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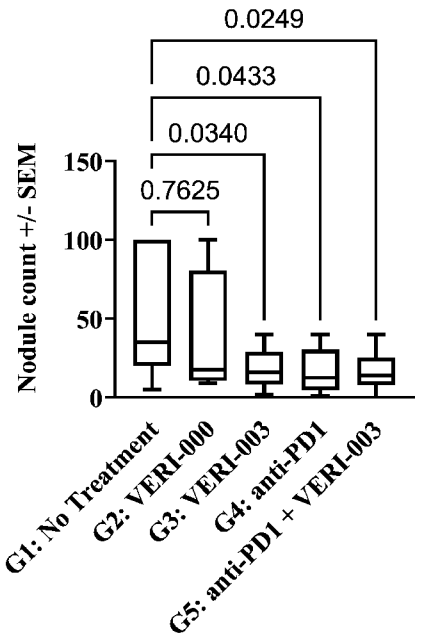

FIG. 30

(57) Abstract: Disclosed are virus-inspired (ViP) compositions and preparation methods thereof, where the compositions comprise mutant papillomavirus LI proteins that spontaneously form capsid backbones and that are conjugated to a peptide comprising an epitope to form immune redirector capsids ("IRCs," also called virus-inspired particles or "ViPs"). The epitopes on the peptides are designed to be recognized by a subject's immune system based on the subject's preexisting immune memory developed from the subject's past exposure to the epitope through infection or vaccination. The mutant papillomavirus LI proteins possess three mutations including an amino-terminal truncation, a carboxy -terminal truncation, and a truncation at helix four. These ViPs can be co-formulated and/or co-administered with an immune checkpoint inhibitor molecule(s) with synergistic effect. Disclosed are uses and methods of using the compositions in treating, reducing the occurrence of, inhibiting the progression and/or metastasis of, a cancer in a subject in need thereof.

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VIRUS-INSPIRED COMPOSITIONS COMPRISING VIRUS-LIKE PROTEINS AND CHECKPOINT INHIBITORS, AND METHODS OF USE THEREOF TO TREAT CANCER

INCORPORATION OF SEQUENCE LISTING

[0001] The application contains a Sequence Listing which has been submitted electronically in .XML format and is hereby incorporated by reference in its entirety. Said .XML copy, created on June 4, 2024, is named "8009PCT.xml" and is 181,912 bytes in size. The sequence listing contained in this .XML file is part of the specification and is hereby incorporated by reference herein in its entirety.

CROSS CITE TO RELATED APPLICATIONS

[0002] This application claims priority to U.S. provisional patent application serial number 63/471,418, filed on June 6, 2023, and U.S. provisional patent application serial number 63/600,580, filed on November 17, 2023, the entire contents of each of which are hereby incorporated by reference.

FIELD OF THE INVENTION

[0003] Disclosed are compositions comprising mutated papillomavirus proteins, especially the L1 major capsid protein, that form capsid backbones and are attached to one or more peptides comprising one or more antigens recognized by a subject's preexisting immune system response memory, and their methods of use in treatment, prevention, and/or reduction in the incidence of cancer in a subject. The compositions disclosed herein additionally comprise one or more checkpoint inhibitors.

BACKGROUND

[0004] Typical cancer treatment includes chemotherapy, radiation, and surgery. However, surgery is highly invasive and often fails, especially after metastasis. Chemotherapy and radiation can be effective, but often yield harsh side-effects that can drastically reduce quality of life for subjects. Despite these treatments, many cancers remain refractory to treatment and the treatments can be ineffective in combating metastatic cancers even when successful in reducing or eliminating the primary tumor. Targeted delivery has become one of the most promising opportunities for improving the treatment of cancer but this approach also

presents the most challenges. Immunotherapies such as cancer vaccines have emerged as an attractive option due to the ability to stimulate the immune system and then use this response to specifically target over-expressed proteins preferentially present on the surface of cancer cells, resulting in targeted elimination of the cancer cells. Such therapies are attractive in that they are target specific and potentially less toxic without nonspecific autoimmunity. These targeted therapies are also considered less invasive or traumatic compared to surgery, radiation, or chemotherapy. However, cancer vaccines based on cancer-associated antigens can have limited success due to poor clinical immunogenicity, immune tolerance, and off target effects, for example. Moreover, such methods typically require identifying a cancer-associated antigen specific to a given patient's cancer to achieve effective targeting of the cancer. Hence, this approach has failed on multiple occasions because most cancer-associated antigens are self-antigens that are tolerated by the immune system, resulting in poor immune responses.

[0005] Other approaches to the treatment and prevention of cancer are based on adoptive transfer of chimeric antigen receptor (CAR)-transduced T cells (CAR-T) or infusion of monoclonal antibodies that require the laborious identification of cancer-specific antigens and are applicable to only a subset of cancer types or subtypes. Finally, adoptive transfer of tumor-specific lymphocytes expanded *ex vivo* is a methodology that aims to take advantage of naturally-occurring antitumor responses. All these approaches are similarly highly personalized and require the identification cancer epitopes of the subject's specific cancer and/or expansion of patient autologous cells *ex vivo*. Importantly, successes demonstrated by these specific cancer antigen approaches in gold standard animal models have not been always translatable to humans. Last, but not least, not all the patients suffering from cancer will express the same antigens on tumors, thus there are some significant limitations to the broad applicability of these approaches.

[0006] A solution to the problem of individualized targeted treatment and elimination of cancer presents itself in the form of viral infection history. In these approaches, a subject's infection history is used to re-initiate a past viral infection immune response through cytotoxic memory T-cells. Such therapies based on past viral infections are finely tune-able to target specific cancers by depositing on the cancer cells an epitope recognized by the subject's own immunological memory. Virus L1 proteins provide key functionality for delivering the epitope label onto the cancer cell target, thereby recruiting and activating the subject's own preexisting immune system components to target and eliminate the labeled cancer cells.

[0007] Mouse papillomavirus L1 proteins are good candidates for addressing this continuing need for better, more personalized cancer treatments. It has been fortuitously

discovered that specific mutations in the mouse papillomavirus L1 protein lead to formation of smaller-sized T=1 virus capsids, called capsid backbones, comprised of twelve (12) capsomeres, that are smaller than the normal T=7 capsids typically formed by virus L1 proteins, for instance as formed with human papillomavirus (HPV). These smaller-sized capsid backbones are very stable, allowing for higher conjugation efficiency, and owing to their smaller size, present less steric hindrance in infiltrating solid tumors or the tumor microenvironment.

[0008] Recently a class of anti-cancer drugs has emerged, called "immune checkpoint inhibitors," (ICIs). For example, some checkpoint inhibitor drugs are used clinically to block Programmed Cell Death Protein 1 ("PD-1") from binding to Programmed Death-Ligand ("PD-L1"). The PD-1 protein is found on the surface of T cells that helps keep the body's immune responses in check. When binding by PD-1 is blocked, the "brakes" on the immune system are released and the ability of T cells to kill cancer cells is increased. Examples of checkpoint proteins found on T cells or cancer cells include PD-1/PD-L1 and Cytotoxic T-lymphocyte Associated Protein 4 ("CTLA-4")/B7-1 (CD80)/B7-2 (CD86). Clinical evidence has shown that ICIs have durable clinical benefit for only a small subset of cancer types, and as a result is currently approved as a first line of treatment for only a small number of cancer indications, such as melanoma and head and neck cancers. Despite the observed clinical benefit, the overall 5-year survival rate for such patients remains less than 30%. Thus, improvements are needed to address the need of a more broadly applicable and effective chemotherapy composition able to treat a wider range of patients and yielding a higher positive outcome.

[0009] Provided are virus-inspired molecules, such as virus-like proteins (VLPs) and synergistic combinations thereof with immune checkpoint inhibitors for enhanced treatment of cancers. Importantly, it is shown here that addition of the disclosed virus-inspired molecules to ICIs synergistically enhances the overall therapeutic effect even in immune checkpoint inhibitor non-responsive tumors, leading to enhanced therapeutic outcome and survival.

SUMMARY

[0010] In various embodiments, compositions comprising: (a) a plurality of mutant mouse papillomavirus L1 proteins, (b) one or more peptides each comprising one or more epitopes from one or more pathogens other than a Papillomaviridae antigenic peptide, and (c) one or more ICI molecules, are disclosed, as well as methods of use of such compositions in the treatment or prevention of cancer or in strategies aimed at reducing the occurrence of such cancers. The compositions further comprise one or more peptides that each comprise one or

more epitopes from one or more pathogens other than a *Papillomaviridae* antigenic peptide. In some embodiments, the mutated amino acid sequence of the *Papillomaviridae* L1 protein of the compositions comprises one or more of the following mutations with respect to the wild type L1 protein sequence: (a) a deletion of at least five amino acid residues from an amino-terminus, (b) a deletion of at least ten amino acid residues from the helix four region, and (c) a deletion of at least thirty amino acid residues from a carboxy-terminus. The one or more peptides are attached to the plurality of virus proteins. In certain embodiments, the plurality of virus proteins spontaneously assemble to form an icosahedron or dodecahedron capsid backbone having a triangulation number *T* equal to 1 that binds to proteoglycan expressed on tumor cells. Thus, the compositions comprise a plurality of mutant *Papillomaviridae* proteins and one or more such peptides. Said differently, the compositions comprise one or more peptides attached to a plurality of mutant *Papillomaviridae* L1 proteins, in combination with one or more ICIs. In some embodiments, these compositions are contemplated as being pharmaceutical compositions, ready for administration to a subject in need thereof.

[0011] In some embodiments the mutant L1 proteins comprise a deletion of at least thirty amino acid residues from the carboxy terminus of the L1 proteins. In some embodiments the peptides are conjugated to the L1 proteins via disulphide, maleimide, or amide bond between the mutant *Papillomaviridae* L1 protein and a residue of the peptide.

[0012] In some embodiments from about 25% to about 85% (w/w) of the L1 proteins are attached to at least one of the peptides. In some embodiments the peptides also comprise a protease cleavage sequence, optionally selected from a furin cleavage sequence, a matrix metalloprotease cleavage sequence, or a disintegrin and metalloprotease (ADAM) cleavage sequence.

[0013] The epitopes are not particularly limited other than that they should be from an antigen that the subject to be treated has been previously exposed to and to which the subject has developed an immune reactivity towards, or has an immune memory of the previous exposure, such that upon re-exposure the subject's immune system will recognize and attack the cells bearing the epitopes. For instance, the epitope may be from a childhood vaccine. In other instances, the epitope may be from a past pathogenic infection the subject recovered from.

[0014] The compositions comprising the Immune Redirector Capsid (IRC) molecules bind to heparin sulfate proteoglycan located on cell surfaces. The IRC molecules do not form T = 7 capsids. In some embodiments, the mutant L1 proteins are from mouse L1 proteins.

[0015] In some embodiments the IRCs, VLPs, or virus-inspired particle (ViP) molecules disclosed herein are combined in a single composition or pharmaceutical

composition co-formulated with an immune checkpoint inhibitor molecule. The immune checkpoint inhibitor is in some instances a small molecule or an antigen-binding protein, such as, but not limited to, an antibody, or combinations of immune checkpoint inhibitor antibodies. The one or more ICIs that are in some embodiments provided in combination with the IRCs function to inhibit the binding of one or more of (i) PD-1 to PD-L1; (ii) CTLA-4 to B7-1 and/or to B7-2, (iii) LAG-3 to its respective ligand(s), (iv) Tim-3 to its respective ligand(s), (v) TIGIT, to its respective ligand(s), and (vi) CD96 to its respective ligand(s). In some embodiments, the one or more ICIs are antibodies or antigen-binding fragments thereof. In some embodiments, the one or more ICIs are one or more of pembrolizumab, nivolumab, durvalumab, atezolizumab, ipilimumab, and relatlimab.

[0016] As mentioned, contemplated herein also are methods of treating, preventing, and/or reducing the occurrence of (inhibiting the progression of or metastasis of) cancer in a subject in need thereof, which comprises administering to the subject a pharmaceutically effective, or therapeutically effective amount (terms used interchangeably herein), of the compositions described herein. Also provided are methods of inhibiting cancer tumor growth, progression, and/or metastasis in a subject in need thereof, which comprises administering to the subject a pharmaceutically effective amount of the compositions described herein, such as co-administration of the IRCs, VLPs, or virus-inspired (ViP) molecules described herein with an immune checkpoint inhibitor molecule(s). Uses of the described compositions in the described methods are also contemplated herein.

[0017] In some embodiments, the IRCs, VLPs, and/or ViPs are administered to the subject first, and then followed at a later time by administration of the one or more ICIs in a second composition. In some embodiments, the IRCs, VLPs, and/or ViPs are administered simultaneously with the one or more ICIs to the subject in need thereof. Contemplated herein are pharmaceutical compositions comprising: (i) the IRCs, VLPs, and/or ViPs, either together or separately from, (ii) the one or more ICIs, such that a user could administer the two components simultaneously by first mixing them together, or separately, for example. Such packaging is, in some embodiments, designed specifically for single dose administration of both components. In other embodiments, the dosages packaged a such are in an amount of from about 10 μg to about 2000 μg and have a peptide content of about 10 ng to about 150 μg or more. Such methods further comprise in some embodiments obtaining from the subject a tumor tissue sample and identifying in the tumor tissue a sequence of one or more MHC molecules expressed by one or more tumor cells in the tumor tissue sample.

[0018] In certain embodiments, the one or more epitopes are capable of complexing with one or more MHC molecules expressed by a tumor cell in a tumor tissue sample obtained from the subject. Secondary uses of the described compositions are also contemplated, as in the use for manufacture of a medicament useful for such methods.

[0019] Further provided herein are processes for producing the described compositions. The processes include various steps, such as: (a) transforming a prokaryotic cell with an expression vector encoding the L1 protein's nuclei acid sequence; (b) culturing the transformed prokaryotic cell under conditions that promote expression of the L1 protein; (c) lysing the transformed prokaryotic cells to release expressed L1 protein; (d) separating cell debris from the expressed L1 protein and recovering the L1 protein as inclusion bodies; (e) optionally washing the L1 protein inclusion bodies; (f) solubilizing the L1 protein inclusion bodies; (g) refolding the L1 protein in refolding buffer in the presence of reducing agent; and (h) forming the icosahedron or dodecahedron capsid having a triangulation number T equal to 1 in the same refolding buffer. Such processes, in some embodiments, further include conjugating in a conjugation buffer the one or more peptides to the assembled L1 protein by incubating the assembled L1 protein under reducing conditions in the presence of one or more peptides and/or removing denaturant from the assembly buffer but maintaining reducing agent when forming the icosahedron or dodecahedron capsid having a triangulation number T equal to 1.

[0020] This Summary is neither intended nor should it be construed as being representative of the full extent and scope of the present disclosure. Moreover, references made herein to "the present disclosure," or aspects thereof, should be understood to mean certain embodiments of the present disclosure and should not necessarily be construed as limiting all embodiments to a particular description. The present disclosure is set forth in various levels of detail in this Summary as well as in the attached drawings and the Description of Embodiments and no limitation as to the scope of the present disclosure is intended by either the inclusion or non-inclusion of elements, components, etc. in this Summary. Additional aspects of the present disclosure will become readily apparent from the Detailed Description, particularly when taken together with the figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIG. 1A is a schematic gene map of wildtype mouse papillomavirus L1 (MPV L1) and associated regions.

[0022] FIG. 1B is a schematic gene map of triple truncated mouse papillomavirus L1 protein (MPV.10.34.d), multiple copies of which combine together to form the T=1 icosahedral papillomavirus capsid backbone.

[0023] FIG. 2 is an amino sequence alignment of MusPV L1 protein sequence (SEQ ID NO: 127) aligned with the corresponding human HPV16 L1 protein sequence (SEQ ID NO: 128).

[0024] FIG. 3 is a flow chart of process steps leading to the manufacture of MPV.10.34.d capsid backbones, which upon formation subsequently are conjugated to peptides to form immune regulatory capsids (IRCs).

[0025] FIG. 4 is a photograph of a stained SDS-PAGE gel showing the incremental purification of the MPV.10.34.d capsid backbone from host cell protein contaminants through the manufacturing process (as described in Figure 3 and Example 1) demonstrating that host cell protein impurities are significantly reduced throughout the purification process (MW = standard molecular weight bands, numbers on the left-hand side of the gel indicate molecular weight in kiloDaltons (kDa), and top row numbers indicate amount of protein loaded in micrograms).

[0026] FIG. 5A is a dynamic light scattering (DLS) plot showing the intensity of the particle size distribution of the MPV.10.34.d capsid backbone after the refolding step but before the two-column chromatography purification. The X-axis shows diameter size distribution (nm) and the Y-axis provides percent intensity data.

[0027] FIG 5B is a dynamic light scatting plot showing the population size of purified MPV.10.34.d capsid backbone after the refolding step but before two-column chromatography purification. The X-axis shows diameter size distribution (nm) and the Y-axis provides volume percent data.

[0028] FIG. 6 is a graph of ELISA data obtained by binding a conformational MPV L1-A4 conformation-specific monoclonal antibody to refolded MPV.10.34.d capsid backbone showing that soluble and refolded MPV.10.34.d capsid backbone have the same T=1 conformation under different pH conditions. The Y-axis provides optical density at 450 nm, and the X-axis provides amount of MPV.10.34.d capsid backbone (ng).

[0029] FIG. 7 is a schematic representation of the steps to produce IRCs, i.e., conjugated MPV.10.34.d L1 capsid backbones.

[0030] FIG. 8 shows a photograph of a stained SDS-PAGE gel showing unconjugated MPV.10.34.d capsid backbones (lane 3) and IRCs (lane 4). Lane 1 shows molecular weight standards and lane 2 was intentionally left blank.

[0031] FIG. 9A is a flow cytometer histogram of cell count vs. fluorescence intensity showing detection of the binding of MPV.10.34.d capsid backbones and a variety of MPV.10.34.d IRCs showing that the IRCs retain specificity to tumor cells via binding heparin sulfate proteoglycan (HSPG). Data include: MPV.10.34.d and corresponding CMV pp65 IRC (solid line), MPV.10.34.d and corresponding E7 IRC (thick solid line), MPV.10.34.d and corresponding OVA IRC (thick dashed line), and MPV.10.34.d capsid backbones (dashed-line). Samples exhibited specificity for tumor cells as evidenced by the shift of the peak to the right. The positive control in these experiments was wildtype MPV capsid backbone (dotted line). The negative control included samples containing no IRC and no L1 (long-dashed line).

[0032] FIG. 9B is a flow cytometer histogram of cell count vs. fluorescence intensity showing that all MPV.10.34.d IRCs do not bind to cells in which HSPG is not expressed. Data include: MPV.10.34.d and corresponding CMV pp65 IRC (solid line), MPV.10.34.d and corresponding E7 IRC (thick solid line), MPV.10.34.d and corresponding OVA IRC (thick dashed line), and MPV.10.34.d capsid backbones (dashed-line). All samples exhibited specificity for tumor cells as evidenced by the shift of the peak to the right. The positive control in these experiments was wildtype MPV capsid backbone (dotted line). The negative control included no IRC and no L1 (long-dashed line).

[0033] FIG. 10 is a flow cytometer histogram of cell count vs. fluorescence intensity showing detection of the tumor cell surface display of OVA (SIINFEKL, SEQ ID NO: 95) / Kb (MHC-I) complex. The results show OVA (SIINFEKL, SEQ ID NO: 95)-conjugated MPV.10.34.d IRCs are able to load more epitopes onto tumor cell MHC receptors as compared with OVA (SIINFEKL, SEQ ID NO: 95)-conjugated HPV16 IRCs, when equivalent molarities of OVA-conjugated HPV16 IRCs (solid line) and OVA-conjugated MPV.10.34.d IRCs (thick solid line) were compared side-by-side. The negative control is represented by a long-dashed line. The positive control containing free peptide (SIINFEKL, SEQ ID NO: 95) at 1 μg/mL is represented by a short-dashed line.

[0034] FIG. 11A is a flow cytometer histogram of cell count vs. fluorescence intensity showing detection of the tumor cell surface cell count of CMV (NLAPMVATV, SEQ ID NO: 129) /HLA-A*0201 (MHC-I) complex showing CMV-conjugated MPV.10.34.d IRCs are able to load human CMV viral epitopes onto human tumor cell MHC receptors in HCT116 cells. Data points include: unrelated control peptide (thin dashed line), MPV.10.34.d capsid backbone (thin solid line), MPV.10.34.d and corresponding CMV pp65 IRC (thick solid line), and HCMV free peptide (thick dashed line).

[0035] FIG. 11B is a flow cytometer histogram of cell count vs. fluorescence intensity showing detection of the tumor cell surface cell count of CMV (NLAPMVATV) (SEQ ID NO: 129) /HLA-A*0201 (MHC-I) complex.

[0036] FIG. 12A is a flow cytometer histogram of cell count vs. fluorescence intensity showing detection of the showing competitive inhibition of binding of OVA-conjugated MPV.10.34.d IRCs to MC38 tumor cells with 10 mg/mL soluble heparin (dashed line) pre-mixed into the sample. The solid line is a negative control showing no OVA peptide loading. The solid line that overlaps the dashed line is the negative control showing no OVA peptide loading.

