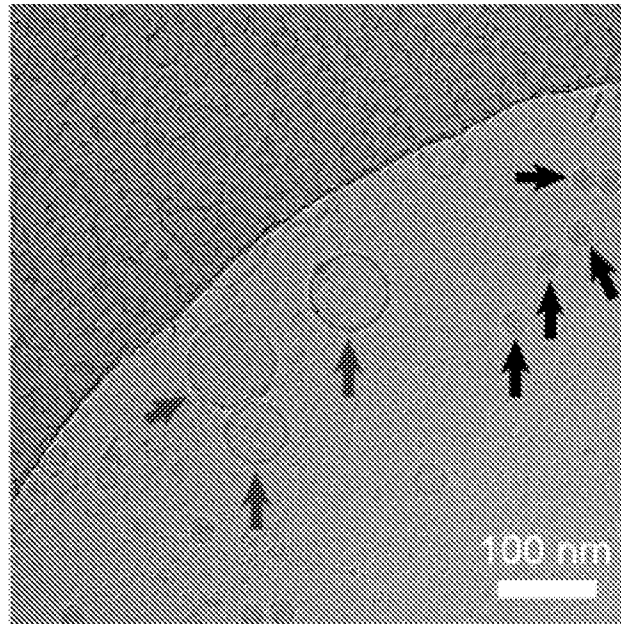


FIGURE 9