[0037] FIG. 12B is a flow cytometer histogram of cell count vs. fluorescence intensity showing detection of the competitive inhibition of binding of OVA-conjugated MPV.10.34.d IRCs to MC38 tumor cells with 5 mg/mL soluble heparin (dashed line) pre-mixed into the sample. The solid line is a negative control showing no OVA peptide loading. The solid line that overlaps the dashed line is the negative control showing no OVA peptide loading. The solid line that overlaps the dashed line is the negative control showing no OVA peptide loading.

[0038] FIG. 12C is a flow cytometer histogram of cell count vs. fluorescence intensity showing detection of the competitive inhibition of binding of OVA-conjugated MPV.10.34.d IRCs to MC38 tumor cells with 1 mg/mL soluble heparin (dashed line) pre-mixed into the sample. The solid line is a negative control showing no OVA peptide loading. The solid line that overlaps the dashed line is the negative control showing no OVA peptide loading.

[0039] FIG. 13A is a bar graph showing that OVA-conjugated MPV.10.34.d IRCs elicit an immune redirection of OVA-specific murine T-cells similar to OVA-conjugated HPV16 IRC in murine tumor cell line ID8-Luc.

[0040] FIG. 13B is a bar graph showing that OVA-conjugated MPV.10.34.d IRCs elicit an immune redirection of OVA-specific murine T-cells similar to OVA-conjugated HPV16 IRC in murine tumor cell lines B16-Luc.

[0041] FIG. 14A is a bar graph showing that E7-conjugated HPV16 IRCs elicit an immune redirection of OVA-specific murine T-cells similar to OVA-conjugated HPV16 IRC in murine tumor cell line ID8-Luc.

[0042] FIG. 14B is a bar graph showing that E7-conjugated HPV16 IRCs elicit an immune redirection of OVA-specific murine T-cells similar to OVA-conjugated HPV16 IRC in murine tumor cell lines B16-Luc.

[0043] FIG. 15A is a bar graph showing that human CMV-conjugated MPV.10.34.d IRCs elicit an immune redirection of CMV-specific CD8 T-cells similar to CMV-conjugated HPV16 IRCs in human tumor cell line HCT-116.

[0044] FIG. 15B is a bar graph showing that human CMV-conjugated MPV.10.34.d IRCs elicit an immune redirection of CMV-specific CD8 T-cells similar to CMV-conjugated HPV16 IRCs in human tumor cell line Ovarcar3.

[0045] FIG. 15C is a bar graph showing that human CMV-conjugated MPV.10.34.d IRCs elicit an immune redirection of CMV-specific CD8 T-cells similar to CMV-conjugated HPV16 IRCs in human tumor cell line MCF7.

[0046] FIG. 16 is a graph demonstrating high statistical correlation of binding of OVA-conjugated MPV.10.34.d IRCs to tumor cells and peptide loading of OVA (SIINFEKL, SEQ ID NO:95) onto tumor cell surface MHC-I molecules via OVA-conjugated MPV.10.34.d IRCs.

[0047] FIG. 17A is a graph of percent cytotoxicity vs. E:T ratio showing blocking of binding of 0.625 μg/mL OVA-conjugated MPV.10.34.d IRCs by incubation with heparin at concentration of 10 mg/mL, an immune redirection response of OVA-specific murine T-cells was not elicited (dashed line). The dotted line represents a negative control of MPV.10.34.d capsid backbones. The solid line represents the positive control sample with no heparin.

[0048] FIG. 17B is a graph of percent cytotoxicity vs. E:T ratio showing blocking of binding of 0.3125 μg/mL OVA-conjugated MPV.10.34.d IRCs by incubation with heparin at concentration of 10 mg/mL, an immune redirection response of OVA-specific murine T-cells was not elicited (dashed line). The dotted line represents a negative control of MPV.10.34.d capsid backbones. The solid line represents the positive control sample with no heparin.

[0049] FIG. 17C is a graph of percent cytotoxicity vs. E:T ratio showing blocking of binding of 0.156 μg/mL OVA-conjugated MPV.10.34.d IRCs by incubation with heparin at concentration of 10 mg/mL, an immune redirection response of OVA-specific murine T-cells was not elicited (dashed line). The dotted line represents a negative control of MPV.10.34.d capsid backbones. The solid line represents the positive control sample with no heparin.

[0050] FIG. 18 is a table of data obtained from a dose titration of the cell-binding assays and cytotoxicity assays. The study was repeated twice (with at least 3 replicates each). The mean values of geometric mean fluorescent intensity (MFI) are reported from the two experiments. These data were used to assess the statistical correlation of OVA-conjugated MPV.10.34.d IRC binding and cytotoxicity.

[0051] FIG. 19 is a graph of percent cytotoxicity vs. MFI demonstrating high statistical correlation of OVA-conjugated MPV.10.34.d IRC binding and cytotoxicity.

[0052] FIG. 20 is a graph of geometric mean fluorescence intensity vs. concentration of OVA-conjugated MPV.10.34.d IRCs (ng/mL) detecting tumor cell surface display of OVA(SIINFEKL, SEQ ID NO:95) / Kb (MHC-I) complex. The results show that OVA-conjugated MPV.10.34.d IRCs extra-cellularly load OVA peptides onto MHC receptors on the surface of tumor cells that are deficient in the MHC intracellular processing pathway.

[0053] FIG. 21A shows the percentage of $CD8^+$ T cells in the blood that stain positive for either the M38 or M45 tetramer at the 3 time points blood was collected post mCMV infection: day 21, day 58 and day 73, and demonstrates that pre-existing mCMV immunity exists in these mouse models.

[0054] FIG. 21B shows tumor volume of each tumor type at the time of harvesting, day 73.

[0055] FIG. 21C shows s total count of M38 $CD8^+$ T cells in the blood and tumor from both tumor types at the time of harvesting, demonstrating that mCMV T cells are present in both tumor types.

[0056] FIG. 21D shows the percentage of $CD8^+$ T cells that were M38 positive in the blood and tumor from both tumor types at the time of harvesting, demonstrating that mCMV T cells are present in both tumor types.

[0057] FIG. 22A shows average MC38 tumor growth curves of the treatment groups. The arrows on the x-axis indicate the day the mice were treated with vehicle, VERI-000 or VERI-003, post-tumor implantation. On day 18, Groups 5 and 7 were mistakenly not treated, thus receiving six treatments, while all other groups received seven treatments.

[0058] FIG. 22B shows average MC38 tumor growth curves of the treatment groups. The arrows on the x-axis indicate the day the mice were treated with vehicle, VERI-000 or VERI-003, post-tumor implantation. On day 18, Groups 5 and 7 were mistakenly not treated, thus receiving six treatments, while all other groups received seven treatments.

[0059] FIG. 23A shows MC38 survival curves for each of the treated groups.

[0060] FIG. 23B shows MC38 survival curves for each of the treated groups.

[0061] FIG. 24A shows average TC-1 tumor growth curves of treatment groups ("Grp"), as disclosed in Example 17 herein. The arrows on the x-axis indicate the day the mice were treated with vehicle, VERI-000 or VERI-003, post-tumor implantation. On day 17, Groups 5 and 7 were mistakenly not treated, thus receiving six treatments, while all other groups received seven treatments.

[0062] FIG. 24B shows average TC-1 tumor growth curves of treatment groups ("Grp"). The arrows on the x-axis indicate the day the mice were treated with vehicle, VERI-

000 or VERI-003, post-tumor implantation. On day 17, Groups 5 and 7 were mistakenly not treated, thus receiving six treatments, while all other groups received seven treatments.

[0063] FIG. 25A shows tumor growth curves of tumor-free mice (complete responders post first study) and 7 age-matched naïve mice were injected with 3 x 10^5 MC38 cells into the opposite flank more than 3 months after the original tumor implantation. No additional VERI-003 treatment was given.

[0064] FIG. 25B shows survival curves of tumor-free mice (complete responders post first study) and 7 age-matched naïve mice were injected with 3x10^5 MC38 cells into the opposite flank more than 3 months after the original tumor implantation. No additional VERI-003 treatment was given.

[0065] FIG. 26 shows tumor volumes of each treatment group prior to treatment on day 10. The range of tumor volume was approximately 75mm^3 to 125mm^3 resulting in an average tumor volume per group of 100mm^3.

[0066] FIG. 27A shows average MC38 tumour growth curves of each treatment group until day 27. Average tumor growth curves were not analyzed beyond day 27 as several mice in the control groups met the tumor endpoint by this point.

[0067] FIG. 27B shows average MC38 tumour growth curves of each treatment group until day 27. Average tumor growth curves were not analyzed beyond day 27 as several mice in the control groups met the tumor endpoint by this point.

[0068] FIG. 28A shows survival curves of each treatment group until day 60.

[0069] FIG. 28B shows survival curves of each treatment group until day 60.

[0070] FIG. 29 is a table showing treatment groups and dosing frequency from the B16 pilot intravenous efficacy study.

[0071] FIG. 30 shows metastatic nodule count of lung lesions from each mice, with the p values listed as a comparison of each group to the no treatment control using a One-way ANOVA adjusted for multiple comparisons.

DETAILED DESCRIPTION

Definitions

[0072] This specification describes exemplary embodiments and applications of the disclosure. This disclosure, however, is not limited to these exemplary embodiments and applications or to the manner in which the exemplary embodiments and applications operate or are described herein. Various embodiments, features, objects, and advantages of the present

teachings will be apparent from the description and accompanying drawings, and from the claims. As used herein, the terms "comprise," "comprises," "comprising," "contain," "contains," "containing," "have," "having," "include," "includes," and "including," and their variants, are not intended to be limiting, are inclusive or open-ended, and do not exclude additional, unrecited additives, components, integers, elements, or method steps. For example, a process, method, system, composition, kit, or apparatus that comprises a list of features is not necessarily limited only to those features but may include other features not expressly listed or inherent to such process, method, system, composition, kit, or apparatus.

[0073] "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.

[0074] The terms “immune redirector capsid” or “IRC” or “virus-inspired particle or “ViP” are used interchangeably herein, and these terms refer to a capsid backbone that also comprises a peptide bound, attached, or conjugated, to the capsid backbone. “AIR-VLP” is a more general term that can refer to T=7 capsids or T=1 capsids. The term “AIR-ViP” refers to a T=1 capsid.

[0075] “Cleavage sequence” as used herein includes, for example, specific peptide sequences, or more often, peptide motifs at which site-specific proteases cleave or cut the protein. Cleavage sites are used, for example, to cleave off an affinity tag, thereby restoring the natural protein sequence, or to inactivate a protein, or to activate proteins. In the present disclosure “cleavage” refers to proteolytic cleavage. In various embodiments, proteolytic cleavage is catalyzed by peptidases, proteases, or proteolytic cleavage enzymes before the final maturation of the protein. Proteins are also known to be cleaved as a result of intracellular processing of, for example, misfolded proteins. Another example of proteolytic processing of proteins is secretory proteins or proteins targeted to organelles, which have their signal peptide removed by specific signal peptidases before release to the extracellular environment or specific organelle. In one embodiment of the present disclosure, the cleavage sequence is specifically recognized by furin which cleaves and releases the peptides from the IRC, making the peptide available for loading onto or binding by the tumor cell surface receptors. In various embodiments, the cleavage sequence is comprised of cysteine, lysine, and/or arginine residues, that not only allow the peptide to be cleaved from the capsid backbone, but also serve as anchors to conjugate the peptide to the capsid protein until release by the cleavage protein, such as furin, which are in some instances enriched in, or selectively present at, the site of the tumor, i.e., in the tumor microenvironment.

[0076] "Epitope" or "antigen" or "antigenic epitope" is a set of amino acid residues that create recognition by or are recognized by a particular immunoglobulin or, in the context of T cells, those residues necessary for recognition by T cell receptor proteins and/or major histocompatibility (MHC) receptors. The amino acid residues of an epitope need not be contiguous/consecutive. In an immune system setting, *in vivo* or *in vitro,* an epitope are in some instances a composite of the collective features of a molecule, such as primary, secondary, and tertiary peptide structure, and charge, that together form a three-dimensional structure recognized by an immunoglobulin, T cell receptor, and/or human leukocyte (HLA) molecule.

[0077] "HPV" and "human papillomavirus" refer to the members of the family *Papillomaviridae* that are capable of infecting humans. There are two major groups of HPVs defined by their tropism (genital/mucosal and cutaneous groups), each of which contains multiple virus "types" or "strains/genotypes," e.g., HPV 16, HPV 18, HPV 31, HPV 32, etc.

[0078] "MusPV," "MMuPV1," "MPV," and "mouse papillomavirus," all alternatively and interchangeably refer to the known members of the family *Papillomaviridae* that are capable of infecting mice (*Mus musculus*).

[0079] "Human vaccine" as used herein means a biological preparation that improves immunity to a particular disease in a human. A vaccine typically contains an antigenic agent(s) that resembles a disease-causing agent (pathogen), and is often made from weakened or killed forms of the microbe, its toxins, or one or multiple immunogenic surface proteins of the disease-causing agent. The antigenic agent stimulates the body's immune system to recognize the disease-causing agent as foreign, destroy it, and "remember" it, so that the immune system can more easily recognize and destroy any of these pathogens should an actual future infection/exposure occur. Human vaccines include vaccines against viral diseases and bacterial diseases. In various embodiments, vaccines against viral diseases include hepatitis A, B, E virus, human papillomavirus, influenza virus, Japanese encephalitis virus, measles virus, mumps virus, polio virus, rabies virus, rotavirus, rubella virus, tick-borne encephalitis virus, varicella zoster virus, variola virus, and yellow fever virus. Human vaccines against viral diseases that are under development include, for example, dengue vaccine, eastern equine encephalitis virus, HTLV-1 T lymphocyte leukemia vaccine, and respiratory syncytial virus vaccine. Such a vaccine includes, in some embodiments, current vaccines in development or currently United States Food and Drug Administration (FDA)-approved vaccinations. A non-limiting list of examples of vaccines that are compatible with the compositions and methods described herein is provided in Table 2. The embodiments described herein, however, are not

limited to these listed vaccines, and are contemplated to apply to any vaccine developed to provide immunity in a human subject.

[0080] "Inhibiting," "reducing," "prevention," or "reducing the occurrence of," and similar terms, when used herein, includes any measurable decrease or complete inhibition/reduction or elimination to achieve a desired result, such as inhibiting, reducing, or preventing, or reducing the occurrence of, or reducing tumor mass, progression, and/or metastasis.

[0081] "MHC" or "major histocompatibility complex" is a group of genes that encode proteins found on the surfaces of cells that help the immune system recognize foreign substances. MHC proteins (receptors, or molecules) are expressed by all higher vertebrates. There are two main types of MHC molecules, MHC class I and MHC class II. In humans there are three different genetic loci that encode MHC class I molecules (human MHC-molecules are also designated as human leukocyte antigens (HLA)): HLA-A, HLA-B, and HLA-C. HLA-A*0l, HLA-A *02, and HLA-A * 11 are examples of different MHC class I alleles that can be expressed from these loci.

[0082] "Papillomavirus" (PV) refers to all members of the papillomavirus family (*Papillomaviridae*). An extensive list of papillomavirus types and the ability to make the respective capsid backbones can be referenced using this publication: "Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments," de Villers et al., *Virology,* 401(1):70-79, 2010, PMID: 20206957 (all the tables specifically incorporated herein by reference for all purposes). "Preferentially cleaved protein" as used herein means that the peptide is preferentially cleaved from the capsid or capsomere or L1 protein at the site of a tumor or tumor microenvironment. Without wishing to be bound by any particular theory, the preferential tumor-site cleavage is in some instances due to: (1) the unique cleavage sequence on the peptide, and/or (2) the unique tumor microenvironment. For example, in one embodiment, the peptide comprises a cleavage sequence that is preferentially cleaved by the enzyme furin, which is known to be expressed in relatively higher concentrations around tumor cells as compared with elsewhere in an organism.

[0083] "Protein," "polypeptide," and "peptide," as used herein, are not restricted to any particular number of amino acids; these terms are sometimes used interchangeably herein. The properties and amino acid sequences of the proteins described herein, and of the nucleic acids encoding them, are well-known and are determined routinely, as well as downloaded from various known databases. (See, e.g., the NCBI GenBank databases). Some peptide sequences are provided herein. However, some peptide sequence information is routinely updated, e.g.,

to correct mistakes in the previous entries, so updated (corrected) information about the proteins and nucleic acids encoding them is included in this application. Information provided in the sequence databases discussed herein is incorporated by reference. An immune "response" is a humoral and/or cellular response of the subject's immune system in which, in a cellular response, an antigen-primed cytotoxic T cell, Th1 T cell, Th2 T cell, and/or B cells primed by a vaccine or other pathogen present in the subject, or that the subject was previously exposed to, binds the epitope or antigen.

[0084] The term "preexisting immune response" as used herein means an immune response that is present in an individual prior to initiation of the inventive cancer treatment methods described herein. Thus, an individual having a preexisting immune response has an immune response capacity stored within their memory T cells or other immune system components against an antigen, prior to the initiation of a method of treatment as described herein with the antigen to treat cancer. A preexisting immune response is in some instances a naturally-occurring immune response. In other instances, the preexisting immune response is an induced immune response. As used herein, a naturally-occurring preexisting immune response is an immune response in an individual that was elicited in response to an antigen, such as a bacterial, fungal, parasitic, or viral antigen, with which the individual unintentionally contacted or contracted. That is, an individual having a preexisting immune response was, in some instances, not exposed to an antigen with the intent to generate an immune response to the antigen. An induced preexisting immune response is an immune response resulting from an intentional exposure to an antigen, such as when receiving a vaccine. The preexisting immune response is in some instances a naturally-occurring immune response, or in other instances the preexisting immune response is an induced immune response.

[0085] A "subject," or "subject in need thereof," as used herein, includes any animal or human that has a tumor/cancer or has had a tumor/cancer or has a precancerous medical condition or cell or has a genetic or other susceptibility, predisposition, or occupational risk of developing cancer or a tumor. Suitable subjects, also referred to herein as patients, include laboratory animals, such as mouse, rat, rabbit, guinea pig, or pig, farm animals, such as cattle, sporting animals, such as dogs or horses, domesticated animals or pets, such as a horse, dog, or cat, nonhuman primates, and humans.

[0086] "T cell response" as used herein refers to the immune response elicited by T cells as they encounter antigens. Naïve mature T cells are activated upon encountering antigen presented by B cells, macrophages, and dendritic cells, and then thereby produce armed effector T cells. Effector T cells are, in some instances, either CD8+ T cells that differentiate

into cytotoxic T cells, or CD4+ T cells that primarily induce the humoral immune response. The T cell immune response further generates immunological memory that gives protection from the subsequent challenge of the subject by the same or a similar pathogen comprising the same or similar epitopes. In various embodiments, the T cell response is at a threshold of at least 2-fold above the baseline of total CD8+ T cells. In various embodiments, the CD8+ T cells are CD69+ as well.

[0087] "Therapeutic compositions" are compositions that are designed and administered to patients for the use of treatment of a disease, such as cancer. Therapeutic compositions, e.g., therapeutic IRC-containing compositions, are used to treat benign or malignant tumors or patients/subjects at risk for such tumors, as well as non-solid cancers. In some embodiments, the IRCs, ViPs, or AIR-ViPs, with or without, an immune checkpoint inhibitor substance, are administered to a subject who previously had a tumor and is currently apparently tumor/cancer free, in an effort to enhance the inhibition or the recurrence of the tumor/cancer.

[0088] An "immune checkpoint inhibitor" ("ICI") is a substance or molecule that inhibits the binding of immune checkpoint proteins with their respective ligands or targets to form immunologically effective complexes. For example, the immune checkpoint inhibitor blocks PD-1 from binding to PD-L1; or blocks CTLA-4 and/or CD28 from binding to B7-1 and/or B7-2; or blocks lymphocyte activation gene 3 (LAG-3), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), T cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibitory motif (ITIM) domains (TIGIT), or CD96, from binding to their respective ligands. For example, the immune checkpoint inhibitor can be an antigen-binding protein that blocks PD-1 from binding to PD-L1; or blocks CTLA-4 and/or CD28 from binding to B7-1 and/or B7-2; or blocks LAG-3, TIM-3, TIGIT, or CD96, from binding to their respective ligands. The term "antigen binding protein" (ABP) includes antibodies or antibody fragments, as defined herein, that specifically bind a target ligand or antigen or target of interest, such an immune checkpoint target protein of interest.

[0089] In some embodiments of the invention the immune checkpoint inhibitor is an antibody. The term "antibody", or interchangeably "Ab," is used in the broadest sense and includes fully assembled antibodies, monoclonal antibodies (including human, humanized or chimeric antibodies), monomeric, homodimeric, and heterodimeric antibodies, polyclonal antibodies, multi-specific antibodies, e.g., bispecific antibodies, single domain antibodies (sdAbs), and antibody fragments that can bind antigen, e.g., Fab, Fab', $F(ab')_2$, Fv, single chain antibodies, diabodies, comprising complementarity determining regions (CDRs) of the

foregoing as long as they exhibit the desired biological activity. Multimers or aggregates of intact molecules and/or fragments, including chemically derivatized antibodies, are contemplated. The term “antibody” encompasses genetically engineered and/or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies, and heteroconjugate antibodies, triabodies, and tetrabodies, tandem di-scFv, tandem tri-scFv. Unless otherwise stated, the term "antibody" should be understood to encompass functional antibody fragments thereof. The term also encompasses intact or full-length antibodies, including antibodies of any class or sub-class, including IgG and sub-classes thereof, IgM, IgE, IgA, and IgD. Antibodies of any isotype class or subclass, including IgG, IgM, IgD, IgA, and IgE, IgGl, IgG2, IgG3, IgG4, IgAl and IgA2, or any allotype, are contemplated. Different isotypes have different effector functions; for example, IgGl and IgG3 isotypes have antibody-dependent cellular cytotoxicity (ADCC) activity.

[0090] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies that are antigen binding proteins are highly specific binders, being directed against an individual antigenic site or epitope, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different epitopes. Nonlimiting examples of monoclonal antibodies include murine, rabbit, rat, chicken, chimeric, humanized, or human antibodies, fully assembled antibodies, multispecific antibodies, including bispecific antibodies, antibody fragments that can bind an antigen, including, Fab, Fab', $F(ab)_2$, Fv, single chain antibodies, diabodies, maxibodies, nanobodies, and recombinant peptides comprising CDRs of the foregoing as long as they exhibit the desired biological activity, or variants or derivatives thereof.

[0091] The modifier "monoclonal" indicates the character of the sdAb or antibody as being obtained from a substantially homogeneous population of sdAb or antibodies, and is not to be construed as requiring production of the sdAb or antibody by any particular method. For example, monoclonal antibodies may be made by the hybridoma method first described by Kohler et al., *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567, the contents of which is expressly incorporated herein by reference). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., *Nature*, 352:624-628 (1991) and Marks et al., *J. Mol. Biol.*, 222:581-597 (1991), for example.

[0092] In a conventional "antibody" (i.e., homodimeric antibody), each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" chain of about 220 amino acids (about 25 kDa) and one "heavy" chain of about 440 amino acids (about 50-70 kDa). The amino-terminal portion of each chain includes a "variable" ("V") region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. The variable region differs among different antibodies. The constant region is the same among different antibodies. Within the variable region of each heavy or light chain, there are three hypervariable subregions that help determine the antibody's specificity for antigen in the case of an antibody that is an antigen binding protein. The variable domain residues between the hypervariable regions are called the framework residues and generally are somewhat homologous among different antibodies. Immunoglobulins can be assigned to different classes depending on the amino acid sequence of the constant domain of their heavy chains. Human light chains are classified as kappa (κ) and lambda (λ) light chains. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. (See generally, Fundamental Immunology, Ch. 7, Paul, W., ed., 2nd ed. Raven Press, N.Y., 1989). An "antibody" also encompasses a recombinantly made antibody, and antibodies that are glycosylated or lacking glycosylation.

[0093] The term "light chain" or "immunoglobulin light chain" includes a full-length light chain and fragments thereof having sufficient variable region sequence to confer binding specificity. A full-length light chain includes a variable region domain, V_L, and a constant region domain, C_L. The variable region domain of the light chain is at the amino-terminus of the polypeptide. Light chains include kappa chains and lambda chains.

[0094] The term "heavy chain" or "immunoglobulin heavy chain" includes a full-length heavy chain and fragments thereof having sufficient variable region sequence to confer binding specificity. A full-length heavy chain includes a variable region domain, V_H, and three constant region domains, C_{H1}, C_{H2}, and C_{H3}. The V_H domain is at the amino-terminus of the polypeptide, and the C_H domains are at the carboxyl-terminus, with the C_{H3} being closest to the carboxy-terminus of the polypeptide. Heavy chains are classified as mu (μ), delta (δ), gamma (γ), alpha (α), and epsilon (ε), and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Heavy chains may be of any isotype, including IgG (including IgG1, IgG2, IgG3 and IgG4 subtypes), IgA (including IgA1 and IgA2 subtypes), IgM and IgE. Several of these may be further divided into subclasses or isotypes, e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and

IgA2. Different IgG isotypes may have different effector functions (mediated by the Fc region), such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). In ADCC, the Fc region of an antibody binds to Fc receptors (Fc.gamma.Rs) on the surface of immune effector cells such as natural killers and macrophages, leading to the phagocytosis or lysis of the targeted cells. In CDC, the antibodies kill the targeted cells by triggering the complement cascade at the cell surface.

[0095] An "Fc region", or used interchangeably herein, "Fc domain" or "immunoglobulin Fc domain", contains two heavy chain fragments, which in a full antibody comprise the C_{H1} and C_{H2} domains of the antibody. The two heavy chain fragments are held together by two or more disulfide bonds and by hydrophobic interactions of the C_{H3} domains.

[0096] For a detailed description of the structure and generation of antibodies, see Roth et al., *Cell*, 94:411-414, 1998, incorporated herein by reference in its entirety. Briefly, the process for generating DNA encoding the heavy and light chain immunoglobulin sequences occurs primarily in developing B-cells. Prior to the rearranging and joining of various immunoglobulin gene segments, the V, D, J and constant (C) gene segments are found generally in relatively close proximity on a single chromosome. During B-cell-differentiation, one of each of the appropriate family members of the V, D, J (or only V and J in the case of light chain genes) gene segments are recombined to form functionally rearranged variable regions of the heavy and light immunoglobulin genes. This gene segment rearrangement process appears to be sequential. First, heavy chain D-to-J joints are made, followed by heavy chain V-to-DJ joints and light chain V-to-J joints. In addition to the rearrangement of V, D and J segments, further diversity is generated in the primary repertoire of immunoglobulin heavy and light chains by way of variable recombination at the locations where the V and J segments in the light chain are joined and where the D and J segments of the heavy chain are joined. Such variation in the light chain typically occurs within the last codon of the V gene segment and the first codon of the J segment. Similar imprecision in joining occurs on the heavy chain chromosome between the D and J_H segments and may extend over as many as 10 nucleotides. Furthermore, several nucleotides may be inserted between the D and J_H and between the V_H and D gene segments which are not encoded by genomic DNA. The addition of these nucleotides is known as N-region diversity. The net effect of such rearrangements in the variable region gene segments and the variable recombination which may occur during such joining is the production of a primary antibody repertoire.

[0097] The term "hypervariable" region refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid

residues from a complementarity determining region or CDR, i.e., residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain as described by Kabat et al., Sequences of Proteins of Immunological Interest, 8th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991. Even a single CDR may recognize and bind antigen, although with a lower affinity than the entire antigen binding site containing all of the CDRs.

[0098] An alternative definition of residues from a hypervariable "loop" is described by Chothia et al., *J. Mol. Biol.*, 196: 901-917 (1987), as residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain.

[0099] "Framework" or "FR" residues are those variable region residues other than the hypervariable region residues.

[0100] "Antibody fragments" comprise a portion of an intact full length antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies (see, Zapata et al., *Protein Eng.*, 8(10):1057-1062, 1995); single-chain antibody molecules; and multi-specific antibodies formed from antibody fragments.

[0101] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment which contains the constant region. The Fab fragment contains all of the variable domain, as well as the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. The Fc fragment displays carbohydrates and is responsible for many antibody effector functions (such as binding complement and cell receptors), that distinguish one class of antibody from another.

[0102] Pepsin treatment yields an $F(ab')_2$ fragment that has two "Single-chain Fv" or "scFv" antibody fragments comprising the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Fab fragments differ from Fab' fragments by the inclusion of a few additional residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Preferably, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains that enables the Fv to form the desired structure for antigen binding. For a review of scFv see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 1 13, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0103] A "Fab fragment" is comprised of one light chain and the C_{H1} and variable regions of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule.

[0104] A "Fab' fragment" contains one light chain and a portion of one heavy chain that contains the V_H domain and the C_{H1} domain and also the region between the C_{H1} and C_{H2} domains, such that an interchain disulfide bond can be formed between the two heavy chains of two Fab' fragments to form an $F(ab')_2$ molecule.

[0105] A "$F(ab')_2$ fragment" contains two light chains and two heavy chains containing a portion of the constant region between the C_{H1} and C_{H2} domains, such that an interchain disulfide bond is formed between the two heavy chains. A $F(ab')_2$ fragment thus is composed of two Fab' fragments that are held together by a disulfide bond between the two heavy chains.

[0106] "Fv" is the minimum antibody fragment that contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the VH VL dimer. A single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0107] "Single-chain antibodies" are Fv molecules in which the heavy and light chain variable regions have been connected by a flexible linker to form a single polypeptide chain, which forms an antigen-binding region. Single chain antibodies are discussed in detail in International Patent Application Publication No. WO 88/01649 and U.S. Pat. No. 4,946,778 and 5,260,203, the disclosures of which are incorporated by reference in their entireties.

[0108] "Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain, and optionally comprising a polypeptide linker between the V_H and V_L domains that enables the Fv to form the desired structure for antigen binding (see, Bird et al., Science 242:423-426, 1988, and Huston et al., *Proc. Nati. Acad. Sci. USA*, 85:5879-5883, 1988). An "Fd" fragment consists of the V_H and C_{H1} domains.

[0109] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites.

Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

[0110] A "domain antibody" is an immunologically functional immunoglobulin fragment containing only the variable region of a heavy chain or the variable region of a light chain. In some instances, two or more V_H regions are covalently joined with a peptide linker to create a bivalent domain antibody. The two V_H regions of a bivalent domain antibody may target the same or different antigens.

[0111] In general, an antigen binding protein, e.g., an immunoglobulin protein, or an antibody or antibody fragment, "specifically binds" to a target ligand or antigen of interest when it has a significantly higher binding affinity for, and consequently is capable of distinguishing, that target ligand or antigen, compared to its affinity for other unrelated proteins, under similar binding assay conditions. There are two expressions called "equilibrium dissociation constant" (abbreviated herein "K_D" or "K_d"), which are commonly used to define the affinity of a protein for a ligand. The value of K_D is a kinetic term; it is the ratio of the off-rate (k_{back}) and on-rate ($k_{forward}$) constants. The formula for the value of K_D is the following:

(I) $K_D = k_{back}/k_{forward} = k_{off}/k_{on} = (k_d/k_a)$, where

(II) $k_{back} = k_{off} = k_d$, i.e., the "dissociation rate constant," and

(III) $k_{forward} = k_{on} = k_a$, i.e., the "association rate constant."

[0112] On the other hand, the value of K_d is calculated by the following formula:

(IV) $K_d = ([\text{total binding sites}] \times [\text{total ligand}])/[PL]$.

[0113] For an antigen-binding protein with a single binding site, K_d can be calculated from the concentrations of the antigen-binding protein (P), the ligand (L) and the P-L complex (PL), at equilibrium, with $K_d = [P][L]/[PL]$.

[0114] The values of K_D and K_d are typically equivalent for a binding protein having a single binding site. Typically, an antigen binding protein is said to "specifically bind" its target antigen when the equilibrium dissociation constant (K_d or K_D) is 10^{-8} M or lower. The antigen binding protein specifically binds antigen with "high affinity" when the equilibrium dissociation constant is 10^{-9} M or lower, and with "very high affinity" when the K_d or K_D is 10^{-10} M or lower.

[0115] “Capsid backbone" refers to a multi-protein structure comprised of viral structural proteins, such as envelop or capsid proteins, such as an L1 protein, that in some instances self-assemble into a capsomere that resembles a virus but lack viral genetic material. Capsid backbones are non-infectious and non-replicating, yet morphologically similar to viruses. The capsid backbones disclosed herein bind to, or possess an inherent tropism for, tumor cells.

Capsid Structure

[0116] Viruses exist in many different morphologies and are generally smaller in size than bacteria, with a diameter between 20 nm and 300 nm, although some filoviruses possess filament lengths of up to 1400 nm. Visualization of viruses or virus capsid backbones requires transmission electron microscopes (TEM) that are more powerful than optical microscopes. Viruses are particle in shape and exist as virions having a nucleic acid surrounded by a protective coat of proteins called the capsid. These capsids are also in turn in some instances surrounded by a protective lipid bilayer that may include surface proteins, receptors, and the like.

[0117] Capsids are formed from a plurality of identical capsomeres. Capsids generally fall into helical or icosahedral structures, with the exception of bacteriophages that possess more complex structures. The most common icosahedral shape is composed of 20 equilateral triangular faces and resembles a three-dimensional sphere in overall shape. Helical capsids resemble a common spring shape in the form of a three-dimensional cylinder. Each face of the capsid is comprised of anywhere from one to three different proteins or monomer units (protomers). Capsids, when not surrounding papillomavirus genomes, are commonly referred to in the art as virus-like particles, or herein referred to as capsid backbones. That is, an empty capsid with no viral genomic material is referred to herein at times as a capsid backbone. Capsid backbones are excellent delivery molecules for treatment and/or prevention of various diseases, especially in the human body, because they are non-infectious and are optionally re-engineered to specifically target or bind to tumor cells, although most capsid backbone, as described above, possess an inherent tissue tropism without further engineering.

[0118] Capsomeres are formed from individual subunits or protomers. Native L1 protomers self-assemble through intermolecular disulfide bonds to form pentamers (capsomeres). As noted above, the capsid is comprised of many capsomeres. As used herein, the term “capsomere” is intended to mean a pentameric assembly of papillomavirus L1 polypeptides, including full-length L1 protein, or fragments and mutants thereof. A standard

icosahedral capsid is comprised of twenty faces and is a polyhedron including twelve vertices. The vertices are comprised of pentagonal capsomeres and the faces of the capsid are comprised of hexagonal capsomeres. There are always twelve pentagons (pentons) and a varying number of hexagons (hexons) in any given capsid depending on the virus type. Capsids that do not have an exogenous peptide attached thereto are termed "capsid backbones" herein.

[0119] The icosahedral structure found in most viruses is very common and consists of twenty triangular faces and twelve fivefold vertexes as noted above. The number of capsomeres included in a capsid follows well-known mathematical principles, such as found in the Goldberg polyhedron first described by Michael Goldberg in 1937. The structures can be indexed by two integers h and k, with h being greater than or equal to one and k being greater than or equal to zero, the structure is visualized by taking h steps from the edge of a pentamer, turning 60 degrees counter-clockwise, then taking k steps to get to the next pentamer. The triangulation number "T" for this type of capsid is therefore defined as $T = h^2 + h \bullet k + k^2$. In this scheme, icosahedral capsids contain twelve pentamers plus $10(T - 1)$ hexamers. (See, Carrillo-Tripp et al., *Nuc. Acids Res.*, 37 (Database issue):D436-D442, 2009). Thus, it can be seen that the "T" number, or triangulation number, is representative of the size and complexity of a given capsid. However, there are many known exceptions to this general "rule of thumb" found in, for instance, the Papillomaviridae family of viruses that can at times possess pentamers instead of hexamers in hexavalent positions, for instance in a *quasi* T=7 lattice. Outside of the canonical T=7 capsid structure, other structures such as T=1, T=2, and T=3, are known. A T=1 triangulation value indicates that the capsid is either only an icosahedron or a dodecahedron.

[0120] Some viruses are enveloped and further comprise a lipid membrane coating surrounding the capsid structure. The envelope is acquired from the host intracellular membrane. The nucleic acid material is either DNA or RNA and can be either single stranded or double stranded.

[0121] The Papillomaviridae family of viruses is a non-enveloped double-stranded DNA virus. There are several hundred family members within the Papillomaviridae family, each of which is referred to as a "type" that infect most known mammals and other vertebrates such as birds, snakes, turtles, and fish. The Papillomaviridae family members are considered to be relatively highly host- and tissue-tropic, meaning that its members usually possess a specific tissue tropism (preference for infection target) and a preference for host type, and are rarely transmitted between species. For example, it is known that the Papillomaviridae family member human papillomavirus (HPV) type 1 exhibits tropism for the soles of the feet, whereas

HPV type 2 prefers tissues in the palms of the hands. Papillomaviruses replicate exclusively in keratinocytes.

[0122] There are over 170 known human papillomavirus types that have been sequenced and are divided into five genera, including: Alphapapillomavirus, Betapapillomavirus, Gammapapillomavirus, Mupapillomavirus, and Nupapillomavirus. Many more human papillomaviruses have been identified but not yet sequenced.

[0123] The papillomavirus has but a single protomer called L1 protein, or major capsid protein L1, that is both necessary and sufficient to form its capsid which is comprised of 72 star-shaped capsomers. The papillomavirus family member capsids are non-enveloped and icosahedral. The papillomavirus genome also includes a second structural protein called L2 that is less abundantly expressed than L1. The presence of L2 in the capsid is optional and not necessary for virus function or for formation of the capsid. All of the capsomeres of the Papillomaviridae family are made of pentamer interactions between proteins.

[0124] As described herein, when describing mutant L1 proteins and the like, such mutants, and capsomers, and capsids made therefrom, are meant to include all Papillomaviridae family members and not just human or mouse family members. Thus, mutant L1 proteins as described herein are meant to encompass all L1 proteins in general, and in some instances specifically Papillomaviridae family L1 proteins in particular.

[0125] The amino acid domains and sequences of the human papillomavirus L1 protein and its mouse counterpart are presented in Figures 1 and 2. A fairly high level of sequence conservation is generally observed across all such L1 proteins of the Papillomaviridae family and is also reflected in this alignment. Further shown in Figure 2 are sites of possible mutation of the L1 sequence. Some of these mutations are known historically, such as the deletion of ten amino acids from the amino- or N-terminus of the L1 protein. (See, for instance, Conway et al., *J. Dent. Res.*, 88(4):307-317, 2009). Other structural mutations of the peptide sequence of the L1 protein in Papillomaviridae family members are known, such as the removal of the carboxy- or C-terminal residues in a truncation mutation.

[0126] The study of an N-terminal truncation mutant of L1 was begun partly in order to obtain stable crystal structures of the protein for high resolution structural analysis of the capsid. Thus, it was found that full length HPV16 L1 were unable to be crystallized under most tested conditions, but upon removal of the ten N-terminal residues, a crystal was able to be formed for further studies. (Conway et al., 2009). Surprisingly, it was found that upon removal of these ten N-terminal residues, the capsomers formed a T=1 capsid structure comprising icosahedral lattices made from twelve L1 pentamers (for a total of 60 protomers). As noted

above, the natural structure of the Papillomaviridae family member capsid is that of 72 L1 pentamers to form a T=7 structure. The T=1 structure of the N-terminal truncation mutant of HPV16 lacks certain disulfide bonds normally formed during capsid formation in wild type HPV16 capsids. Studies have shown that serine to cysteine mutation of C428 or deletion of the helix 4 region on human papillomavirus L1 capsid protein results in disrupting both the T=1 or the T=7 capsid backbone formation. (See, Varsani et al., *Virus Res.*, 122(1-2):154-163, 2006, and Schädlich et al., *ibid.*).

[0127] The overall structure of the papillomavirus L1 protein is presented in Figure 1A and Figure 1B and has a tertiary structure consisting generally of various secondary structures including a core of β-strands that form a classic "jelly roll" β-sandwich and five C-terminal α-helices that support five surface-exposed loop regions generally designated in the art as loops BC, DE, EF, FG, and HI. (See, Chen et al., *Mol. Cell*, 5:557-567, 2000, and Bissett et al., *Scientific Reports*, 6:39730, 2016). Three of the α-helices, commonly referred to as h2, h3, and h4, form the surface of contact with other monomers and pentamers (Figure 1B). (See, Chen et al., Figure 4, page 561). The five α-helices generally reside at the carboxy-terminus of the L1 sequence.

Design and Production of Mutant L1 Proteins

[0128] Deletions of the MPV L1 sequence were made to facilitating the formation of 10 nm to 15 nm capsomeres made from five L1 proteins. It was previously shown that truncation of the amino-, helix-four, and carboxy-terminus residues of the HPV16 L1 protein results in capsomere formation. (See, Bishop et al., *Virol. J.*, 4:3, 2007, and Schädlich et al., *J. Virol.*, 83(15):7690-7705, 2009). On the other hand, it was shown in HPV11 and HPV16 that truncation of the amino-terminal ten residues of L1, alone, would yield T=1 icosahedral capsid backbones. These T=1 icosahedral capsid backbones are approximately 20 nm to 30 nm in diameter and consist of 60 L1 proteins (or 12 capsomers). Deletion of up to 34 amino acids at the carboxy-terminus did not inhibit T=1 formation. However, if deletions in the helix-four region of L1 occurred (amino acids 411 to 436), the formation of T=1 would be ablated, even in the presence of N-terminal or C-terminal truncations. In all permutations, capsomers would be observed. (Chen et al., *Mol. Cell*, 5(3):557-567, 2000, and WO 2000054730). These results were consistent with papillomavirus type 16 L1 produced in *E. coli* or in insect cells. (See, Schädlich et al., *J. Virol.*, 83(15):7690-7705, 2009). Taken together, the authors of this study concluded that the helix-4 structure was needed for the assembly of capsomers into both higher ordered T=1 and T=7 icosahedral structures. (See, Bishop et al., *Virol.*, 4:3, 2007).

[0129] Various deletions of the MPV L1 sequence were generated, resulting in the construct called "MPV.10.34.d" shown schematically in Figure 1B. This construct was designed to create an MPV capsomere as a therapeutic platform. MPV L1 proteins were selected as the carrier vehicle construct instead of HPV L1 because humans have for the most part not been exposed to MPV and therefore it was postulated that virion-derived capsids from MPV would not be sensitive to innate immune response as is seen with HPV L1 proteins.