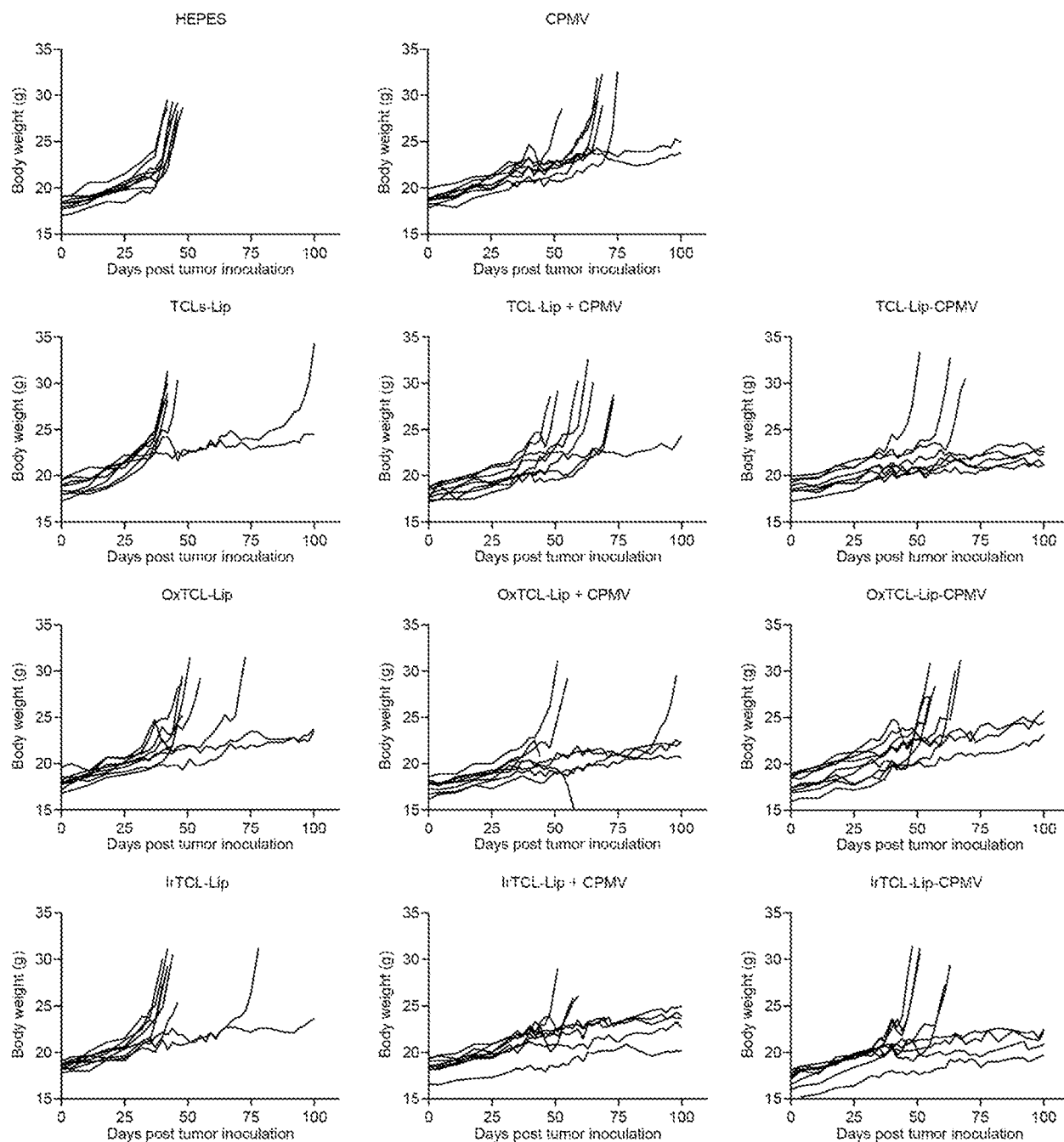
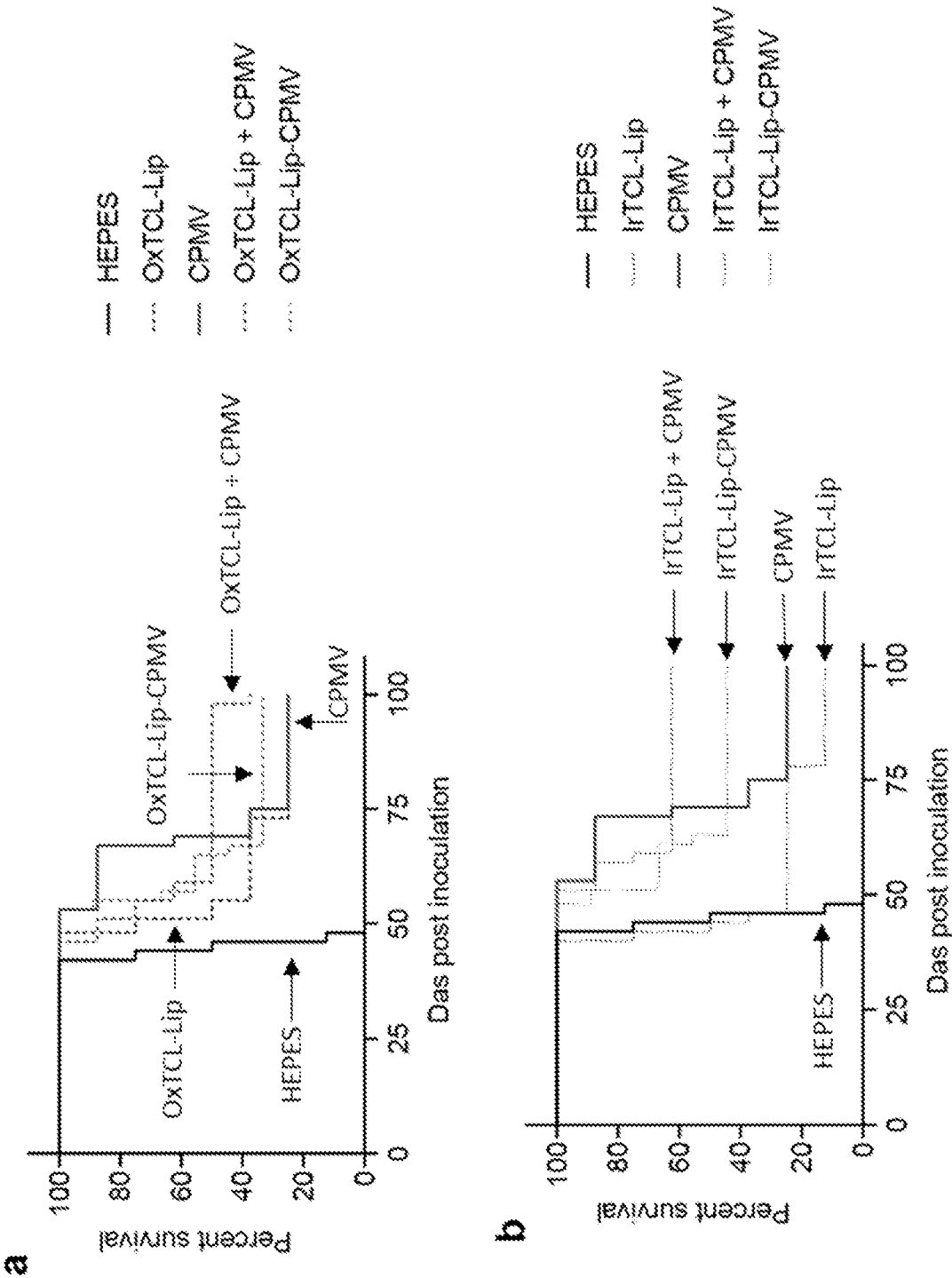