[0130] Recently a ΔN10 deletion of HPV16 L1, in which the amino-terminal ten residues of the HPV L1 sequence are removed, was crystallized and found to conform to the shape of a T=1 capsid backbone. (See, Chen et al., 2000). The structure revealed that the carboxy terminal segment from residue 384 to 446 of L1 folds into three helices with connecting loops and turns. These helices are the primary inter-pentamer bonding contacts in the assembled T=1 capsid backbone. To test whether these helices also affect capsid backbone assembly, L1 proteins comprising the ΔN10 deletion were generated with a specific deletion of helix 4 for both HPV16 (residues 408 to 431) and HPV11 (residues 409 to 429). The pentamers were purified by FPLC and were shown to possess a "donut" shape as observed by electron microscopy (EM). No assembly of capsid backbones from these pentamers was found under any condition tested, suggesting that this carboxy-terminal helical domain is essential for T=1 or T=7 capsid backbone assembly. Crystallographic analysis of the T=1 capsid backbone revealed that inter-pentameric contacts are established by hydrophobic interactions between the α-helices 2 and 3 of one capsomere and α-helix 4 of a neighboring capsomere. (Chen et al., 2000). Consequently, a mutant L1 with helix 4 deleted formed homogenous capsomeres but failed to form T=1 and T=7 capsid backbones. (See, Bishop et al., 2006). The constructs with helix 4 deleted did not exhibit any ability to self-assemble, consistent with previous reports. (See, Schadlich et al., *J. Virol.*, 83(15):7690–7705, 2009).

[0131] For the purposes of this description, the term mutant L1 protein means an L1 protein or protomer comprising one or more non-wild type sequences. Such non-wild type sequences include truncations or deletions (internal or at the ends of the sequences), single residue substitutions, and the like. For instance, a mutant L1 protein includes an L1 protein in which any of the following are true: 1) a certain number of the N-terminal residues are deleted, a certain number of the C-terminal residues are deleted, and/or 3) a certain number of internal residues are deleted, in some instances in more than one location internally within the sequence.

[0132] The mutant L1 protein is in some embodiments derived from a wild type papillomavirus L1 protein. Any papillomavirus L1 protein is useful in the presently described compositions. L1 protein sequences are relatively conserved. Thus, description of mouse

papillomavirus mutant L1 proteins, below, are exemplary and it is contemplated that the same mutations made in other L1 proteins of the papillomavirus family is expected to yield similar results. In various embodiments, a capsid backbone is provided comprising a papillomavirus L1 protein and/or a papillomavirus L2 protein. Thus, the capsid backbone in some embodiments comprises both papilloma L1 and L2 proteins. In other embodiments, the capsid backbone is comprised of only L1 proteins. In some embodiments the L1 protein is a hybrid or chimeric protein comprised of L1 sequences from more than one source merged together into a single L1 sequence.

[0133] The L1 protein sequences are known for substantially all papillomavirus genotypes identified to date, and any of these L1 sequences or fragments are contemplated as being included in the present compositions. Examples of L1 polypeptides include, without limitation, full-length L1 polypeptides, e.g., HPV16 L1 polypeptide, SEQ ID NO: 128, L1 truncations that lack any one or more residues of the native C-terminus, L1 truncations that lack any one or more residues of the native N-terminus, and L1 truncations that lack any one or more internal domain residues in any one or more internal locations. The L1 protein is in some instances exemplified as a modified L1 protein, e.g., a modified HPV16 or MPV16 L1 protein, wherein the HPV16 L2 amino acids 17 to 36 (the RG1 epitope) are inserted within the DE-surface loop of HPV16 L1. (See, Schellenbacher et al., *J. Invest. Dermatol.,* 133(12):2706-2713, 2013; Slupetzky et al., *Vaccine,* 25:2001-2010, 2007; Kondo et al., *J. Med. Virol.*, 80:841-6, 2008; Schellenbacher et al., *J. Virol.*, 83:10085-10095, 2009; and Caldeira et al., *Vaccine*, 28:4384-93, 2010).

[0134] The L2 polypeptide is in some embodiments full-length L2 protein or an L2 polypeptide fragment. The L2 sequences are known for substantially all papillomavirus genotypes identified to date, and any of these L2 sequences or fragments can be employed in the present disclosure. Examples of L2 polypeptides include, without limitation, full-length L2 polypeptides, e.g., HPV16 L2 polypeptide (SEQ ID NO:1), or mouse papillomavirus L2 (SEQ ID NO:2), L2 truncations that lack any one or more of the native C-terminus, L2 truncations that lack any one or more of the native N-terminus, and L2 truncations that lack any one or more internal domain residues in any one or more locations.

[0135] The papillomavirus capsid backbone is in some embodiments formed using the L1 and optionally L2 polypeptides from any animal papillomavirus, or derivatives or fragments thereof. Thus, any known (or hereafter identified) L1 and optionally L2 sequences of human, bovine, equine, ovine, porcine, deer, canine, feline, rodent, rabbit, etc., papillomaviruses are employed to prepare the capsid backbones described herein. (See, de Villiers et al., *Virology*,

324:17-27, 2004, for a current description of papillomavirus genotypes and their relatedness, incorporated herein by reference for all purposes).

[0136] In certain embodiments, the L1 and optionally L2 polypeptides that are used to form the capsid backbones are from a non-human papillomavirus or a human papillomavirus genotype other than HPV6, HPV11, HPV16, and HPV18. For example, the L1 and/or L2 proteins are in some embodiments from HPV 1, 2, 3, 4, 5, 6, 8, 9, 15, 17, 23, 27, 31, 33, 35, 38, 39, 45, 51, 52,58, 66, 68, 70, 76, or 92.

[0137] As described above, in human papillomavirus HPV16, several different mutations of L1 protein have been characterized. (See, for instance, Chen et al., 2000). Some of these mutations include the following in Table 1.

TABLE 1

(Chen et al., 2000, Table 1, page 558)

Deletion	Trypsin Sensitivity	Apparent Diameter of Assembled Particle (Å)[a]
ΔN=0	No	600
ΔN=8	No	600
ΔN=9	No	600
ΔN=10	No	300
ΔN=15	Yes[b]	NA
ΔN=20	Yes	NA
ΔC=16	No	600
ΔC=30	No	600
ΔC=46	Yes	NA
ΔC=86	Yes	NA

[0138] In Table 1, the delta symbol (Δ) designates deletion and the "N" or "C" designated whether the deletion is located at the N-terminus or C-terminus, respectively. The number following these two symbols indicates the number of residues of the L1 sequence that were deleted. It is noted that Chen et al. does not report any double, triple, or higher number of mutations within a single L1 protein.

[0139] Thus, the L1 mutant proteins described herein include N-terminal truncation L1 mutant proteins. The N terminus is truncated by at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids. In some embodiments the N-terminal truncation is 5 amino acids. In some embodiments the N-truncation is 10 amino acids. In some embodiments the N-terminal truncation is 37, 38, 39, or even 40 amino acids.

[0140] The L1 mutant proteins described herein further include C-terminal truncation L1 mutant proteins. The C terminus is truncated by at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids. In some embodiments the C-terminal truncation is 5 amino acids. In some embodiments the C-truncation is 10 amino acids.

[0141] The L1 mutant proteins described herein further include L1 mutants in which any number of internal residues are deleted. Surprisingly, the retention of the helix-4 region is in some embodiments needed for the formation of capsid backbones having a T=1 geometry, whereas in the literature it is reported, as discussed above, that its deletion is not supposed to yield any capsid backbone assembly. Generally, the internal residues deleted in the described mutant L1 proteins are those shown in Figures 1 and 2. Contemplated also are deletions of 34 residues in the helix 4 (H4) region. In some instances, the truncation of internal residues of L1 proteins is 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or even 40 residues in length.

[0142] Also described herein are L1 mutant proteins in which any one or more of the C-terminus and/or N-terminus and/or internal residues are deleted simultaneously. For instance, in some embodiments the mutant L1 protein has both C- and N-terminal truncation mutations of similar or varying length. In other embodiments, the mutant L1 protein has a C-terminal truncation and an internal residue truncation. In some embodiments, the mutant L1 protein has an N-terminal truncation and an internal truncation. In certain embodiments, the mutant L1 protein has truncations simultaneously in all three locations, C-terminus, N-terminals, and internal truncations.

[0143] The mutant L1 proteins described herein are generally produced recombinantly but are also produced by any known protein expression methodology. For instance, the mutant L1 proteins are generated by first designing DNA primers complimentary to the wild type L1 sequence and then performing PCR amplification of the sequence in the presence of the primers designed to truncate or otherwise mutate the wild type L1 sequence, as further explained in detail below, in the Examples section. (See also, Touze et al., *J. Clin. Microbiol.*, 36(7):2046-2051, 1998). The design and implementation of proper primer sequences and PCR protocols are known and such methods are used to ultimately generate the desired mutant L1 protein nucleic acid sequence, from which the mutant L1 proteins are expressed.

[0144] The mutant L1 protein nucleic acid sequence is then in some embodiments codon-optimized for better protein expression and production depending on the organism in which the expression is conducted. Utilization of different codon optimization methods for

certain expression vectors and host expression systems are known in the art. (See, Mauro and Chappell, *Trends in Molecular Medicine*, 20(11):604-613, 2014, for instance).

[0145] The mutant L1 protein nucleic acid sequence is then ligated into an acceptably prepared and commercially-available expression vector designed for protein expression. Expression vectors of various types possessing functionality for certain expression hosts are widely commercially available. Recombinant mutant L1 proteins described herein are expressed in bacterial as well as eukaryotic cells and in certain embodiments are expressible *in vitro*.

[0146] Often expression of recombinant proteins in bacterial hosts results in the formation of inclusion bodies (IBs). Thus, in some instances, recombinant mutant L1 protein expressed as IBs are solubilized using known procedures. In a particular embodiment, the solubilization of IBs of expressed mutant L1 proteins described herein includes the steps set forth, for instance, in Figure 3, and Example 3. The processes include various steps, such as: (a) transforming a prokaryotic cell with an expression vector encoding the L1 protein; (b) culturing the transformed prokaryotic cell under conditions that promote expression of the L1 protein; (c) lysing the transformed prokaryotic cells to release expressed L1 protein; (d) separating cell debris from the expressed L1 protein and recovering the L1 protein in the form of IBs; (e) optionally washing the L1 protein IBs; (f) solubilizing the L1 protein IBs; (g) refolding the L1 protein optionally in the presence of one or more denaturants, reducing agents, and the like; and (h) forming the icosahedron or dodecahedron capsid having a triangulation number T equal to 1 by incubating the refolded L1 protein in assembly buffer. Such processes, in some embodiments, further include conjugating in a conjugation buffer the one or more peptides to the assembled L1 protein by incubating the assembled L1 protein under reducing conditions in the presence of one or more peptides and/or removing denaturant from the conjugation buffer but maintaining reducing agent when forming the icosahedron or dodecahedron capsid having a triangulation number T equal to 1.

[0147] The described methods and processes for creating and purifying the described mutant L1 proteins is different in many aspects from such processes described in the art for assembly of papillomavirus capsids. Indeed, it is known in the art that assembly into higher ordered papillomavirus capsids requires that the L1 protein must first be subjected to a dis-assembly buffer that includes a reducing agent. This step is then often followed by subjecting the L1 protein to an assembly buffer that then removes the reducing agent. This legacy methodology results in stable capsids with improved properties. (See, McCarthy et al.,

10.1128/JVI.72.1.32-41, 1998, Zhao et al., *Virol. J.*, 9:52, 2012, Mach et al., *J. Pharm. Sci.*, 95:2195-2206, 2006, and U.S. Patent No. 6,436,402).

Properties of Capsid Backbones Formed From Mutant L1 Proteins

[0148] It was serendipitously discovered during the studies described herein that certain mutant L1 proteins possess beneficial and unexpected properties. For instance, certain mutant L1 proteins led primarily to the formation of a T=1 capsid backbone possessing helpful and unexpected conjugation properties. The formation of a T=1 capsid backbone instead of a T=7 capsid backbone leads to higher stability under reducing conditions and therefore higher conjugation efficiency as compared with wild type sequences that form T=7 capsid backbone.

[0149] For instance, the efficiency with which the mutant capsid backbone, e.g., the MPV.10.34.d backbone, is able to be conjugated with peptide is from 25 to 85% (w/w). In some embodiments, the conjugation efficiency is about 25%. In other embodiments, the conjugation efficiency is about 25, about 35, about 45, about 55, about 65, about 75, or even about 85% (w/w).

[0150] In contrast, wild type T=7 capsid backbones have generally a lower efficiency of conjugation that is less than about 25%. See, for instance, WO 2020/139978. The ability to achieve a higher amount of peptide conjugated to the T=1 capsid backbone compared to T=7 capsid backbone allows for delivery of a higher number of peptides to the target tumor or cancer at an overall lower IRC dose amount compared with IRC forms from T=7 capsid backbones.

[0151] Additionally, T=1 capsid backbones having a smaller geometric shape or size as compared to T=7 capsid backbones allows for less stearic hindrance with the IRC made from T=1 capsid backbones is injected into a subject and the IRC infiltrate tumor microenvironments. This beneficial and unexpected effect then leads to a lower IRC dose needed to achieve the same effect as an equivalent T=7, or higher order, capsid backbone-based IRC.

[0152] These and other additional beneficial features of the T=1 capsid backbone geometry are described in further detail hereinbelow.

Mutant L1 Protein IRC and Mechanism of Action

[0153] In some embodiments, the mutant L1 protein is conjugated to another peptide. To add further beneficial functionality to capsids or capsid backbones comprised of the mutant L1 proteins, additional peptides are conjugated to the surface of such capsids. These peptides

add beneficial functionality to the capsid and result in added functionality such as treatment of cancer in subjects in need thereof.

[0154] In an embodiment, the conjugated papillomavirus capsid backbone comprises an L1 capsid protein and a peptide. In other embodiments, the IRC comprises an (at least one) L1 capsid protein, an (at least one) L2 capsid protein, and at least one peptide. The L1 polypeptide is in some embodiments a full length L1 protein or in other embodiments is an L1 polypeptide fragment. In specific embodiments, the full-length L1 protein or L1 polypeptide fragment is capsid backbone assembly-competent; that is, the L1 polypeptide will self-assemble to form capsomeres under proper conditions that are competent for self-assembly into higher-order structural geometries, thereby forming a capsid backbone. In more specific embodiments, the capsid backbones comprise a T=1 particle, a structure of about 20 nm to 30 nm in diameter, and composed of 12 capsomeres or 60 copies of L1 protein. In other embodiments, the capsid backbones comprise a fully assembled papillomavirus capsid, a structure of about 50 nm and composed of 72 capsomeres or 360 copies of L1 protein.

[0155] In various embodiments, the IRC presented herein bind to, specifically or non-specifically, or otherwise contact, one or more cancer cells. This is in part due to the capsid backbone's selectivity (tropism) for proteins and/or molecules that are in some instances specific to, or expressed in higher abundance by, tumor cells. In various embodiments, the IRC binds to a certain sub-family type of heparin sulfate proteoglycan (HSPG), which is preferentially expressed on tumor cells. As used herein, "binding to a cancer cell" refers to the formation of non-covalent interactions between the capsid protein of the IRC and the tumor cell such that the IRC comes into close proximity to the tumor cell and the peptide is cleaved from the capsid backbone, and then the peptide binds to, or is bound by, or otherwise interacts with, the MHC receptor present on the tumor cell surface.

[0156] In various embodiments, the peptide is an epitope that is recognized by a T cell or T cell population that already exists in the subject. In various embodiments, this existing T cell or T cell population exists because of a prior infection or vaccination. In various embodiments, the peptide is an epitope that is capable of being bound by a T cell. In various embodiments, the peptide is an epitope capable of being bound by a T cell already present in a subject. In this context, "capable of being bound" means that an "epitope" is presented on the surface of a cell, where it is bound to MHC molecules. T cell epitopes presentable by MHC class I receptors are bound by the T cell receptor of cytotoxic CD8 T lymphocytes (CD8 T cells or CTLs). T cell epitopes presentable by MHC class I molecules are typically peptides of about 9 to about 12 amino acids in length. In various embodiments, an IRC is provided that releases

a T cell response-eliciting peptide that upon release is directly bound by and consequently appropriately presentable by one or more MHC molecules expressed on the surface of one or more cancer or tumor cells. As the released peptide does not require processing by the antigen processing machinery in the cytosol, the T cell response-eliciting peptides are presented on the surface of the target cell in a short amount of time. The process of release of such peptides from the IRC and subsequent binding of the peptides by the MHC molecules of target cells is akin to labelling, tagging, or otherwise "marking" these tumor or cancer cells. This tagging or marking leads to ready identification by other components of the subject's immune system, thereby recruiting these components of the subject's immune system to remove the cancer or tumor cells via the various known cell destruction pathways.

[0157] Hence, in one embodiment of the described methods, uses, and compositions described herein, in less than about 8.5 hours after administration of the IRC dose to the subject, the IRC will naturally migrate to the target cell after which the T cell response-eliciting peptide released from the IRC, is bound by the MHC molecule on the cancer cell, and then the peptide is presented on the surface of the target cell via an MHC class I molecule to other components of the subject's immune system for recognition thereby. In another embodiment of the invention, in less than 23.5 hours after introduction of the IRC to the target cell the T cell response eliciting peptide is presented on the surface of the target cell via an MHC class I molecule. In another embodiment of the invention, the IRC is capable of mediating T cell cytotoxicity against the target cell within less than 6 hours after administration of the IRC to the target cell.

[0158] In various embodiments, the peptide comprises one epitope or comprises at least two epitopes. The peptide epitopes are in some instances derived from different proteins, or in other embodiments they are epitopes from the same protein (or antigen). In various embodiments, the pathogen is a virus, a bacterium, a fungus, a parasite, or a combination thereof.

[0159] In various embodiments, the subject's preexisting T cells are specific to a vaccine epitope. In various embodiments the epitope is derived from a childhood, early childhood, adolescent, or elderly (geriatric), vaccine. In various embodiments the subject's preexisting immunity is the result of prior administration of a human vaccine. Antigens described herein that comprise epitopes incorporated into the peptides described herein are found in any of the known infectious agents, such as viruses, bacteria, parasites, fungi, and the like. In various embodiments, the peptide is selected from the list provided by Table 2.

[0160] For instance, non-limiting examples of a viruses from which antigens bearing epitopes that are incorporated in some embodiments into the described peptides include, for instance, a vaccinia virus, a varicella zoster virus, a herpesvirus, e.g., herpes zoster virus or cytomegalovirus or Epstein-Barr virus, rubella, a hepatitis virus, e.g., hepatitis A virus or hepatitis B virus or hepatitis C virus, influenza, e.g., type A or type B, a measles virus, a mumps virus, a polio virus, a variola (smallpox) virus, a rabies virus, a coronavirus, Dengue virus, an Ebola virus, a West Nile virus, a yellow fever virus, or a zika virus.

[0161] For instance, non-limiting examples of a bacteria from which antigens bearing epitopes that are incorporated in some embodiments into the described peptides include, for example, a *Bordetella pertussis*, *chlamydia*, *trachomatis*, *Clostridium tetani*, diphtheria, *Hemophilus influenza*, *Meningococcus*, *pneumococcus*, vibrio cholera, *Mycobacterium tuberculosis*, BCG, typhoid, *E. coli*, *salmonella*, *Legionella pneumophila*, rickettsia, *Treponema pallidum pallidum*, *Streptococcus* group A or group B, *Streptococcus pneumonia*, *Bacillus anthracis*, *Clostridium botulinum*, or a *Yersinia sp* bacteria.

[0162] For instance, non-limiting examples of a parasite from which antigens bearing epitopes that are incorporated in some embodiments into the described peptides include, *Entamoeba histolytica*, *Toxoplasma gondii*, a *Trichinella sp.*, e.g., *Trichinella spiralis*, a *Trichomonas sp.*, e.g., *Trichomonas vaginalis*, a *Trypanosoma sp.*, e.g., *Trypanosoma brucei gambiense*, *Trypanosoma brucei rhodesiense*, or a *Trypanosoma cruzi*, or a plasmodium, e.g., *Plasmodium falciparum*, *Plasmodium vivax*, or *Plasmodium malariae*.

TABLE 2

Epitope Peptide Sequences

Epitope Sequence	SEQ ID NO	Virus Type	MHC allele	Viral Protein
SLPRSRTPI	4	Chicken Pox (VZV)	A*02:01	IE62
SAPLPSNRV	5	Chicken Pox (VZV)	A*02:01	IE62
GSAPLPSNRV	6	Chicken Pox (VZV)	A*02:01	IE62
ALWALPHAA	7	Chicken Pox (VZV)	A*02:01	IE62
SLSGLYVFV	8	Shingles vaccines	A*02:01	Glycoprotein E
YLGVYIWNM	9	Shingles vaccines	A*02:01	Glycoprotein E

KIHEAPFDL	10	Shingles vaccines	A*02:01	Glycoprotein E
LLCLVIFLI	11	Shingles vaccines	A*02:01	Glycoprotein E
DLLLEWLYV	12	Shingles vaccines	A*02:01	Glycoprotein E
SMYYAGLPV	13	Shingles vaccines	A*02:01	Glycoprotein E
ILHDGGTTL	14	Shingles vaccines	A*02:01	Glycoprotein E
WLYVPIDPT	15	Shingles vaccines	A*02:01	Glycoprotein E
VLMGFGIIT	16	Shingles vaccines	A*02:01	Glycoprotein E
CLVIFLICT	17	Shingles vaccines	A*02:01	Glycoprotein E
KEADQPWIV	18	Shingles vaccines	A*02:01	Glycoprotein E
VVSTVDHFV	19	Shingles vaccines	A*02:01	Glycoprotein E
FLICTAKRM	20	Shingles vaccines	A*02:01	Glycoprotein E
VLRTEKQYL	21	Shingles vaccines	A*02:01	Glycoprotein E
HMWNYHSHV	22	Shingles vaccines	A*02:01	Glycoprotein E
TVNKPVVGV	23	Shingles vaccines	A*02:01	Glycoprotein E
FVVYFNGHV	24	Shingles vaccines	A*02:01	Glycoprotein E
WIVVNTSTL	25	Shingles vaccines	A*02:01	Glycoprotein E
VAYTVVSTV	26	Shingles vaccines	A*02:01	Glycoprotein E
FMYMSLLGV	27	measles	A*02:01	m50
SLWGSLLML	28	measles	A*02:01	C protein
LLAVIFVMFL	29	measles	A*02:01	H38
SMYRVFEVGV	30	measles	A*02:01	H250-259
ILPGQDLQYV	31	measles	A*02:01	H516-525
KLWCRHFCV	32	measles	A*02:01	H576
KLWCRHFCVL	33	measles	A*02:01	H576
RLSDNGYYTV	34	measles	A*02:01	M164
KLLRYYTEI	35	measles	A*02:01	F205
KLWESPQEI	36	measles	A*02:01	C 84
RLLDRLVRL	37	measles	A*02:01	N50
KLMPNITLL	38	measles	A*02:01	F57
TLLNNCTRV	39	measles	A*02:01	F64
EMLTLATWV	40	Hep B	A*02:01	C64-72

FLPSDFFPSV	41	Hep B	A*02:01	Core 18
FLPADFFPSV	42	Hep B	A*02:01	Core 19
FLPSDFFPSI	43	Hep B	A*02:01	Core 20
WLSLLVPF	44	Hep B	A*02:01	ENV335
FLLTRILTI or FLLTRILTL	45 or 46	Hep B	A*02:01	ENV183
GLSPTVWLSV	47	Hep B	A*02:01	ENV348
LLDYQGMLPV	48	Hep B	A*02:01	ENV260
LLCLIFLLV	49	Hep B	A*02:01	ENV251
SIVSPFIPLL	50	Hep B	A*02:01	ENV370
FLLTKILTI	51	Hep B	A*02:01	ENV183
ILSPFLPLL	52	Hep B	A*02:01	ENV371
FLLSLGIHL	53	Hep B	A*02:01	POL 575
GLSRYVARL	54	Hep B	A*02:01	POL 455
SLYADSPSV	55	Hep B	A*02:01	POL 816
YMDDVVLGA	56	Hep B	A*02:01	POL 551
ALMPLYACI	57	Hep B	A*02:01	POL 655
VLHKRTLGL	58	Hep B	A*02:01	HBx 92
CLFKDWEEL	59	Hep B	A*02:01	Hbx115
STLPETTVVRR	60	Hep B	A*03, A*11	Core 141
EYLVSFGVW	61	Hep B	A*31, A*68	core 117
FFPSIRDLL	62	Hep B	A*24	Core 23
SWLSLLVPF	63	Hep B	A*24	Env 334
KYTSFPWLL	64	Hep B	A*24	Pol 756
HLSLRGLFV	65	Hep B	A*02:01	HBx 52–60
CLFKDWEEL	66	Hep B	A*02:01	HBx 115–123
LPSDFFPSV	67	Hep B	B*51	Core 19
GILGFVFTL	68	Influenza	HLA-A2	M1
ILGFVFTLTVPSERGLQRRRF	69	Influenza		
LIRHENRMVLASTTAKA	70	Influenza		
LQAYQKRMGVQMQR	71	Influenza		
YVYDHSGEAVK	72	Measles		
WLSLLVPFV	73	Hep B		
(K)GILGFVFTL(T)(V)	74	Influenza		
KLSTRGVQIASNEN	75	Influenza		
RGLQRRRFVQNALNGNG	76	Influenza		
FMYSDFHFI	77	Influenza		
NLVPMVATV	3	Cytomegalovirus	HLA-A2	
VAIIEVDNEQPTTRAQKL	78	Poliovirus		
Any 9-mer sequence of GACV AIIEVDNEQPTTRAQKLF AMWRITYKDTVQLRRKL	79	Poliovirus		
SVRDRLARL	80	EBV		
LLDRVRFMGV	81	EBV		
CLGGLLTMV	82	EBV		
GLCTLVAML	83	EBV		
SVLGPISGHVLK	84	Cytomegalovirus	HLA-A11	

RPHERNGFTVL	85	Cytomegalovirus	HLA-B7	
FTSQYRIQGKL	86	Cytomegalovirus	HLA-A24	
YSEHPTFTSQY	87	Cytomegalovirus	HLA-A1	
EFFWDANDIY	88	Cytomegalovirus	HLA-B44	
TTVYPPSSTAK	90	Cytomegalovirus	HLA-A3	
FVFPTKDVALR	91	Cytomegalovirus	HLA-A68	
QTVTSTPVQGR	92	Cytomegalovirus	HLA-A68	
PTFTSQYRIQGKL	93	Cytomegalovirus	HLA-B38	
FPTKDVAL	94	Cytomegalovirus	HLA-B35	
SIINFEKL	95			
RAHYNIVTF	96			
SSPPMFRV	97			
KLWAQCVQL	98	SARS-CoV-2	A*02:01	
KLPDDFTGCV	99	SARS-CoV-2	A*02:01	
YLQPRTFLL	100	SARS-CoV-2	A*02:01	
LLYDANYFL	101	SARS-CoV-2	A*02:01	
ALWEIQQVV	102	SARS-CoV-2	A*02:01	
LLLDRLNQL	103	SARS-CoV-2	A*02:01	
YLFDESGEFKL	104	SARS-CoV-2	A*02:01	
FTSDYYQLY	105	SARS-CoV-2	A*01:01	
PTDNYITTY	106	SARS-CoV-2	A*01:01	
ATSRTLSYY	107	SARS-CoV-2	A*01:01	
CTDDNALAYY	108	SARS-CoV-2	A*01:01	
NTCDGTTFTY	109	SARS-CoV-2	A*01:01	
DTDFVNEFY	110	SARS-CoV-2	A*01:01	
GTDLEGNFY	111	SARS-CoV-2	A*01:01	
KTFPPTEPK	112	SARS-CoV-2	A*03:01	
KCYGVSPTK	113	SARS-CoV-2	A*03:01	
VTNNTFTLK	114	SARS-CoV-2	A*03:01	
KTIQPRVEK	115	SARS-CoV-2	A*03:01	
KTFPPTEPK	116	SARS-CoV-2	A*11:01	
VTDTPKGPK	117	SARS-CoV-2	A*11:01	
ATEGALNTPK	118	SARS-CoV-2 SARS-CoV-2	A*11:01	
ASAFFGMSR	119	SARS-CoV-2	A*11:01	
ATSRTLSYYK	120	SARS-CoV-2	A*11:01	
QYIKWPWYI	121	SARS-CoV-2	A*24:02	
VYFLQSINF	122	SARS-CoV-2	A*24:02	
VYIGDPAQL	123	SARS-CoV-2	A*24:02	
SPRWYFYYL	124	SARS-CoV-2	B*07:02	
RPDTRYVL	125	SARS-CoV-2	B*07:02	
IPRRNVATL	126	SARS-CoV-2	B*07:02	

[0163] In various embodiments the epitope is found in one or more known human vaccines, such as a childhood vaccine, early childhood, adolescent, or elderly (geriatric),

vaccine. In various embodiments the vaccine is an early childhood vaccine. Certain non-limiting examples of suitable vaccines from which such epitopes are found that are compatible with the described peptides are listed in Table 3.

TABLE 3

Human Vaccines Containing Peptide-Compatible Epitopes

Type	Commercial Name	Form	Source	Responsible National Regulatory Authority
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Hemophilus influenzae type b	Quinvaxem	Liquid: ready to use	Janssen Vaccines Corp.	Ministry of Food and Drug Safety
Diphtheria-Tetanus	Adsorbed DT Vaccine	Liquid: ready to use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia
Diphtheria-Tetanus-Pertussis (whole cell)	DTP Vaccine	Liquid: ready to use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia
Hepatitis B	Hepatitis B Vaccine Recombinant	Liquid: ready to use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia
Polio Vaccine - Oral (OPV) Trivalent	Oral polio	Liquid: ready to use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia
Polio Vaccine - Oral (OPV) Trivalent	Oral polio	Liquid: ready to use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia
Tetanus Toxoid	TT vaccine	Liquid: ready to use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia
Tetanus Toxoid	TT vaccine	Liquid: ready to use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia

Tetanus Toxoid	TT vaccine	Liquid: ready to use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia
Measles	Measles vaccine	Lyophilized Lyophilized active component to be reconstituted with excipient diluent before use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia
Yellow Fever	Yellow Fever	Lyophilized Lyophilized active component to be reconstituted with excipient diluent before use	Bio-Manguinhos/Fiocruz	Agencia Nacional da Vigilancia Sanitaria
Yellow Fever	Yellow Fever	Lyophilized Lyophilized active component to be reconstituted with excipient diluent before use	Bio-Manguinhos/Fiocruz	Agencia Nacional da Vigilancia Sanitaria
Yellow Fever	Yellow Fever	Lyophilized Lyophilized active component to be reconstituted with excipient diluent before use	Bio-Manguinhos/Fiocruz	Agencia Nacional da Vigilancia Sanitaria
Hepatitis B	Heberbiovac HB	Liquid: ready to use	Centro de Ingenieria	Centro para el Control Estatal de

			Genetica y Biotecnologia	la Calidad de los Medicamentos
Hepatitis B	Heberbiovac HB	Liquid: ready to use	Centro de Ingenieria Genetica y Biotecnologia	Centro para el Control Estatal de la Calidad de los Medicamentos
Rabies	Rabipur	Lyophilized active component to be reconstituted with excipient diluent before use	Chiron Behring Vaccines Private Ltd.	Central Drugs Standard Control Organization
Rabies	Rabipur	Lyophilized active component to be reconstituted with excipient diluent before use	GlaxoSmithKline Vaccines GmbH	Paul-Ehrlich-Institut
Haemophilus influenzae type b	Vaxem HIB	Liquid: ready to use	Novartis Vaccines and Diagnostics S.r.l	Agenzia Italiana del Farmaco
Hepatitis B	Engerix	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Hepatitis B	Engerix	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Hepatitis B	Engerix	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products

Polio Vaccine - Oral (OPV) Trivalent	Polio sabin	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Polio Vaccine - Oral (OPV) Trivalent	Polio sabin	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Measles, Mumps and Rubella	Priorix	Lyophilized active component to be reconstituted with excipient diluent before use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Rotavirus	Rotarix	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Polio Vaccine - Oral (OPV) Trivalent	Polioviral vaccine	Liquid: ready to use	Haffkine Bio Pharmaceutical Corporation Ltd	Central Drugs Standard Control Organization
Yellow Fever	Stabilized Yellow Fever Vaccine	Lyophilized active component to be reconstituted with excipient diluent before use	Institut Pasteur de Dakar	Ministère de la Santé publique
Yellow Fever	Stabilized Yellow Fever Vaccine	Lyophilized active component to be reconstituted with excipient	Institut Pasteur de Dakar	Ministère de la Santé publique

		diluent before use		
Yellow Fever	Stabilized Yellow Fever Vaccine	Lyophilized active component to be reconstituted with excipient diluent before use	Institut Pasteur de Dakar	Ministère de la Santé publique
BCG	BCG Freeze Dried Glutamate vaccine	Lyophilized active component to be reconstituted with excipient diluent before use	Japan BCG Laboratory	Chiba Local Government
Hepatitis B	Euvax B	Liquid: ready to use	LG Chem Ltd	Ministry of Food and Drug Safety
Hepatitis B	Euvax B	Liquid: ready to use	LG Chem Ltd	Ministry of Food and Drug Safety
BCG	BCG Vaccine	Lyophilized active component to be reconstituted with excipient diluent before use	Bul Bio - National Center of Infectious and Parasitic Diseases Ltd.	Bulgarian Drug Agency
BCG	BCG Vaccine	Lyophilized active component to be reconstituted with excipient diluent before use	Bul Bio - National Center of Infectious and Parasitic Diseases Ltd.	Bulgarian Drug Agency

Tetanus Toxoid	Tetatox	Liquid: ready to use	Bul Bio - National Center of Infectious and Parasitic Diseases Ltd.	Bulgarian Drug Agency
Tetanus Toxoid	Tetatox	Liquid: ready to use	Bul Bio - National Center of Infectious and Parasitic Diseases Ltd.	Bulgarian Drug Agency
Diphtheria-Tetanus	Diftet	Liquid: ready to use	Bul Bio - National Center of Infectious and Parasitic Diseases Ltd.	Bulgarian Drug Agency
Diphtheria-Tetanus	Diftet	Liquid: ready to use	Bul Bio - National Center of Infectious and Parasitic Diseases Ltd.	Bulgarian Drug Agency
Diphtheria-Tetanus (reduced antigen content)	Tetadif	Liquid: ready to use	Bul Bio - National Center of Infectious and Parasitic Diseases Ltd.	Bulgarian Drug Agency
Diphtheria-Tetanus (reduced antigen content)	Tetadif	Liquid: ready to use	Bul Bio - National Center of Infectious and	Bulgarian Drug Agency

			Parasitic Diseases Ltd.	
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	Easyfive-TT	Liquid: ready to use	Panacea Biotec Ltd.	Central Drugs Standard Control Organization
Diphtheria-Tetanus (reduced antigen content)	IMOVAX dT adult	Liquid: ready to use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Polio Vaccine - Inactivated (IPV)	IMOVAX POLIO	Liquid: ready to use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Polio Vaccine - Oral (OPV) Trivalent	OPVERO	Liquid: ready to use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Polio Vaccine - Oral (OPV) Trivalent	OPVERO	Liquid: ready to use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Polio Vaccine - Oral (OPV) Trivalent	OPVERO	Liquid: ready to use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Tetanus Toxoid	TETAVAX	Liquid: ready to use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Tetanus Toxoid	TETAVAX	Liquid: ready to use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Haemophilus influenzae type b	Act-HIB	Lyophilized active component to be reconstituted with	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé

		excipient diluent before use		
Rabies	VERORAB	Lyophilized active component to be reconstituted with excipient diluent before use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Yellow Fever	STAMARIL	Lyophilized active component to be reconstituted with excipient diluent before use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Meningococcal A+C	POLYSACCHARIDE MENINGOCOCCAL A+C VACCINE	Lyophilized active component to be reconstituted with excipient diluent before use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Polio Vaccine - Oral (OPV) Monovalent Type 1	ORAL MONOVALENT TYPE 1 POLIOMYELITIS VACCINE	Liquid: ready to use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
cholera: inactivated oral	Dukoral	Liquid: ready to use	Valneva Sweden AB	Medical Products Agency
BCG	BCG Vaccine	Lyophilized active component to be reconstitute	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization

		d with excipient diluent before use		
Diphtheria-Tetanus	Diphtheria and Tetanus Vaccine Adsorbed (Paediatric)	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Diphtheria-Tetanus	Diphtheria and Tetanus Vaccine Adsorbed (Pediatric)	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Diphtheria-Tetanus	Diphtheria and Tetanus Vaccine Adsorbed (Pediatric)	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Diphtheria-Tetanus (reduced antigen content)	Diphtheria and Tetanus Vaccine Adsorbed for Adults and Adolescents	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Diphtheria-Tetanus (reduced antigen content)	Diphtheria and Tetanus Vaccine Adsorbed for Adults and Adolescents	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Diphtheria-Tetanus (reduced antigen content)	Diphtheria and Tetanus Vaccine Adsorbed for Adults and Adolescents	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Diphtheria-Tetanus-Pertussis (whole cell)	Diphtheria-Tetanus-Pertussis Vaccine Adsorbed	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization

Diphtheria-Tetanus-Pertussis (whole cell)	Diphtheria-Tetanus-Pertussis Vaccine Adsorbed	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Diphtheria-Tetanus-Pertussis (whole cell)	Diphtheria-Tetanus-Pertussis Vaccine Adsorbed	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B	Diphtheria ,Tetanus, Pertussis and Hepatitis B Vaccine Adsorbed	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B	Diphtheria ,Tetanus, Pertussis and Hepatitis B Vaccine Adsorbed	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B	Diphtheria ,Tetanus, Pertussis and Hepatitis B Vaccine Adsorbed	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Hepatitis B	Hepatitis B Vaccine (rDNA) (Adult)	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Hepatitis B	Hepatitis B Vaccine (rDNA) (Adult)	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Hepatitis B	Hepatitis B Vaccine (rDNA) (Paediatric)	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization

Hepatitis B	Hepatitis B Vaccine (rDNA) (Paedriatic)	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Tetanus Toxoid	Tetanus Toxoid Vaccine Adsorbed	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Tetanus Toxoid	Tetanus Toxoid Vaccine Adsorbed	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Tetanus Toxoid	Tetanus Toxoid Vaccine Adsorbed	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Measles and Rubella	Measles and Rubella Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Measles and Rubella	Measles and Rubella Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Measles and Rubella	Measles and Rubella Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization

Measles and Rubella	Measles and Rubella Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Measles, Mumps and Rubella	Measles, Mumps and Rubella Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Measles, Mumps and Rubella	Measles, Mumps and Rubella Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Measles, Mumps and Rubella	Measles, Mumps and Rubella Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Measles, Mumps and Rubella	Measles, Mumps and Rubella Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization

Measles	Measles Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Measles	Measles Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Measles	Measles Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Measles	Measles Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Rubella	Rubella Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization

Rubella	Rubella Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Rubella	Rubella Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Rubella	Rubella Vaccine, Live, Attenuated	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Tetanus Toxoid	ShanTT	Liquid: ready to use	Shantha Biotechnics Private Limited (A Sanofi Company)	Central Drugs Standard Control Organization
Tetanus Toxoid	ShanTT	Liquid: ready to use	Shantha Biotechnics Private Limited (A Sanofi Company)	Central Drugs Standard Control Organization
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	Shan-5	Liquid: ready to use	Shantha Biotechnics Private Limited (A Sanofi Company)	Central Drugs Standard Control Organization

Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	Shan-5	Liquid: ready to use	Shantha Biotechnics Private Limited (A Sanofi Company)	Central Drugs Standard Control Organization
BCG	BCG Vaccine SSI	Lyophilized active component to be reconstituted with excipient diluent before use	AJ Vaccines A/S	Danish Medicines Agency
Rotavirus	Rotateq	Liquid: ready to use	Merck Vaccines	CBER/FDA
Measles, Mumps and Rubella	rHA M-M-R II	Lyophilized active component to be reconstituted with excipient diluent before use	Merck Vaccines	European Medicines Agency
Rotavirus	Rotarix	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Yellow Fever	-	Lyophilized active component to be reconstituted with excipient diluent before use	Federal State Budgetary Scientific Institution «Chumakov Federal Scientific Center for Reserch & Development of Immune-And Biological	Federal Service on Surveillance in Healthcare (ROSZDRAVNADZOR) of the Russian Federation

			Products», Russian Academy of Sciences	
Yellow Fever	-	Lyophilized active component to be reconstituted with excipient diluent before use	Federal State Budgetary Scientific Institution «Chumakov Federal Scientific Center for Reserch & Development of Immune-And Biological Products», Russian Academy of Sciences	Federal Service on Surveillance in Healthcare (ROSZDRAVNADZOR) of the Russian Federation
Yellow Fever	-	Lyophilized active component to be reconstituted with excipient diluent before use	Federal State Budgetary Scientific Institution «Chumakov Federal Scientific Center for Reserch & Development of Immune-And Biological Products», Russian Academy of Sciences	Federal Service on Surveillance in Healthcare (ROSZDRAVNADZOR) of the Russian Federation
Human Papillomavirus (Quadrivalent)	Gardasil	Liquid: ready to use	Merck Vaccines	European Medicines Agency

Human Papillomavirus (Bivalent)	Cervarix	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Human Papillomavirus (Bivalent)	Cervarix	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Polio Vaccine - Oral (OPV) Monovalent Type 1	Polio Sabin Mono T1	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Polio Vaccine - Oral (OPV) Monovalent Type 1	Polio Sabin Mono T1	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Polio Vaccine - Oral (OPV) Bivalent Types 1 and 3	Polio Sabin One and Three	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Polio Vaccine - Oral (OPV) Bivalent Types 1 and 3	Polio Sabin One and Three	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Haemophilus influenzae type b	Haemophilus influenzae type b Conjugate Vaccine	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Polio Vaccine - Oral (OPV) Monovalent Type 1	Monovalent type 1 Oral Poliomyelitis vaccine, IP (mOPV1)	Liquid: ready to use	Haffkine Bio Pharmaceutical Corporation Ltd	Central Drugs Standard Control Organization
Polio Vaccine - Oral (OPV) Monovalent Type 1	Monovalent Oral Poliomyelitis Vaccine	Liquid: ready to use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia

	Type 1 (mOPV1)			
Tetanus Toxoid	None used on labelling for supply through UN agencies. Also marketed with labelled commercial name BEtt.	Liquid: ready to use	Biological E. Limited	Central Drugs Standard Control Organization
Pneumococcal (conjugate)	Synflorix	Liquid: ready to use	GlaxoSmithKline Biologicals SA	European Medicines Agency
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	Diphtheria, Tetanus, Pertussis, Hepatitis B and Haemophilus influenzae type b Conjugate Vaccine	Lyophilized active component to be reconstituted with liquid active component before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Polio Vaccine - Oral (OPV) Bivalent Types 1 and 3	Bivalent Oral Poliomyelitis Vaccine Type 1&3 (bOPV 1&3)	Liquid: ready to use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia
Meningococcal A Conjugate 10 µg	Meningococcal A Conjugate MenAfriVac	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Haemophilus influenzae type b	Quimi-Hib	Liquid: ready to use	Centro de Ingenieria Genetica y	Centro para el Control Estatal de

			Biotecnologia	la Calidad de los Medicamentos
Pneumococcal (conjugate)	Synflorix	Liquid: ready to use	GlaxoSmithKline Biologicals SA	European Medicines Agency
Influenza, seasonal	Fluvirin	Liquid: ready to use	Seqirus Vaccines Limited	CBER/FDA
Polio Vaccine - Oral (OPV) Bivalent Types 1 and 3	Bivalent type 1&3 Oral Poliomyelitis vaccine, IP (bOPV1&3)	Liquid: ready to use	Haffkine Bio Pharmaceutical Corporation Ltd	Central Drugs Standard Control Organization
Influenza, seasonal	Fluzone	Liquid: ready to use	Sanofi Pasteur-USA	CBER/FDA
Influenza, seasonal	Fluzone	Liquid: ready to use	Sanofi Pasteur-USA	CBER/FDA
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	Diphtheria, Tetanus, Pertussis, Hepatitis B and Haemophilus influenzae type b Conjugate Vaccine	Lyophilized active component to be reconstituted with liquid active component before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Influenza, seasonal	GC FLU Multi inj.	Liquid: ready to use	Green Cross Corporation	Ministry of Food and Drug Safety
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	Diphtheria, Tetanus, Pertussis, Hepatitis B and Haemophilus influenzae type b	Lyophilized active component to be reconstituted with liquid active	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization

	Conjugate Vaccine	component before use		
Influenza, pandemic H1N1	Panvax	Liquid: ready to use	Seqirus Limited	Therapeutic Goods Administration
Influenza, pandemic H1N1	Green Flu-S	Liquid: ready to use	Green Cross Corporation	Ministry of Food and Drug Safety
Influenza, pandemic H1N1	Influenza A (H1N1) 2009 monovalent vaccine	Liquid: ready to use	MedImmune	CBER/FDA
Influenza, pandemic H1N1	Celtura	Liquid: ready to use	Seqirus GmbH	Paul-Ehrlich-Institut
Influenza, pandemic H1N1	Focetria	Liquid: ready to use	Seqirus Vaccines Limited	
Influenza, pandemic H1N1	Fluvirin-H1N1	Liquid: ready to use	Seqirus Vaccines Limited	CBER/FDA
Influenza, pandemic H1N1	Panenza	Liquid: ready to use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Influenza, pandemic H1N1	Influenza A (H1N1) 2009 monovalent vaccine	Liquid: ready to use	Sanofi Pasteur-USA	CBER/FDA
Influenza, pandemic H1N1	Influenza A (H1N1) 2009 monovalent vaccine	Liquid: ready to use	Sanofi Pasteur-USA	CBER/FDA
Diphtheria-Tetanus-Pertussis (whole cell)-Haemophilus influenzae type b	Diphtheria, Tetanus, Pertussis and Haemophilus influenzae type b Conjugate Vaccine	Lyophilized active component to be reconstituted with liquid active	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization

		component before use		
Polio Vaccine - Inactivated (IPV)	Poliorix	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Polio Vaccine - Inactivated (IPV)	Poliorix	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Pneumococcal (conjugate)	Prevenar 13	Liquid: ready to use	Pfizer	European Medicines Agency
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	Diphtheria, Tetanus, Pertussis, Hepatitis B and Haemophilus influenzae type b Conjugate Vaccine Adsorbed	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	Diphtheria, Tetanus, Pertussis, Hepatitis B and Haemophilus influenzae type b Conjugate Vaccine Adsorbed	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	Diphtheria, Tetanus, Pertussis, Hepatitis B and Haemophilus influenzae type b Conjugate	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization

	Vaccine Adsorbed			
Polio Vaccine - Oral (OPV) Monovalent Type 3	Polio Sabin Mono Three (oral)	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Polio Vaccine - Oral (OPV) Monovalent Type 3	Polio Sabin Mono Three (oral)	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Polio Vaccine - Inactivated (IPV)	Poliomyelitis vaccine	Liquid: ready to use	Bilthoven Biologicals	Medicines Evaluation Board (MEB)
Polio Vaccine - Inactivated (IPV)	IPV Vaccine SSI	Liquid: ready to use	AJ Vaccines A/S	Danish Medicines Agency
Influenza, seasonal	GC FLU inj	Liquid: ready to use	Green Cross Corporation	Ministry of Food and Drug Safety
Polio Vaccine - Oral (OPV) Monovalent Type 2	Polio Sabin Mono Two (oral)	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Polio Vaccine - Oral (OPV) Monovalent Type 2	Polio Sabin Mono Two (oral)	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Typhoid (Polysaccharide)	Typhim-Vi	Liquid: ready to use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Influenza, seasonal	Vaxigrip	Liquid: ready to use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Polio Vaccine - Oral (OPV) Bivalent Types 1 and 3	BIOPOLIO B1/3	Liquid: ready to use	Bharat Biotech International Limited	Central Drugs Standard Control Organization

Diphtheria-Tetanus (reduced antigen content)	none	Liquid: ready to use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia
Polio Vaccine - Oral (OPV) Bivalent Types 1 and 3	none	Liquid: ready to use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	None used on labelling for supply through UN agencies. Also marketed with labelled commercial name ComBE Five (Reconstituted).	Lyophilized active component to be reconstituted with liquid active component before use	Biological E. Limited	Central Drugs Standard Control Organization
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	None used on labelling for supply through UN agencies. Also marketed with labelled commercial name ComBE Five (Reconstituted).	Lyophilized active component to be reconstituted with liquid active component before use	Biological E. Limited	Central Drugs Standard Control Organization
cholera: inactivated oral	Shanchol	Liquid: ready to use	Shantha Biotechnics Private Limited (A Sanofi Company)	Central Drugs Standard Control Organization
Measles, Mumps and Rubella	Priorix	Lyophilized active component to be reconstituted with	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products

		excipient diluent before use		
Measles	Measles vaccine	Lyophilized active component to be reconstituted with excipient diluent before use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia
Polio Vaccine - Oral (OPV) Bivalent Types 1 and 3	Poliomyelitis Vaccine (Oral), Bivalent types 1 and 3	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Influenza, pandemic H1N1	NASOVAC Influenza Vaccine, Live Attenuated (Human) Freeze-Dried	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Influenza, pandemic H1N1	NASOVAC Influenza Vaccine, Live Attenuated (Human) Freeze-Dried	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Tetanus Toxoid	None used on labelling for supply through UN agencies. Also marketed with labelled commercial name BEtt.	Liquid: ready to use	Biological E. Limited	Central Drugs Standard Control Organization

Tetanus Toxoid	None used on labelling for supply through UN agencies. Also marketed with labelled commercial name BEtt.	Liquid: ready to use	Biological E. Limited	Central Drugs Standard Control Organization
Japanese Encephalitis Vaccine (Inactivated) 6µg	JEEV®	Liquid: ready to use	Biological E. Limited	Central Drugs Standard Control Organization
Hepatitis A (Human Diploid Cell), Inactivated (Adult)	Havrix 1440 Adult	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Hepatitis A (Human Diploid Cell), Inactivated (Paediatric)	Havrix 720 Junior	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Diphtheria-Tetanus-Pertussis (acellular)	Boostrix	Liquid: ready to use	GlaxoSmithKline Biologicals SA	Federal Agency for Medicines and Health Products
Meningococcal ACYW-135 (conjugate vaccine)	Menveo	Lyophilized active component to be reconstituted with liquid active component before use	GlaxoSmithKline Vaccines S.r.l.	European Medicines Agency
Meningococcal ACYW-135 (conjugate vaccine)	Menactra	Liquid: ready to use	Sanofi Pasteur-USA	CBER/FDA
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	Easyfive-TT	Liquid: ready to use	Panacea Biotec Ltd.	Central Drugs Standard Control Organization

Japanese Encephalitis Vaccine (live, attenuated)	Japanese Encephalitis Vaccine Live (SA14-14-2)	Lyophilized active component to be reconstituted with excipient diluent before use	Chengdu Institute of Biological Products Co.,Ltd	National Medical Products Administration
Japanese Encephalitis Vaccine (live, attenuated)	Japanese Encephalitis Vaccine Live (SA14-14-2)	Lyophilized active component to be reconstituted with excipient diluent before use	Chengdu Institute of Biological Products Co.,Ltd	National Medical Products Administration
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	None used on labelling for supply through UN agencies. Also marketed with labelled commercial name ComBE Five (Liquid).	Liquid: ready to use	Biological E. Limited	Central Drugs Standard Control Organization
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	None used on labelling for supply through UN agencies. Also marketed with labelled commercial name ComBE Five (Liquid).	Liquid: ready to use	Biological E. Limited	Central Drugs Standard Control Organization
Japanese Encephalitis Vaccine (live, attenuated)	IMOJEV MD	Lyophilized active component to be	GPO-MBP Co., Ltd.	Thai Food and Drug Administration

		reconstituted with excipient diluent before use		
Diphtheria-Tetanus-Pertussis (whole cell)	None used on labelling for supply through UN agencies. Also marketed with labelled commercial name TRIPVAC	Liquid: ready to use	Biological E. Limited	Central Drugs Standard Control Organization
Diphtheria-Tetanus-Pertussis (whole cell)	None used on labelling for supply through UN agencies. Also marketed with labelled commercial name TRIPVAC	Liquid: ready to use	Biological E. Limited	Central Drugs Standard Control Organization
Diphtheria-Tetanus (reduced antigen content)	None used on labelling for supply through UN agencies. Also marketed with labelled commercial name BE Td	Liquid: ready to use	Biological E. Limited	Central Drugs Standard Control Organization
Diphtheria-Tetanus (reduced antigen content)	None used on labelling for supply through UN agencies. Also marketed with labelled	Liquid: ready to use	Biological E. Limited	Central Drugs Standard Control Organization

	commercial name BE Td			
Polio Vaccine - Oral (OPV) Bivalent Types 1 and 3	Poliomyelitis Vaccine (Oral), Bivalent types 1 and 3	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Polio Vaccine - Inactivated (IPV)	Poliomyelitis vaccine multidose, suspension for injection 2.5 mL	Liquid: ready to use	Bilthoven Biologicals	Medicines Evaluation Board (MEB)
Influenza, seasonal	Nasovac-S Influenza Vaccine, Live, Attenuated (Human)	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Diphtheria-Tetanus-Pertussis (acellular)-Hepatitis B-Haemophilus influenzae type b-Polio (Inactivated)	Hexaxim	Liquid: ready to use	Sanofi Pasteur SA	European Medicines Agency
Meningococcal A Conjugate 5 µg	Meningococcal A Conjugate 5 micrograms MenAfriVac 5µg	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	None used on labelling for supply through UN agencies. Also marketed with labelled	Liquid: ready to use	Biological E. Limited	Central Drugs Standard Control Organization

	commercial name ComBE Five (Liquid).			
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	None used on labelling for supply through UN agencies. Also marketed with labelled commercial name ComBE Five (Liquid).	Liquid: ready to use	Biological E. Limited	Central Drugs Standard Control Organization
Polio Vaccine - Inactivated (IPV)	Poliomyelitis Vaccine (Inactivated)	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Polio Vaccine - Oral (OPV) Trivalent	BIOPOLIO	Liquid: ready to use	Bharat Biotech International Limited	Central Drugs Standard Control Organization
Polio Vaccine - Oral (OPV) Trivalent	BIOPOLIO	Liquid: ready to use	Bharat Biotech International Limited	Central Drugs Standard Control Organization
Influenza, seasonal	Influenza Vaccine (Split virion, inactivated)	Liquid: ready to use	Hualan Biological Bacterin Co., Ltd	National Medical Products Administration
Influenza, seasonal	IL-YANG FLU Vaccine INJ.	Liquid: ready to use	IL-YANG PHARMACEUTICAL CO., LTD.	Ministry of Food and Drug Safety
BCG	BCG vaccine (Freeze Dried) - Intradermal	Lyophilized active component to be reconstituted with excipient	GreenSignal Bio Pharma Limited	Central Drugs Standard Control Organization

		diluent before use		
Influenza, seasonal Quadrivalent	Fluzone Quadrivalent	Liquid: ready to use	Sanofi Pasteur-USA	CBER/FDA
Influenza, seasonal Quadrivalent	Fluzone Quadrivalent	Liquid: ready to use	Sanofi Pasteur-USA	CBER/FDA
Polio Vaccine - Oral (OPV) Bivalent Types 1 and 3	Bivalent Oral Poliomyelitis Vaccine Type 1&3 (bOPV 1&3)	Liquid: ready to use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia
cholera: inactivated oral	Euvichol	Liquid: ready to use	EuBiologics Co., Ltd.	Ministry of Food and Drug Safety
Polio Vaccine - Oral (OPV) Monovalent Type 2	ORAL MONOVALENT TYPE 2 POLIOMYELITIS VACCINE (mOPV2)	Liquid: ready to use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Polio Vaccine - Oral (OPV) Monovalent Type 3	ORAL MONOVALENT TYPE 3 POLIOMYELITIS VACCINE	Liquid: ready to use	Sanofi Pasteur SA	Agence nationale de sécurité du médicament et des produits de santé
Meningococcal ACYW-135 (conjugate vaccine)	Nimenrix	Lyophilized active component to be reconstituted with excipient diluent before use	Pfizer	European Medicines Agency
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-	Eupenta	Liquid: ready to use	LG Chem Ltd	Ministry of Food and Drug Safety

Haemophilus influenzae type b				
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	Eupenta	Liquid: ready to use	LG Chem Ltd	Ministry of Food and Drug Safety
Human Papillomavirus (Ninevalent)	Gardasil 9	Liquid: ready to use	Merck Vaccines	European Medicines Agency
Influenza, seasonal Quadrivalent	GCFLU Quadrivalent inj.	Liquid: ready to use	Green Cross Corporation	Ministry of Food and Drug Safety
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	Pentabio	Liquid: ready to use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia
Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b	Pentabio	Liquid: ready to use	PT Bio Farma (Persero)	National Agency of Drug and Food Control Indonesia
Hepatitis A (Human Diploid Cell), Inactivated (Adult)	HEALIVE	Liquid: ready to use	Sinovac Biotech Co. Ltd	National Medical Products Administration
Varicella	Varivax	Lyophilized active component to be reconstituted with excipient diluent before use	Merck Vaccines	CBER/FDA
Rotavirus (live, attenuated)	Rotavac	Liquid: ready to use	Bharat Biotech International Limited	Central Drugs Standard Control Organization

Diphtheria-Tetanus-Pertussis (acellular)	Adacel	Liquid: ready to use	Sanofi Pasteur Limited	Health Canada - Santé Canada
Influenza, seasonal	AGRIFLU	Liquid: ready to use	Seqirus Vaccines Limited	Health Canada - Santé Canada
Pneumococcal (conjugate)	Prevenar 13 Multidose Vial	Liquid: ready to use	Pfizer	European Medicines Agency
Typhoid (Conjugate)	Typbar-TVC	Liquid: ready to use	Bharat Biotech International Limited	Central Drugs Standard Control Organization
Polio Vaccine - Oral (OPV) Bivalent Types 1 and 3	Poliomyelitis Vaccine (live, oral attenuated, human Diploid Cell), type 1 and 3	Liquid: ready to use	Beijing Bio-Institute Biological Products Co.,Ltd	National Medical Products Administration
Japanese Encephalitis Vaccine (Inactivated) (3µg Pediatric)	JEEV®	Liquid: ready to use	Biological E. Limited	Central Drugs Standard Control Organization
Rotavirus (live, attenuated)	ROTASIIL	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Polio Vaccine - Inactivated (IPV)	Poliomyelitis Vaccine (Inactivated)	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Polio Vaccine - Inactivated (IPV)	Poliomyelitis Vaccine (Inactivated)	Liquid: ready to use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization

Influenza, seasonal Quadrivalent	GCFLU Quadrivalent Multi inj.	Liquid: ready to use	Green Cross Corporation	Ministry of Food and Drug Safety
Influenza, seasonal	Serinflu	Liquid: ready to use	Abbott Biologicals BV	Medicines Evaluation Board (MEB)
Polio Vaccine - Inactivated (IPV)	ShanIPV	Liquid: ready to use	Shantha Biotechnics Private Limited (A Sanofi Company)	Central Drugs Standard Control Organization
Polio Vaccine - Oral (OPV) Bivalent Types 1 and 3	Bivalent OPV Type 1 and 3 Poliomyelitis Vaccine, Live (Oral)	Liquid: ready to use	Panacea Biotec Ltd.	Central Drugs Standard Control Organization
cholera: inactivated oral	Euvichol-Plus	Liquid: ready to use	EuBiologics Co., Ltd.	Ministry of Food and Drug Safety
Polio Vaccine - Oral (OPV) Bivalent Types 1 and 3	BIOPOLIO B1/3	Liquid: ready to use	Bharat Biotech International Limited	Central Drugs Standard Control Organization
BCG	BCG Freeze Dried Glutamate vaccine	Lyophilized active component to be reconstituted with excipient diluent before use	Japan BCG Laboratory	Chiba Local Government
Pneumococcal (conjugate)	Synflorix	Liquid: ready to use	GlaxoSmithKline Biologicals SA	European Medicines Agency
Rotavirus (live, attenuated)	Rotavac	Liquid: ready to use	Bharat Biotech International Limited	Central Drugs Standard Control Organization

Hepatitis A (Human Diploid Cell), Inactivated (Paediatric)	HEALIVE	Liquid: ready to use	Sinovac Biotech Co. Ltd	National Medical Products Administration
Typhoid (Conjugate)	Typbar-TVC	Liquid: ready to use	Bharat Biotech International Limited	Central Drugs Standard Control Organization
Rotavirus (live, attenuated)	ROTASIIL	Lyophilized active component to be reconstituted with excipient diluent before use	Serum Institute of India Pvt. Ltd.	Central Drugs Standard Control Organization
Japanese Encephalitis Vaccine (Inactivated) 6µg	JEEV®	Liquid: ready to use	Biological E. Limited	Central Drugs Standard Control Organization
Japanese Encephalitis Vaccine (Inactivated) (3µg Pediatric)	JEEV®	Liquid: ready to use	Biological E. Limited	Central Drugs Standard Control Organization
SARS-CoV-2	PFIZER-BIONTECH COVID-19 VACCINE-bnt162b2	Liquid: ready to use	Pfizer-BIONTECH	CEBR/FDA
SARS-CoV-2	Moderna COVID-19	Liquid: ready to use	Moderna Inc	CEBR/FDA
SARS-CoV-2	COVID-19 Vaccine (ChAdOx1-S [recombinant])	Liquid: ready to use	Astra Zeneca	European Medicines Agency
SARS-CoV-2	Janssen COVID-19 Vaccine	Liquid: ready to use	Johnson & Johnson	CEBR/FDA
SARS-CoV-2	CoronaVac , COVID-19 Vaccine	Liquid: ready to use	Sinovac	National Medical Products Administration

	(Vero Cell), Inactivated			
SARS-CoV-2	Novavax COVID-19 Vaccine,	Liquid: ready to use	Novavax	CEBR/FDA
Respiratory Syncytial Virus (RSV)	Arexvy	Liquid: ready to use	Glaxosmith kline (GSK)	CEBR/FDA
Respiratory Syncytial Virus (RSV)	Abrysvo	Liquid: ready to use	) Pfizer	CEBR/FDA
Respiratory Syncytial Virus (RSV)	Moderna RSV	Liquid: ready to use	Moderna Inc	CEBR/FDA

[0164] In various embodiments, the epitope is released following proteolytic cleavage of the peptide from the IRC. After proteolytic cleavage of the peptide from the IRC, the epitope binds to an MHC, optionally an MHC class I, molecule. The MHC molecule is in some embodiments from the HLA-A, B, and/or HLA C families. The specific epitope that binds to the MHC class I molecule is any of those recited in Table 2 or Table 3 or found elsewhere in the art. The MHC class I molecule itself is, in some embodiments, one or more of the following non-limiting examples: HLA-A*02:01, HLA-A*03:01, HLA-A*11:01, HLA-A*201, HLA-A*020101, HLA-A*0203, HLA-A*0206, HLA-A2, HLA-A2.1, or HLA-A*02.