FIGURE 10



FIGURES 11A-11B

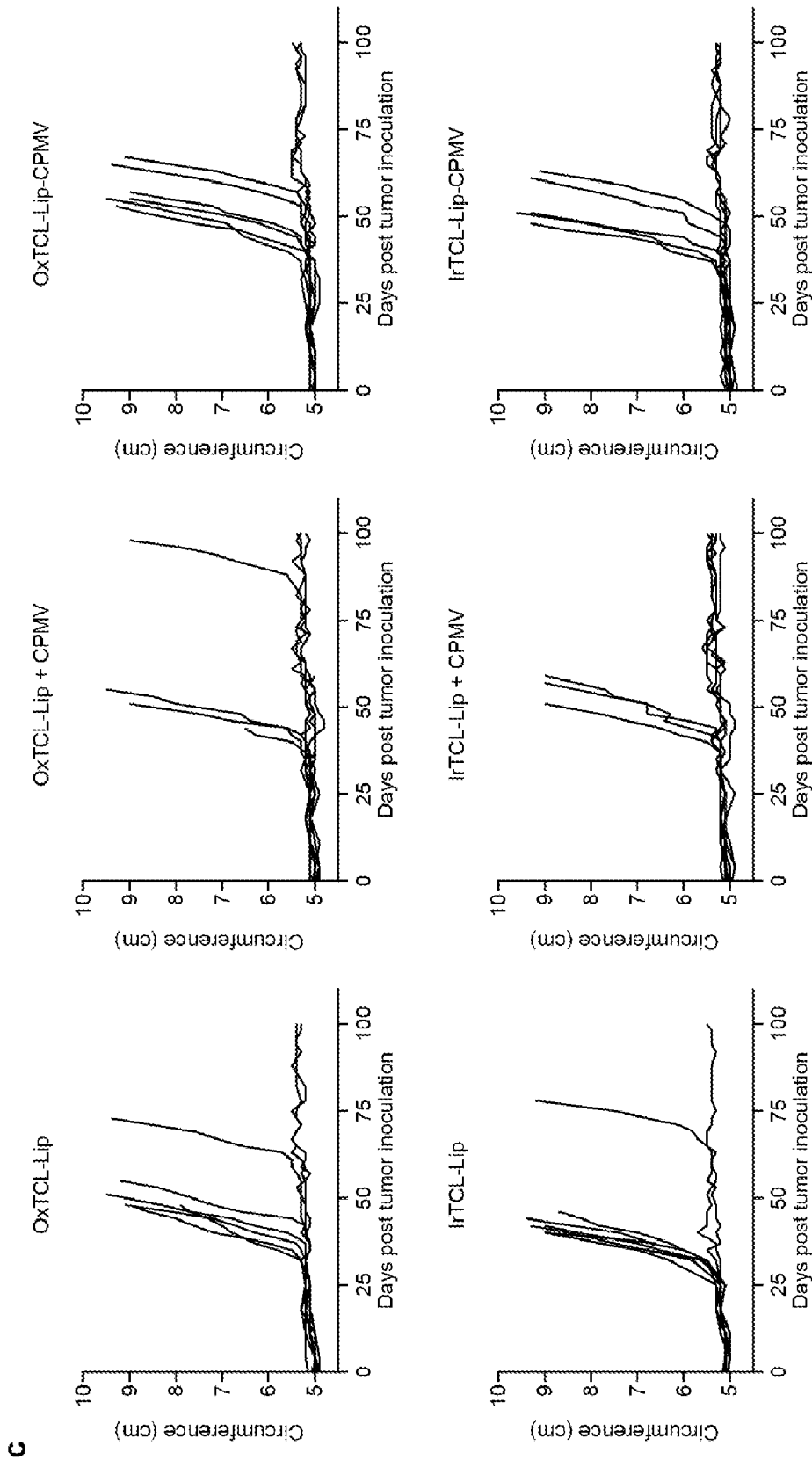