[0165] In an aspect the described methods, uses, and compositions, the epitope is about 8 amino acid to about 50 amino acids in length, or about 8 amino acid to about 45 amino acids in length, or about 8 amino acid to about 40 amino acids in length, about 8 amino acid to about 35 amino acids in length, or about 8 amino acid to about 30 amino acids in length, about 8 amino acid to about 25 amino acids in length, about 8 amino acid to about 20 amino acids in length, or is about 8 amino acid to about 15 amino acids in length. In an aspect of the invention the peptide is about 13 amino acid to about 50 amino acids in length, or about 13 amino acid to about 45 amino acids in length, or about 13 amino acid to about 40 amino acids in length, about 13 amino acid to about 35 amino acids in length, or about 13 amino acid to about 30 amino acids in length, about 13 amino acid to about 25 amino acids in length, about 13 amino acid to about 20 amino acids in length, or is about 13 amino acid to about 15 amino acids in length. In some embodiments, the CD8+ T cell epitope is, e.g., about 8, 9, 10, 11, 12, 13, 14, 15, 16, or about 17 amino acids in length.

[0166] Cleavage Sequence. In various embodiments, one or more protease cleavage sequences are incorporated into the IRC that, upon cleavage, allows the peptide to be released from the IRC so that the peptide then is free to bind to the MHC on the tumor or cancer cell surface. In various embodiments, the IRC must escape the endosome, disassemble, and release their therapeutic cargo to the cytosol in a functional form. In various embodiments the IRC and/or peptide of the IRC is susceptible to cleavage by a proteolytic enzyme within the tumor microenvironment, i.e., in the nearby interstitial space surrounding tumors or tumor cells, and the position of the target cleavage sequence in the IRC or peptide is such that the cleavage of the target site releases all or a portion of the peptide comprising the CD8+ T cell epitope from the IRC, which then is free to bind to, and/or form a complex with, an MHC molecule expressed on the surface of the tumor cell in the subject. Pharmaceutically effective, or therapeutic amounts of IRC required to achieve this end goal are determined by the skilled artisan by known clinical methods utilizing *in vitro* cell culture techniques, animal model studies, and small scale to large scale human clinical trials. It will be appreciated that the amount of IRC administered to the subject in need thereof in the described methods and uses herein will depend on, e.g., the characteristics of the subject, e.g., age, weight, gender, and/or medical condition/history, genetic makeup, and other factors pertinent to the subject or class of subjects, and that the characteristics of the tumor, e.g., type, volume, and developmental status will also be taken into account when designing the dosage range finding clinical studies.

[0167] The proteolytic cleavage sequence is in some embodiments recognized by any protease present in, on, around, or nearby a tumor cell. At least about 569 known proteases have been described. (See, Lopez-Otin, et al., *Nature Reviews Cancer*, 7(10):800–808, 2007). All human proteolytic enzymes identified to date are classifiable into five catalytic classes: metalloproteinases, serine, threonine, cysteine, and aspartic proteases. A non-limiting list of potential proteases is demonstrated in Table 4, which is a table summarizing exemplars of the most well-studied proteases distributed into the five noted classes. (See Choi et al., *Theranostics*, (2)2:156-78, 2012). Several of these proteases have been found to be over-expressed in cancer cells relative to healthy cells.

[0168] In various embodiments, the proteolytic cleavage sequence is recognized by the protease furin, a matrix metalloproteinase (MMP), of which several different members are identified, e.g., MMP, 1, 2, 3, 7, 8, 9, 11, 13, 14, or 19, an ADAM (a disintegrin and metalloproteinase), e.g., ADAMS 8, 9, 10, 15, 17, or 28, a cathepsin, e.g., cathepsin D, G, H, or N. Also contemplated herein are the proteases elastase, proteinase-3, azurocidin, and ADAMTS-1. In various embodiments, the cleavage sequence is recognized by any one or more

of the aforementioned proteases, and in a certain embodiment the sequence is recognized by a human furin protease. In various embodiments, the cleavage sequence comprises at least about 4 amino acid residues, at least about three of which are arginine residues. In various embodiments, the cleavage sequence comprises at least 4 amino acid residues, at least three of which are arginine residues and one of which is either a lysine residue or an arginine residue. In various embodiments, the cleavage sequence is R-X-R/K-R (SEQ ID NO:89). In various embodiments, the cleavage sequence comprises additional residues. In various embodiments, the cleavage sequence further comprises about 1, 2, 3, 4, 5, 6, 7, 8, or about 9 additional arginine residues. It is known that arginines are positively charged and it has been discovered that a longer chain of positive charged arginine residues will bring the peptides closer to the surface of the capsid backbone which is more negatively charged.

TABLE 4

Proteases and cancers associated with overexpressed proteases

Choi et al., *Theranostics*, (2)2:156-78, 2012

Family	Protease	Location	Cancer	Ref.	Other Diseases	Ref.
Cysteine Cathepsins	General	Intracellular, lysosomes	Most	Table in [121]		
	Cathepsin K	Extracellular, bone	Breast	[178]	Artherosclerosis, osteoporosis	[179-182]
	Cathepsin B	Extracellular and pericellular under pathological conditions	Breast, cervix, colon, colorectal, gastric, head and neck, liver, lung, melanoma, ovarian, pancreatic, prostate, thyroid	[31, 38, 81, 183-196]		
	Cathepsin L		Breast, colorectal	[28]	AD	[197]
Aspartic Cathepsins	Cathepsin E	Endosomal structures, ER, Golgi	Cervical, gastric, lung, pancreas adenocarcinomas	[51-55]		
	Cathepsin D	Lysosome	Breast, colorectal, ovarian	[47-49, 198-200]	Atherosclerosis	[121]
Kallikreins (hK)	General	Intracellular, secreted	Most	Table in [15, 58]		
	hK1				Hypertension, inflammation	[24]
	PSA (hK 3)		Prostate, ovarian	[201-202]		
	hK10		Colon, ovarian, pancreatic, head and neck	[203-206]		
	hK15		Ovarian, prostate	[207-208]		
Serine Proteases	uPA, uPAR	Membrane, Pericellular	Cervical, colorectal, gastric, prostate	[86, 116, 209-210]		
Caspases		Intracellular			Neurodegenerative disorders	[82]
MMPs	General	Extracellular	Most	Table in [211]		
	MMP-1, -8, -13		Breast	[85, 102-104, 211-212]	Artherosclerosis, RA	[213-214]
	MMP-2, -9		Breast, colorectal, lung, malignant gliomas, ovarian	[91-94] [95-98]	Bronchiectasis, chronic asthma, COPD, cystic fibrosis, HIV associated dementia, hypertension, stroke	[87, 113-117]
	MMP-14	Membrane	Breast	[212]		
ADAM		Extracellular			AD	[105, 107, 112]

*Abbreviations: AD: Alzheimer's disease; ADAM: a disintegrin and metalloproteinase domain protease; COPD: chronic obstructive pulmonary disease; ER: endoplasmic reticulum; RA: rheumatoid arthritis

[0169] In various embodiments, the peptide is bound to the capsid backbone, as described in more detail below. There are multiple known means by which the peptide is able to be associated with, or bound to, the capsid backbone. In various embodiments of the present disclosure the cleavage sequence is chemically conjugated by way of a maleimide linkage or an amide linkage (discussed below). The peptide is generally linked to any residue on the capsid backbone; however, disulfide linkages, maleimide linkages, and amide linkages are formed by conjugating the peptide to cysteine, lysine, or arginine residues of the mutant L1 proteins that comprise the capsid backbones.

[0170] In various embodiments the peptide comprises at least one protease cleavage sequence. In some embodiments, the protease cleavage sequence is any sequence capable of being preferentially cleaved by or near a tumor cell. The insertion of this cleavage sequence into the peptide allows the protein to remain attached to the capsid backbone carrier until the IRC enters the tumor microenvironment. By taking advantage of the elevated activities of particular proteases in cancer tissues or tumor microenvironments, the peptide is to a large extent not released from the capsid backbone and able to actively coat MHC receptors until the peptide enters the tumor microenvironment. Several proteases are known in the art to be active in the tumor microenvironment. For example, several metallo-, cysteine and serine proteases are known. From the standpoint of cancer therapy, an additional attraction is that because the proteases responsible for prodrug cleavage may come not just from cancer cells but also from the stromal components of tumors, release of the active drug direction into the tumor microenvironment does not depend on a target expressed only by the cancer cells. Instead, it is the entire tumor ecosystem that represents the target.

Methods of Attaching Peptides to the L1 Protein

[0171] The capsid backbones described herein are in some embodiments first functionalized to deliver an epitope containing on one or more peptides associated with the capsid backbone to the target cells, thereby labeling the tumor or cancer cells for destruction. In various embodiments, peptides are conjugated to the capsid backbone through cysteine residues on the capsid protein. Such cysteine molecule are presented naturally, or by mutation, on the surface of the capsid backbone. In various embodiments, the capsid backbone is subjected to reducing conditions sufficient to reduce the sulfhydryl groups of cysteine residues on the surface of the capsid backbone while maintaining the capsid-like icosahedron structures of the capsid backbone. Because of its free sulfhydryl group, cysteine will readily and spontaneously form disulfide bonds with other sulfhydryl–containing ligands under oxidative conditions. Alternatively, a series of compounds are known to add a maleimide moiety to receptive substrates that readily and irreversibly form thioester linkages with cysteine residues at a pH between about 6.5 and about 7.5. Thus, in one embodiment, the peptide is associated with the capsid backbone via a maleimide linkage.

[0172] In various embodiments, the peptide is conjugated to a lysine residue on the capsid backbone. Lysine residues are easily modified because of their primary amine moiety. Using reactions termed n-hydroxysuccinimide (NHS) ester reactions (because NHS is released as prat of the reaction), amide bonds are formed at surface-exposed lysine residues on the

capsid backbone. The NHS reaction occurs spontaneously between about pH 7.2 and about pH 9.

[0173] In various embodiments, the peptide is conjugated to an aspartate or glutamate residue. Unlike chemical coupling strategies involving cysteine and lysine groups, chemically coupling to aspartate or glutamate residues requires multiple steps. First, the carboxylic acid of the aspartate or glutamate is activated using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), or similar chemical cross-linking reagent. Once activated, this adduct will react with NHS to form an NHS ester. The NHS ester is then reacted with a ligand with an exposed primary amine to from a stable amide bond.

[0174] In various embodiments, the capsid backbone comprises a region of negatively charged amino acids on a surface-exposed area that is capable of binding to the peptide comprising a region of positively charged amino acids. In various embodiments, the region of negatively charged amino acids is flanked, on one or on both sides, by one or more cysteine residues, referred to as polyanionic: cysteine or more specifically, polyglutamic acid:cysteine or polyaspartic acid:cysteine. In such cases, the conjugation of the capsid backbone and the peptide would result from non-covalent binding between the complementary amino acid charges of the capsid backbone and the peptide and a disulfide bond between the cysteines. In various embodiments, the cysteine(s) are one or more amino acids away from the region of charged amino acids such that any secondary/tertiary structure would bring the charged amino acid region in close proximity to the cysteine(s). In various embodiments, the peptide comprises at least one peptide and a polyionic:cysteine for attaching the peptide to the capsid backbone comprising a complementary polyionic:cysteine sequence and an enzyme cleavage site positioned between the terminal cysteine and the CD8+ T cell epitope. In various embodiments, the peptide comprises, a terminal cysteine, at least one peptide, and an enzyme cleavage sequence positioned between the terminal cysteine and the peptide(s).

[0175] Negatively charged amino acids that are useful in producing the described IRC include, e.g., glutamic acid and aspartic acid. These amino acids are used singly in some embodiments, e.g., polyglutamic acid, or in combination. In a specific embodiment, the negatively charged region comprises glutamic acid. The number of negatively charged amino acids can vary and can include about 4 to about 20 amino acids, about 6 to about 18 amino acids, or about 8 to about 16 amino acids, and the like. In a specific embodiment, the negatively charged region comprises about 8 negatively charged amino acids. In a more specific embodiment, the negatively charged region comprises EEEEEEEEC (E8C, SEQ ID NO:130). In another embodiment, the negatively charged region comprises CEEEEEEEEC (SEQ ID

NO:131). Methods for conjugating peptides to a capsid backbones via disulfide bonding are known. For instance, the presence of a polyarginine-cysteine moiety on the peptide allows docking of the peptide to the polyanionic site (EEEEEEEEC, E8C, SEQ ID NO:130) present in the various loops of the capsid backbone. Covalent cross-linking between the two cysteine residues should render this association irreversible under oxidizing conditions. For the conjugation reactions, purified capsid backbones are dialyzed in conjugation buffer (20 mM Tris/HCl, pH 7.5, 150 mM NaCl, 5% glycerol, 0.5 mM $CaC1_2$) and then the peptide and the oxidizing reagents are added, allowing the reaction to proceed for 16 hrs at 4 °C. At the end of the incubation, the reaction mixtures are applied to a size-exclusion column (such as SEPHADEX® G-100, Pharmacia, New Jersey, US, volume 20 ml, flow rate 1 ml/min, 10 mM Tris/HCl (pH 7.4), 150 mM NaCl, 0.5 mM $CaCl_2$) to remove unconjugated peptide and exchange buffer. IRCs that elute in the void volume are identified by the presence of the L1 protein on SDS-PAGE. The conjugated capsid backbones (IRC) are than optionally analyzed by electron microscopy.

[0176] In various embodiments, the peptide is genetically fused to the L1 protein. In various embodiments, the peptide is either covalently or non-covalently linked to the capsid backbone. Rather than attaching the peptide to the capsid backbone via, e.g., binding of negatively and positively charged amino acids, or via maleimide based conjugation, a nucleic acid sequence encoding the peptide is inserted in some embodiments into the nucleic acid encoding the L1 protein such that upon expression a peptide is produced that is inserted into a loop of the capsid protein and displayed on the surface of the capsid backbone.

[0177] In various embodiments, non-natural amino acids are used to conjugate the peptide to the capsid backbone. Beyond the 20 natural amino acids, many non-natural amino acids have been used for site-specific protein conjugation reactions. For example, an azidohomoalanine (AHA) or a p-amino-phenylalanine (pAF) may be incorporated into the capsid backbone coat protein for conjugation. These amino acids are incorporated into proteins in two ways: global methionine replacement and amber stop codon suppression. Because AHA is very similar to methionine, AHA will be incorporated at each AUG codon if the methionine supply is rate limiting, this is termed global methionine replacement. Bacteria auxotrophic for methionine or cell-free protein synthesis can be used to limit-methionine availability. Amber stop codon suppression will incorporate pAF. Amber stop codon suppression uses nonnative synthetases and tRNAs that do not react with the natural amino acids to incorporate the non-natural amino acid at the amber stop codon UAG. AHA, displaying an azide, will participate

in in copper(I)-catalyzed azide-alkyne cycloaddition ("click" reaction) and form covalent triazole rings with alkyne-containing ligands.

[0178] In various embodiments, the IRC comprises, at least one-tenth of the L1 proteins display a peptide. In various embodiments, at least one-fifth of the L1 proteins display a peptide. In various embodiments, about half of the L1 proteins display a peptide. In various embodiments, about two-thirds of the L1 proteins display a recall peptide. In various embodiments, nearly all of the L1 proteins display a peptide.

IRCs and Uses Thereof in Clinical Therapies

[0179] In various embodiments, the capsid backbone binds preferentially to tumor cells. The capsid backbones' tumor preference originates, in some embodiments, from several sources such as the capsid backbone's charge (positive or negative), shape and size (different aspect ratio filaments and diameter spheres), shielding (self-proteins/peptides and polymers of various sizes and densities), and targeting (ligands for receptors or environmental factors displayed on different linkers at various densities).

[0180] In terms of charge, in various embodiments, the capsid backbone contains a positive surface charge. Positively charged capsid backbones have been shown in some studies to remain longer in circulation when injected into a subject. Due to the abundant presence of proteoglycan in cell membranes that confer a negative charge to cell membranes, and collagen within the tumor interstitial space conferring a positive charge, positively charged IRCs are more likely to possess enhanced binding to mammalian cells as compared with non-charged or negatively charged IRCs, and therefor are better able to avoid aggregation and as a result, are able to better penetrate tumor tissue. Some examples demonstrating these charge-based effects include polyarginine-decorated cowpea mosaic virus (CPMV) found to be taken up eight times more efficiently than native CPMV in a human cervical cancer. (Wen et al., *Chem. Soc. Rev.*, 45(15):4074-4126, 2016).

[0181] With regards to shape, the shape and flexibility of the capsid backbone in some instances plays an additional functional role in the ability of capsid backbones to diffuse throughout a tumor. A comparison between the diffusion profiles of a spherical and rod-shaped particle was performed with CPMV and TMV using a spheroid model. It was shown in this study that the CPMV (spherical) experienced a steady diffusion profile, but the TMV (rod shaped) exhibited a two-phase diffusion behavior that entailed an extremely rapid early loading phase that could be attributed to its movement axially, like a needle. (Wen et al., *Chem. Soc.*

Rev., 45(15):4074-4126, 2016). Some other advantageous properties that are conferred by elongated particles include better margination toward the vessel wall and stronger adherence due to greater surface area for interaction, which not only have implications for tumor homing but also for enhanced targeting of cardiovascular disease.

[0182] Besides passive tumor homing properties, natural interactions of viruses with certain cells can also be exploited. CPMV in particular exhibits unique specificity in interacting with surface vimentin, which is found on endothelial, cancer, and inflammatory cells. (Wen et al., *Chem. Soc. Rev.*, 45(15):4074-4126, 2016). The native affinity of CPMV for surface vimentin allows for high-resolution imaging of microvasculature up to 500 μm in depth, which cannot be achieved through the use of other nanoparticles, as they tend to aggregate and block the vasculature. This interaction can be utilized for a range of applications, such as delivery to a panel of cancer cells including cervical, breast, and colon cancer cell lines, delineation of atherosclerotic lesions, and intravital imaging of tumor vasculature and angiogenesis. Another example of an existing endogenous association is canine parvovirus (CPV) with transferrin receptor (TfR), an important receptor for iron transport into cells and highly upregulated by numerous cancer cell lines. Even after dye labelling, CPV retains its specificity for TfR and was shown to bind to receptors found on HeLa cervical cancer cells, HT-29 colon cancer cells, and MDA-MB-231 breast cancer cells. (Wen et al., *Chem. Soc. Rev.*, 45(15):4074-4126, 2016).

[0183] In various embodiments, the capsid backbone targets a protein expressed preferentially on the tumor cell surface in the subject. Such proteins are typically overexpressed on the surface of tumor cells, but some if not all, are also found in the blood, i.e., serum. Non-limiting examples of such surface markers include: CEA (carcinoembryonic antigen), E-cadherin, EMA (epithelial membrane antigen; aka MUC-1), vimentin, fibronectin, Her2/neu (human epidermal growth factor receptor type 2, also called Erb b2), αvβ3 integrin, EpCAM (epithelial cell adhesion molecule), FR-α (folate receptor-alpha), PAR (urokinase-type plasminogen activator receptor), and transferrin receptor (over expressed in tumor cells).

[0184] Peptides are often used to label cancerous cells based on recognition of their transmembrane proteins. The most commonly used peptide is arginylglycylaspartic acid (RGD), which is composed of L-arginine, glycine, and L-aspartic acid. RGD was first isolated from the cell-binding domain of fibronectin, a glycoprotein that binds to integrins, and is involved in cell–cell and cell–extracellular matrix (ECM) attachment and signaling by binding collagen, fibrin, and proteoglycans. RGD peptides have the highest affinity for a type of cell surface integrins, αvβ which are highly expressed in tumoral endothelial cells, but not in normal endothelial cells. In various embodiments such a peptide sequence is incorporated into the IRC.

[0185] Methods of treating cancers in a subject in need thereof by administering an IRC to patient in need thereof, and related uses of the described IRC compositions, are described herein. The methods described herein comprise, for instance, administering the IRCs described herein to a subject in need thereof in an amount sufficient to inhibit tumor growth, progression or metastasis, i.e., a therapeutic amount or dose. In various embodiments, the IRC is administered to a subject in need thereof in amount sufficient to stimulate cytokine production and/or cellular immunity, particularly innate immunity, including stimulation of the cytotoxic activity of macrophages and natural killer cells. In various embodiments described herein, a subject in need thereof is a subject who has been previously treated for a tumor and is currently deemed cancer-free or disease free in accordance with medical standards.

[0186] Briefly, various understood aspects of what is believed to be the mechanism of action of the described IRCs are described and supported by the examples, below. The IRC first bind to a tumor cell, in some embodiments the binding is specific. (See, Example 5, Figures 9A and 9B). The peptide epitope on the IRC is then proteolytically cleaved by furin, in some embodiments, or by any other resident protease nearby the tumor cell, which is over-expressed in the tumor microenvironment. This in turn leads to release of the peptide from the IRC and the loading, or binding, of the peptide by an MHC molecule expressed on the surface of the tumor cell ("epitope coating"). (See, Example 6, and Figures 10, 11,12 and 20). The epitope-coated tumor cell is then recognized as a pathogen-infected cell by one or more T-cells responsive to the specific peptide bound in the MHC molecules, and pre-existing CD8 T cells, yielding a triggered immune redirection response. (See, Examples 8 and 9 as well as 11 and 12). That is, this recognition event leads to triggering or activation of the subject's preexisting immune memory against pathogens and childhood vaccines against the tumor, leading to the attacking and destroying of the subject's tumor cells.