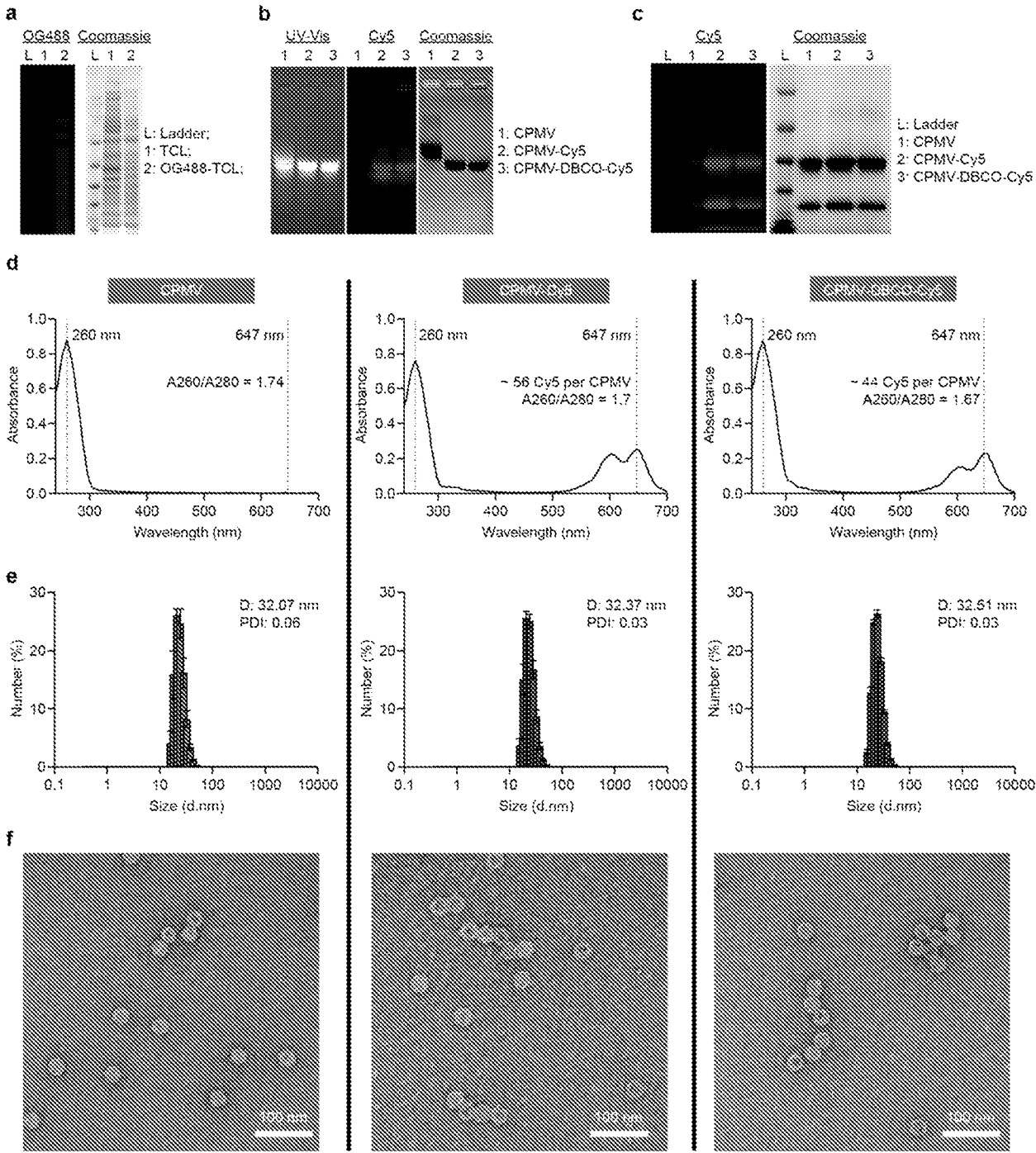
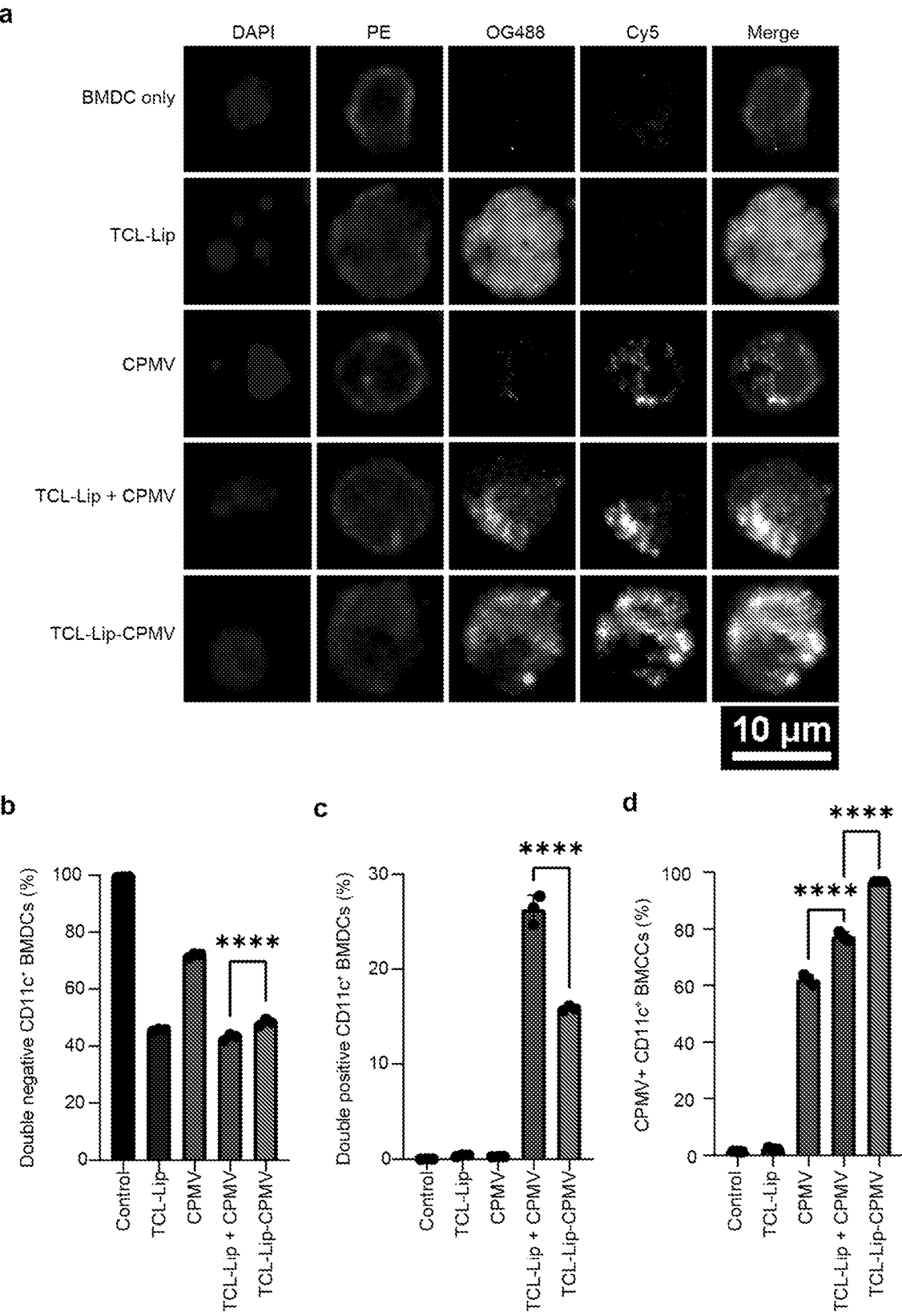


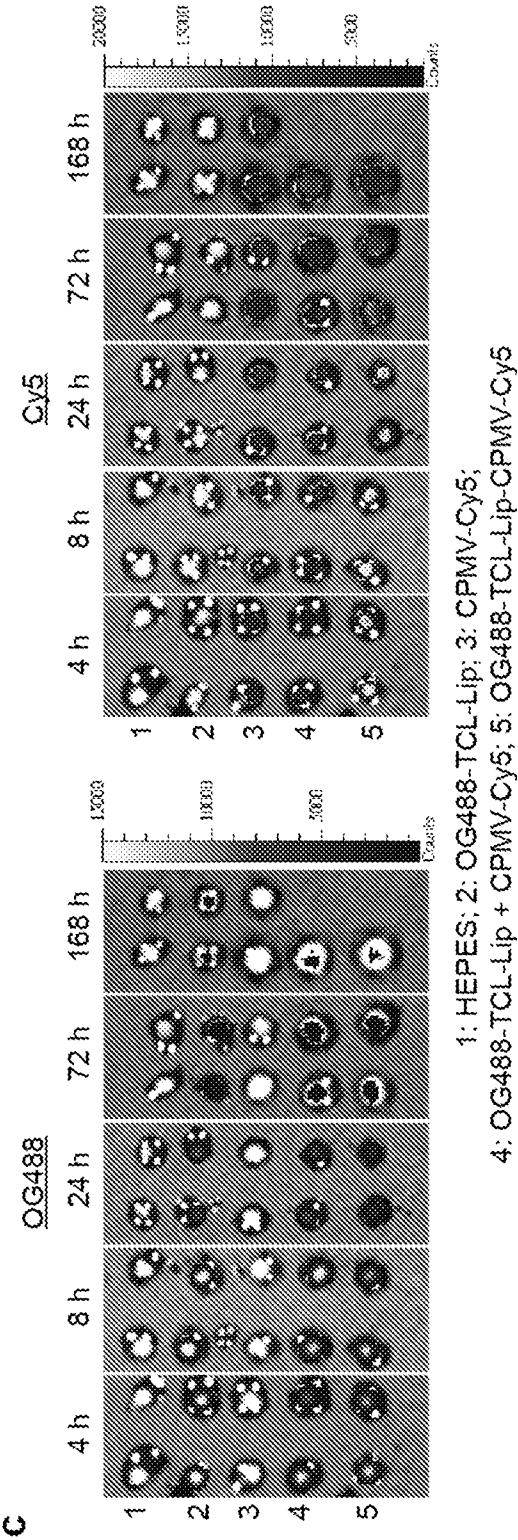
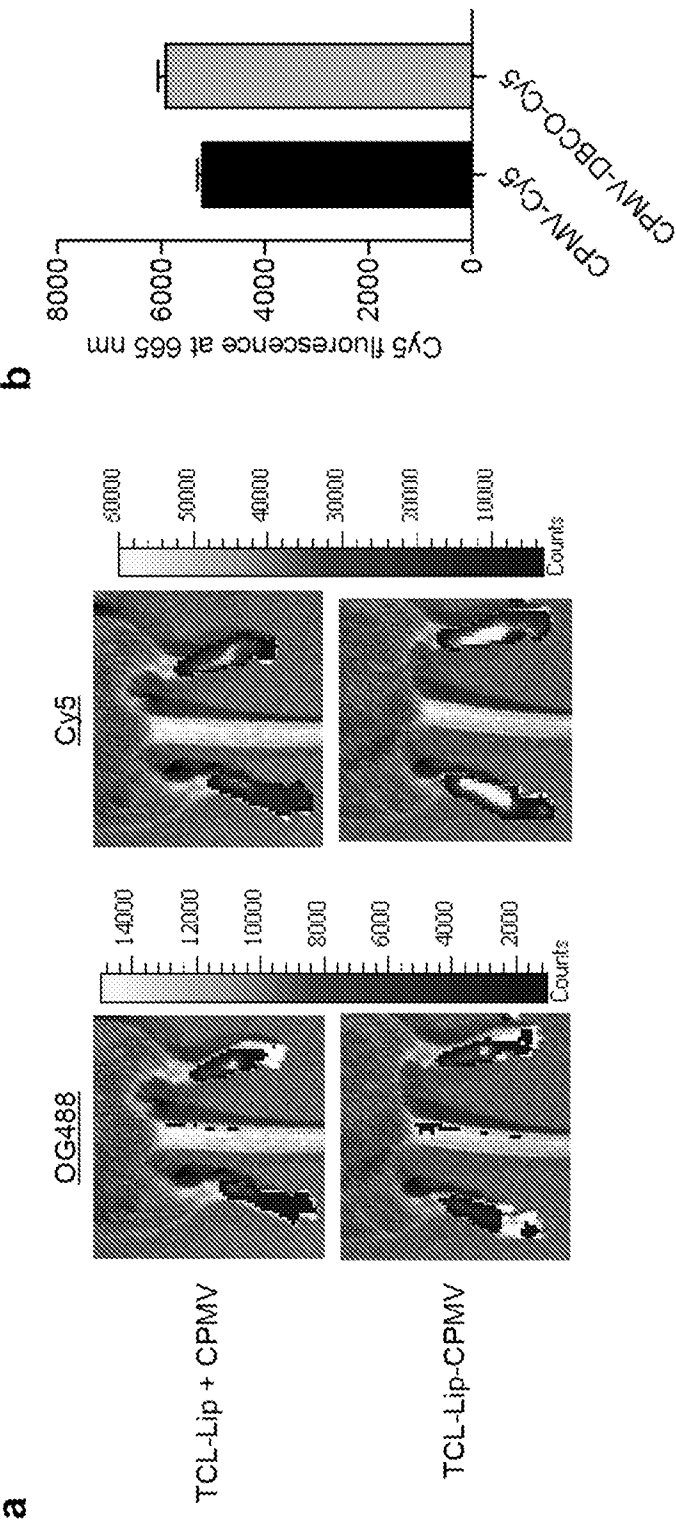
FIGURE 11C



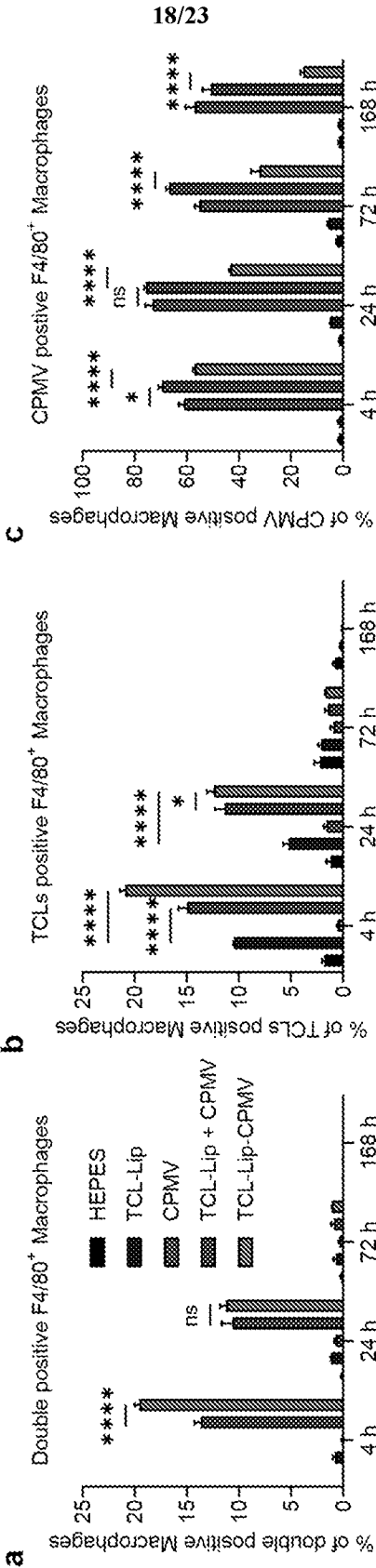
FIGURES 12A-12F



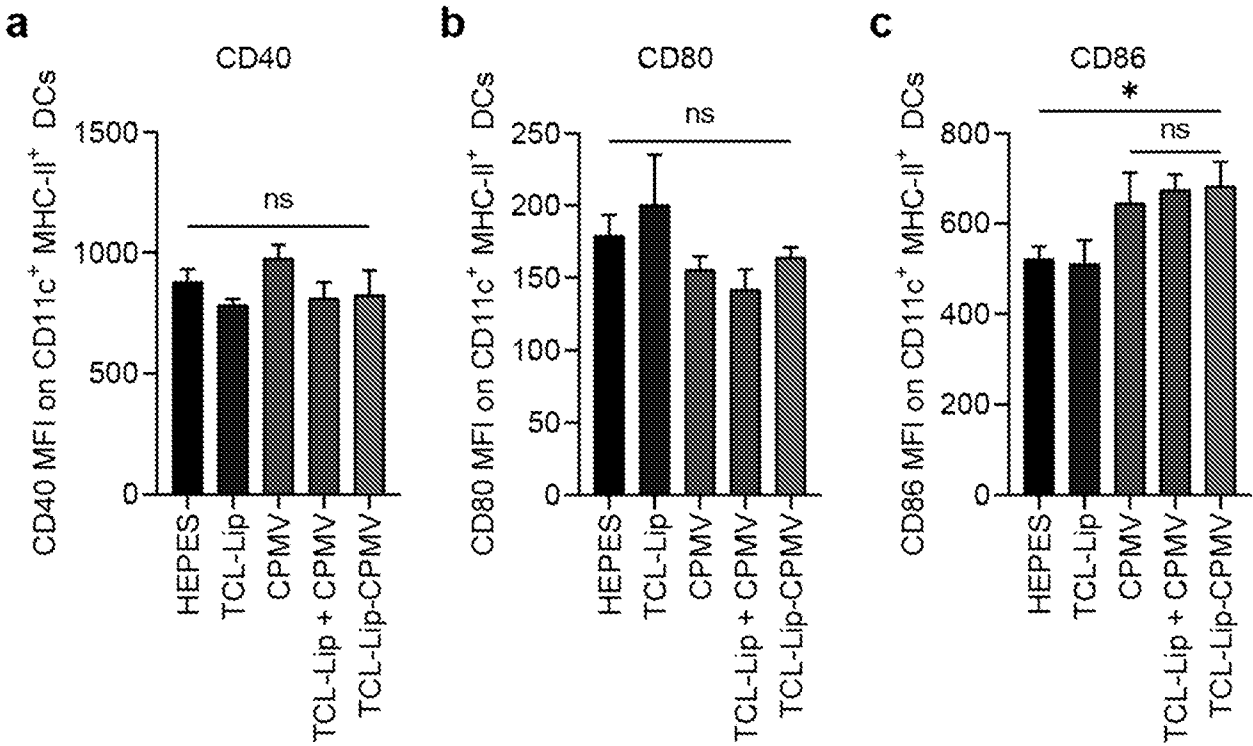
FIGURES 13A-13D



FIGURES 14A-14C

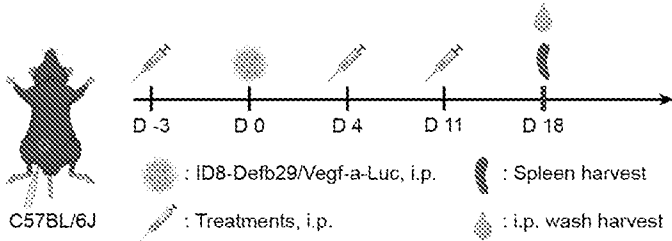


FIGURES 15A-15C

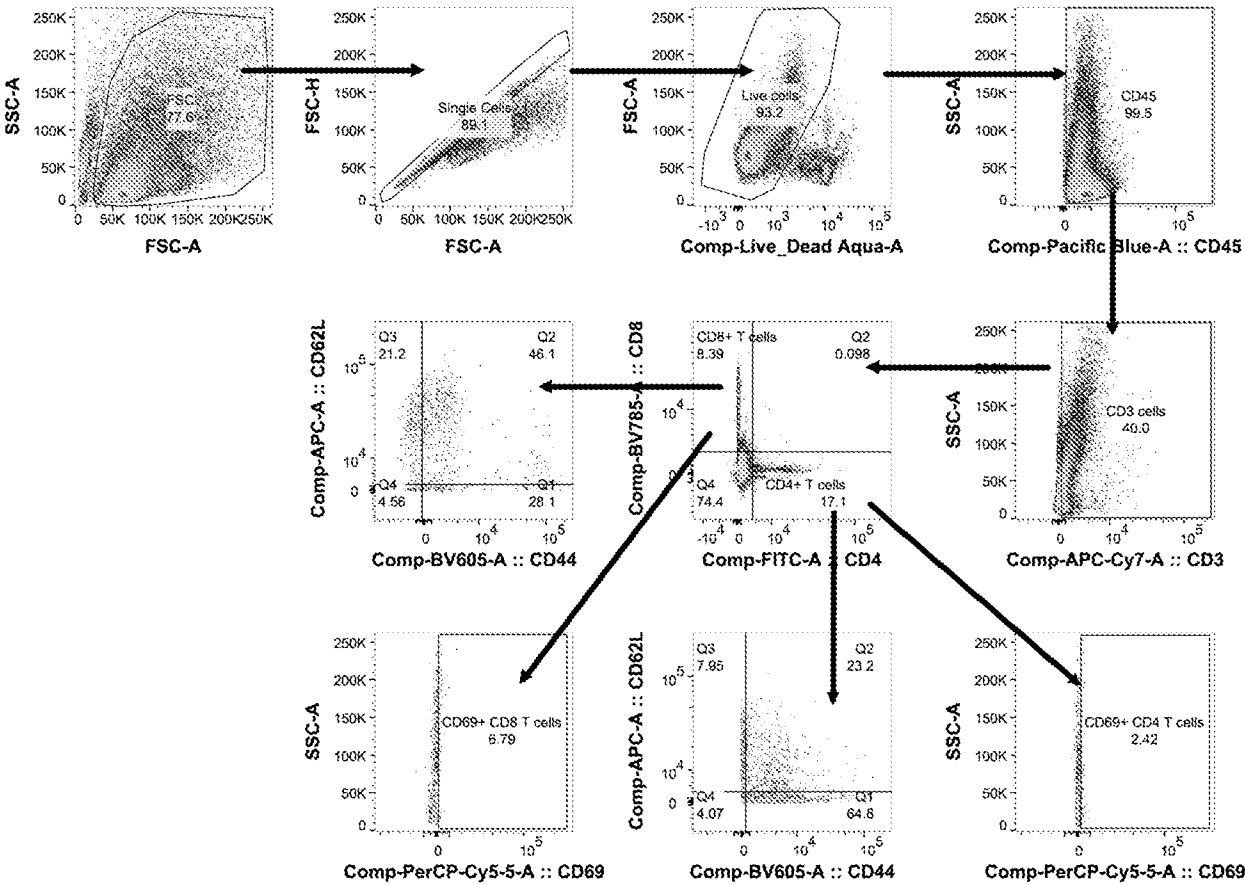


FIGURES 16A-16C

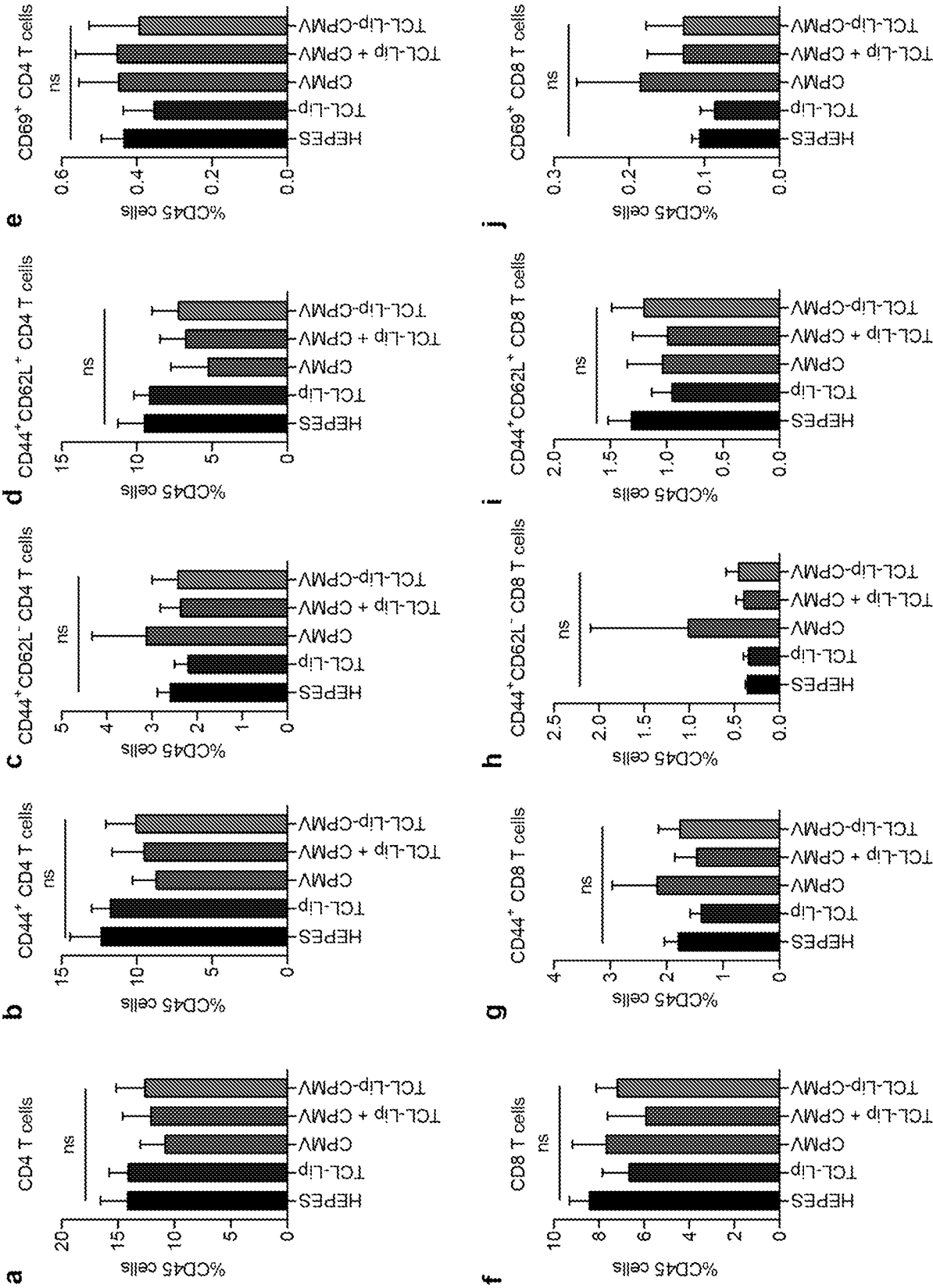
a



b



FIGURES 17A-17B



FIGURES 18A-18J

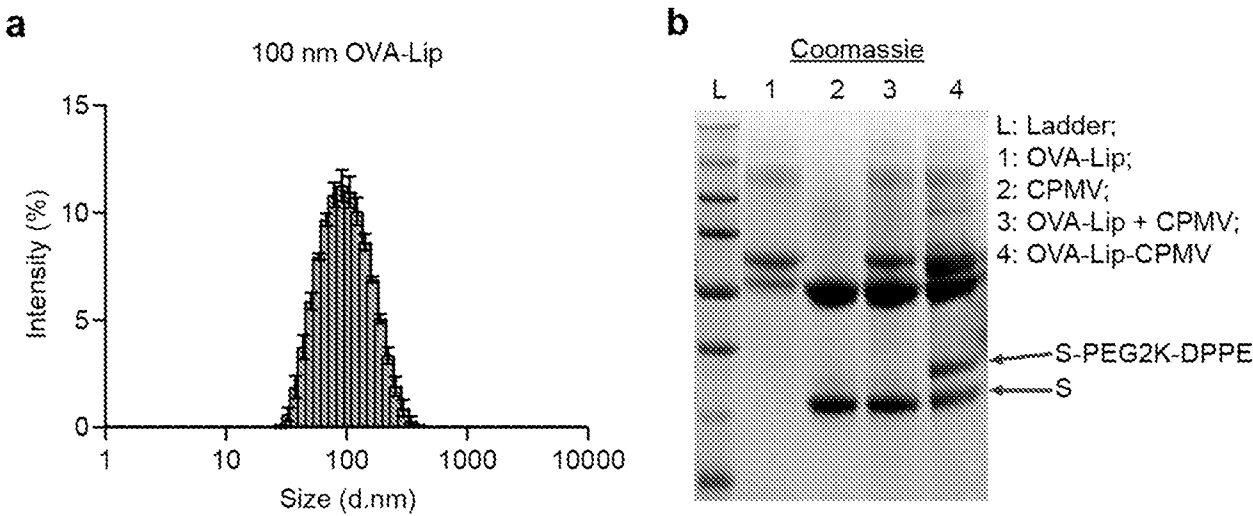


FIGURE 19A-19B

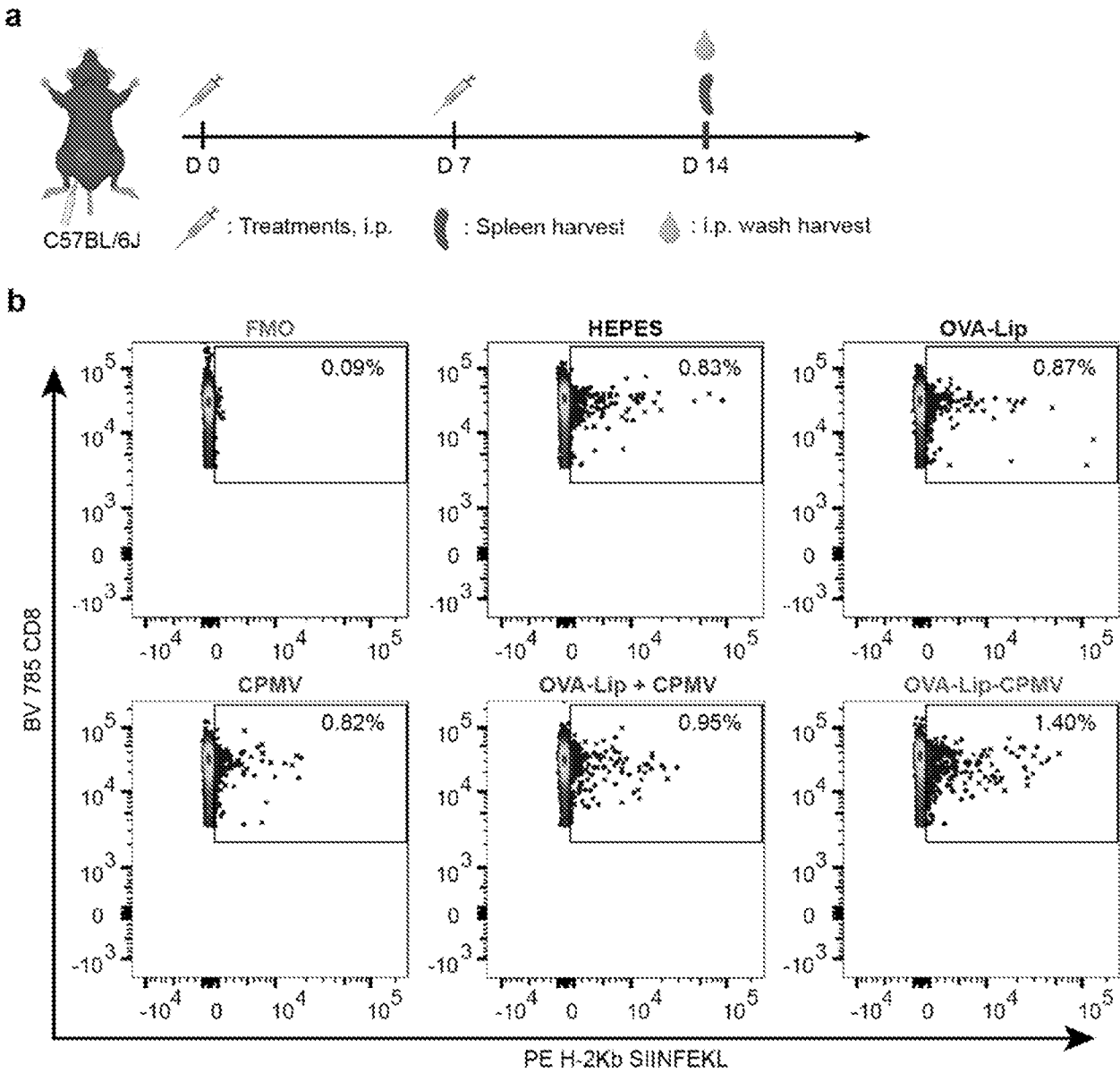


FIGURE 20A-20B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/035871

A. CLASSIFICATION OF SUBJECT MATTERIPC: **A61K 9/127** (2024.01); **A61P 35/00** (2024.01); **C07K 14/005** (2024.01); **C12N 7/00** (2024.01); **A61K 35/76** (2024.01); **A61K 39/12** (2024.01); **A61K 39/385** (2024.01)CPC: **A61K 9/1271**; **A61K 39/0011**; **A61K 39/385**; **C07K 14/005**; **C12N 7/00**; **A61K 39/12**; **A61K 35/76**; **A61P 35/00**; **A61K 2039/5258**; **A61K 2121/00**; **A61K 2039/5555**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2011/149061 A1 (JAPAN AS REPRESENTED BY THE NATIONAL INSTITUTE OF INFECTIOUS DISEASES et al.) 01 December 2011 (01.12.2011) entire document	1-3, 7, 20
Y	US 2017/0021003 A1 (CASE WESTERN RESERVE UNIVERSITY) 26 January 2017 (26.01.2017) entire document	1-3, 7, 20
A	US 10,052,370 B2 (ICON GENETICS GMBH et al.) 21 August 2018 (21.08.2018) entire document	1-3, 7, 20



Further documents are listed in the continuation of Box C.



See patent family annex.

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“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

17 August 2024 (17.08.2024)

Date of mailing of the international search report

03 September 2024 (03.09.2024)

Name and mailing address of the ISA/US

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MATOS
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Telephone No. 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/035871

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: **4-6, 8-19**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).