[0187] Destruction of tumor cells can result in components of the preexisting immune response being exposed to cancer cell antigens. Thus, antigens released from the killed tumor cells will initiate a further immune response to recruit additional tumor-specific CD8 T cells, or a "second wave" of T cells that then proceed to attack additional tumor cells in the area. This can result in elicitation of an endogenous immune response against the cancer cell antigens (referred in some instances to "epitope spreading") and leads to anti-tumor immune memory.

[0188] Thus, the methods and uses disclosed herein are methods of treating cancer in a subject in need thereof that occurs through utilizing, or the re-orienting of, the subject's own preexisting adaptive memory immune system to attack cancer cells. The methods and uses described herein make use of the fact that subjects, in some instances, possess preexisting

immune responses that were not originally elicited in response to a cancer, but that were elicited instead by routine vaccination or via natural infection by a parasite or pathogen. Because the cancer cells would not normally express such epitopes that elicit preexisting immune responses, it would not be expected that such an immune response would not normally, without exogeneous intervention, be capable of attacking any cancer cell. However, by way of the present methods and uses described herein, such preexisting immune responses are readily recruited to attack, kill, and clear a cancer in a subject. This recruitment or repurposing effect is therefore achieved by way of the present IRC compositions since these IRC, upon injection or other means of delivery into the subject, introduce into or onto the surface of the cancer one or more epitopes known to be recognized by the preexisting immune response in the subject, resulting in cells of the immune response attacking antigen-displaying cancer cells.

[0189] Thus, without wishing to be bound by any specific theory, the methods, uses, and compositions described herein act by recruiting a preexisting immune response in a subject to the site of a cancer, such that the preexisting immune response attacks and kills the cancer cells. Thus, there are generally four or five steps involved in the described methods, including: 1) binding IRC to the tumor cells, 2) cleavage of the epitope from the IRC, 3) MHC binding of the epitopes for display on the tumor cell surface, 4) recognition of the loaded MHC by the subject's pre-existing recalled immunity against the epitope, and optionally 5) triggering of a second wave and longer-term anti-tumoral immunity thereafter.

[0190] Data obtained from cell culture assays and animal studies are often used in formulating a range of dosages for use in humans. The dosages of such compositions lie preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending on the dosage form employed and the route of administration utilized. For any composition used in the methods described herein, the therapeutically effective dose is capable of being estimated initially from cell culture assays. A dose is formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (the concentration of the test composition that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information is then used to accurately determine useful doses in humans. Levels in plasma are measured, for example, by high performance liquid chromatography. In some embodiments the range of effective dosages of the IRCs is between 10 μg and 2000 μg. In some embodiments, the range of effective dosage values is between 20 μg and 1000 μg, 30 μg to 1500 μg, and the like. Effective dosage ranges in some embodiments begin at, for instance, about 10 μg, 20 μg, 30 μg, 40 μg, 50 μg, 60 μg, 70 μg, 80 μg, 90, μg, 100 μg, 110 μg, 120 μg, 130 μg, 140 μg, 150 μg, and are effective to

approximate upper limits of about 200 μg, 300 μg, 400 μg, 500 μg, 600 μg, 700 μg, 800 μg, 900 μg, 1000 μg, 1200 μg, 1400 μg, 1600 μg, 1800 μg, or even 2000 μg, depending on the size of the tumor and/or the subject, per dosage.

[0191] In many instances, it will be desirable to have multiple administrations of the IRC-containing compositions, usually at most, at least, or not exceeding 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more doses including all ranges therebetween. The administrations will normally be at 1, 2, 3, 4, 5, 6, to 5, 6, 7, 8, 9, 10, 11, to 12 week/month/year intervals, including all values and ranges there between, more usually from three- to five-week intervals.

[0192] In various embodiments, a method is provided for stimulating the cytotoxic activity of macrophages and natural killer (NK) cells by administering to a subject in need thereof an effective amount of an IRC described herein. The macrophages and natural killer cells are in some instances those that are present in the tumor microenvironment. In one aspect, the IRCs are administered to the subject in an amount effective to stimulate the cytotoxic activity of macrophages and natural killer cells already present in the tumor microenvironment. In various other embodiments, the IRCs are administered to the subject in an amount effective to attract macrophages and natural killer cells to the tumor microenvironment. In various embodiments, the IRCs are administered to the subject in an amount effective to bind sufficient numbers of antibodies to the peptide or IRC capsid itself to attract and stimulate macrophages, neutrophils and natural killer cells.

[0193] In various embodiments, methods and uses are provided for redirecting the cytotoxic activity of an existing memory CD8+ T cell to a tumor cell or tumor microenvironment by administering to a subject in need thereof an effective amount of the IRC described herein. Preferably, the T cell epitope of the peptide of the IRC is from a pathogen for which the subject has been vaccinated or from a pathogen that has previously infected the subject and the subject has memory CD8+ T cells that recognize the T cell epitope in complex with an MHC class I molecule on the tumor cells. In an aspect described herein, the effective or therapeutic amount of the IRC compositions described herein is an amount sufficient to attract the memory CD8+ T cell to the tumor microenvironment. In another alternative aspect, the effective amount of the IRC is an amount sufficient to stimulate the memory CD8+ T cell present in the tumor microenvironment.

[0194] In various embodiments, the subject or patient is in need of treatment for a tumor that is a small lung cell cancer, a non-small cell lung cancer (NSCLC), hepatocellular carcinoma, liver cancer, hepatocellular carcinoma, melanoma, metastatic melanoma, adrenal cancer, anal cancer, aplastic anemia, bile duct cancer, bladder cancer, bone cancer, brain/CNS

cancer, breast cancer, cancer of unknown primary origin, Castleman disease, cervical cancer, colon/rectum cancer, endometrial cancer, esophagus cancer, Ewing family of tumors, eye cancer, gallbladder cancer, gastrointestinal carcinoid tumors, gastrointestinal stromal tumor (gist), gestational trophoblastic disease, Hodgkin disease, Kaposi sarcoma, kidney cancer, laryngeal and hypopharyngeal cancer, leukemia, liver cancer, lung cancer, lymphoma, malignant mesothelioma, multiple myeloma, myelodysplastic syndrome, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer. neuroblastoma, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma, skin cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, Wilms tumor, non-Hodgkin lymphoma, Hodgkin lymphoma, Burkitt's lymphoma, lymphoblastic lymphomas, mantle cell lymphoma (MCL), multiple myeloma (MM), small lymphocytic lymphoma (SLL), splenic marginal zone lymphoma, marginal zone lymphoma (extra-nodal or nodal), mixed cell type diffuse aggressive lymphomas of adults, large cell type diffuse aggressive lymphomas of adults, large cell immunoblastic diffuse aggressive lymphomas of adults, small non-cleaved cell diffuse aggressive lymphomas of adults, or follicular lymphoma, head and neck cancer, endometrial or uterine carcinoma, osteosarcoma, glioblastoma, or metastatic cancer. In a preferred embodiment, the cancer is a breast cancer, a cervical cancer, an ovarian cancer, a pancreatic cancer, a lung cancer, or a melanoma,

[0195] The term "cancer" as used herein refers to proliferative diseases, such as lymphomas, lymphocytic leukemias, lung cancer, non-small cell lung (NSCL) cancer, bronchioloalveolar cell lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, gastric cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, neoplasms of the central nervous system (CNS), spinal axis tumors, brain stem glioma, glio-blastoma multiforme, astrocytomas, schwanomas, ependymonas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenoma

and Ewing's sarcoma, including refractory versions of any of the above cancers, or a combination of one or more of the above cancers.

[0196] An aspect described herein is a method for treating a cancer in a subject in need thereof by administering an IRC described herein to the subject wherein the CD8+ epitope of the peptide is of a failed therapeutic cancer vaccine against a viral-induced cancer, e.g., HPV cervical cancer, HPV+ oral cancer, EBV nasopharyngeal cancer (the "therapeutic vaccine"). The methods and uses described herein therefore comprise determining whether the subject has been actively vaccinated but did not respond with an anti-tumor effect to the treatment. The IRC composition is then administering to the subject an effective amount of an IRC of this invention wherein the CD8+ epitope of the peptide is of the antigenic determinant in the vaccine previously administered to the subject that infected the subject.

[0197] Capsid backbones have inherent adjuvant properties. In some embodiments, the immunogenicity of the IRC compositions described herein are further enhanced by the combination with additional nonspecific stimulators of the immune response, known as adjuvants. Suitable adjuvants include all acceptable immunostimulatory compounds, such as, but not limited to, cytokines, toxins, or synthetic compositions such as alum.

[0198] Adjuvants include, but are not limited to, oil-in-water emulsions, water-in-oil emulsions, mineral salts, polynucleotides, and natural substances. Specific adjuvants that may be used include IL-1, IL-2, IL-4, IL-7, IL-12, y-interferon, GM-CSF, BCG, aluminum salts, such as aluminum hydroxide or other aluminum compound, methylenedioxyphenyl (MDP) compounds, such as thur-MDP and nor-MOP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL), or inactivated microbial agents. RIBI, which contains three components extracted from bacteria, MPL, trehalose dimycolate (TOM), and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion. MHC antigens may even be used.

[0199] Various methods of achieving adjuvant affect for the IRC compositions includes use of agents such as aluminum hydroxide or phosphate (alum), commonly used as about 0.05 to about 0.1 % solution in phosphate buffered saline, admixture with synthetic polymers of sugars (CARBOPOL®) used as an about 0.25% solution, aggregation of a protein in the composition by heat treatment with temperatures ranging between about 70°C to about 101 °C. for a 30-second to 2-minute period, respectively. Aggregation by reactivating with pepsin-treated (Fab) antibodies to albumin; mixture with bacterial cells, e.g., C. *parvum*, endotoxins or lipopolysaccharide components of Gram-negative bacteria; emulsion in physiologically acceptable oil vehicles, e.g., mannide monooleate (Aracel A™), or emulsion with a 20% solution of a perfluorocarbon (FLUOSOL-DA®) used as a block substitute may also be

employed to produce an adjuvant effect. A typical adjuvant is complete Freund's adjuvant (containing killed *Mycobacterium tuberculosis*), incomplete Freund's adjuvants, and aluminum hydroxide.

[0200] For administration to humans, a variety of suitable adjuvants will be evident to a skilled worker. These include, e.g., Alum-MPL as adjuvant, or the comparable formulation, ASO4, which is used in the approved HPV vaccine CERVARIX®, AS03, AS02, MF59, montanide, saponin-based adjuvants such as GPI-0100, CpG-based adjuvants, or imiquimod. In embodiments of the invention, an adjuvant is physically coupled to the capsid backbone, or encapsulated by the capsid backbone, rather than simply mixed with them. In addition to adjuvants, it may be desirable to co-administer biologic response modifiers (BRM) to enhance immune responses. BRMs have been shown to upregulate T cell immunity or downregulate suppresser cell activity. Such BRMs include, but are not limited to, Cimetidine (CIM; 1200 mg/d) (Smith/Kline, PA, US); or low-dose Cyclophosphamide (CYP; 300 mg/ml) (Johnson/Mead, NJ, US) and cytokines such as γ-interferon, IL-2, or IL-12 or genes encoding proteins involved in immune helper functions, such as B-7. In embodiments described herein, these genes are encapsulated by the capsid backbone to facilitate their delivery into a subject.

[0201] The preparation of compositions that contain polypeptide or peptide sequence(s) as active ingredients is generally well understood in the art. Typically, such compositions are prepared as injectables either as liquid solutions or suspensions: solid forms suitable for solution in or suspension in liquid prior to injection may also be prepared. The preparation is in some instances emulsified. The active immunogenic ingredient is in some embodiments mixed with excipients that are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the compositions may contain amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants that enhance the effectiveness of the vaccines. In specific embodiments, vaccines are formulated with a combination of substances.

[0202] The compositions comprising the IRCs of the present disclosure are intended to be in a biologically-compatible form that is suitable for administration *in vivo* to subjects. The pharmaceutical compositions described herein further comprise one or more optional pharmaceutically acceptable carriers. The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government, e.g., the FDA, or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle

with which the capsid backbone is administered. Such pharmaceutical carriers include, for example, sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, including but not limited to peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a carrier in some instances when the pharmaceutical composition described herein is administered orally. Saline and aqueous dextrose are carriers, for example, when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions are employed, for instance, as liquid carriers for injectable solutions. Suitable pharmaceutical excipients include, without limitation, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried slim milk, glycerol, propylene, glycol, water, ethanol and the like. The pharmaceutical composition in some embodiments optionally contains minor amounts of wetting or emulsifying agents, or pH buffering agents.

[0203] The pharmaceutical compositions comprising the IRCs of the present disclosure take the form of, for example, solutions, suspensions, emulsions, tablets, pills, capsules, powders, sustained-release formulations, and the like. Oral formulation includes in some embodiments standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. In a specific embodiment, a pharmaceutical composition comprises an effective amount of an IRC of the present disclosure together with a suitable amount of a pharmaceutically acceptable carrier so as to provide the form for proper administration to the subject. The formulation should suit the mode of administration.

[0204] The pharmaceutical compositions of the present disclosure are administered by any particular route of administration including, but not limited to, intravenous, intramuscular, intraarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intraosseous, intrapelvic, intrapericardial, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, oral, parenteral, subcutaneous, vaginal, rectal, buccal, sublingual, intranasal, iontophoretic means, or transdermal means. Most suitable routes are intravenous injection or oral administration. In particular embodiments, the compositions are administered at or near the target area, e.g., intratumoral injection.

[0205] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with

sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, intratumoral, subcutaneous, and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in isotonic NaCl solution and either added to hypodermoclysis fluid or injected at the proposed site of infusion. (See, for example, Remington's Pharmaceutical Sciences, 1990). Some variation in dosage necessarily occurs depending on the condition of the subject. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

[0206] The IRC-containing compositions described herein, in some embodiments, are administered by inhalation. In certain embodiments a composition is administered as an aerosol. As used herein the term "aerosol" or "aerosolized composition" refers to a suspension of solid or liquid particles in a gas. These terms are used generally to refer to a composition that has been vaporized, nebulized, or otherwise converted from a solid or liquid form to an inhalable form including suspended solid or liquid drug particles. Such aerosols can be used to deliver a composition via the respiratory system. As used herein, "respiratory system" refers to the system of organs in the body responsible for the intake of oxygen and the expiration of carbon dioxide. The system generally includes all the air passages from the nose to the pulmonary alveoli. In mammals it is generally considered to include the lungs, bronchi, bronchioles, trachea, nasal passages, and diaphragm. For purposes of the present disclosure, delivery of a composition to the respiratory system indicates that a drug is delivered to one or more of the air passages of the respiratory system, in particular to the lungs.

[0207] Additional formulations that are suitable for other modes of administration include suppositories (for anal or vaginal application) and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkalene glycols or triglycerides: such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10%, preferably about 1 % to about 2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations, or powders and contain about 10% to about 95% of active ingredient, preferably about 25% to about 70%.

[0208] The IRC compositions described herein are, in some instances, formulated into a vaccine as neutral or salt forms. Pharmaceutically-acceptable salts include the acid addition

salts (formed with the free amino groups of the peptide) and those that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

[0209] The pharmaceutical compositions of the present disclosure also include, in certain embodiments, an effective amount of an additional adjuvant. As noted herein, papillomavirus capsid backbones have adjuvant properties. Suitable additional adjuvants include, but are not limited to, Freund's complete or incomplete, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, and potentially useful human adjuvants such as Bacille Calmette-Guerin (BCG), *Corynebacterium parvum*, and non-toxic cholera toxin.

[0210] Under ordinary conditions of storage and use, the described IRC compositions in some embodiments also contain a preservative to prevent the growth of microorganisms. In all cases the pharmaceutical form must be sterile and must be fluid to the extent that it may be easily injected. It also should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[0211] The carrier is in some embodiments a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity is maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. The prevention of the action of microorganisms is brought about in some instances by incorporation of various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions is achieved by the addition to the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0212] Sterile injectable solutions are prepared by incorporating the IRCs in the required amount in the appropriate solvent with various ingredients enumerated above, as required may be followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the

basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques, which yield a powder of the active ingredient, plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0213] Different aspects of the present disclosure involve administering an effective amount of a composition comprising the IRCs to a subject in need thereof. In some embodiments of the present disclosure, an IRC comprising a target peptide comprising a CD8+ T cell epitope is administered to the patient to treat a tumor or prevent the recurrence of such tumor. Such compositions will generally be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium.

Design of IRC for Specific Uses

[0214] In various embodiments, a method for providing an IRC to a subject in need thereof is provided comprising: (1) measuring the preexisting immunity in a subject, and (2) selecting the appropriate IRC for administration of a subject in need. The appropriate IRC to administer to the subject will depend upon the patients T cell profile. The appropriate IRC will be one that is capable of eliciting a T cell response that is at least twice the baseline total of CD8+ cells. In various embodiments, the appropriate IRC will be one that is capable of eliciting a T cell response that is twice the baseline total of CD8+ or total CD8+ CD69+ T cells. The goal is to choose the appropriate IRC based on the subject's vaccination history or prior exposure to a pathogen. Determining which IRC is appropriate is, for example, achieved through: (1) subject interviews; (2) review of a subject's medical records; and/or (3) assessing the subject's T cell profile.

[0215] In various embodiments, more than one peptide is suitable for eliciting an immune response directed at a tumor. In various embodiments, an IRC carrying either peptide or a mixture of both peptides will be appropriate. In various embodiments, more than one peptide is expressed and bound to the capsid backbone. In various embodiments, a single peptide will comprise more than one peptide. In various embodiments, multiple peptides comprising different peptides will be conjugated to the capsid backbone. In various embodiments, the invention comprises a population of IRCs as described herein and a pharmaceutically acceptable excipient. In various embodiments, the IRCs administered to the subject are identical. In various embodiments, IRCs carrying different peptide(s) are administered to a subject.

[0216] Selection Based on Prior Vaccination. In various embodiments of the methods and uses described herein, contemplated is also a method of selecting an appropriate IRC to administer to a subject in need thereof. In various embodiments this involves ascertaining if the subject has been actively vaccinated against a given pathogen, e.g., a parasite, a bacterium, or virus, e.g., measles or polio, and then selecting and administering to the subject an IRC as disclosed herein wherein the CD8+ T cell epitope of the peptide is from the pathogen against which the subject has been immunized in the past. In various embodiments, a subject's vaccination history is obtained by reviewing the subject's medical record. In various embodiments, a subject's vaccination history is obtained by interviewing the subject.

[0217] Selection Based on Prior Infection. In various embodiments, the method of selecting an appropriate IRC for administration to a subject in need thereof involves ascertaining if a subject has been previously infected with a given pathogen, e.g., a parasite, a bacterium, or virus, e.g., measles or polio, and resolved the infection. In various embodiments, the subject is then administered an IRC comprising a peptide which comprises said pathogen for which the subject has been previously infected.

[0218] One may ascertain if a subject has been infected with a particular pathogen by reviewing the subject's medical records or interviewing the subject. Non-limiting examples of CD8+ T cell epitopes that bind to particular MHC class I molecules are set forth in Table 1. The method also comprises, in certain embodiments, determining which MHC class I determinant(s) the subject's cells express and then administering an IRC described herein wherein the CD8+ T cell epitope of the peptide is a CD8+ T cell epitope of the antigenic component of the pathogen in the vaccine or of the pathogen that previously infected the subject that forms a complex with the subject's MHC class I determinant(s).

[0219] Measuring T cell Responses. In various embodiments, a subject's T cell profile is also assessed in order to select an appropriate IRC using various techniques known in the art. This profile is then used to guide selection of the appropriate IRC to administer to the subject. Such techniques include, for example, measuring interferon-γ levels, using flow cytometry to isolate Ag-specific CD8+ T cells, and/or cytotoxicity assays. To measure interferon-γ (a marker of T cell activation), intracellular staining of isolated T cells. Alternatively, an enzyme-linked immunosorbent spot (ELISPOT) assay for interferon-γ may be conducted. This technique allows for a high throughput assessment of a patient's T cell profile. This method can potentially detect one in 100,000-300,000 cells. Briefly, a monoclonal antibody for a specific cytokine is pre-coated onto a polyvinylidene difluoride (PVDF)-backed microplate. CD8+ T cells are pipetted into the wells along with dendritic cells and individual

peptides and the microplate is placed into a humidified 37°C CO_2 incubator for a period ranging from 24 to 48 h. During incubation, the immobilized antibody binds the cytokine secreted from the cells. After washing a detection antibody specific for the chosen analyte is added to the wells. Following the washes, enzyme conjugated to streptavidin is added and a substrate is added. A colored precipitate forms, according to the substrate utilized and appears as spot at the sites of cytokine secretion, with each individual spot representing a single producing cell.

[0220] In various embodiments, provided are methods of determining the appropriate IRC to administer to a subject in need thereof, by assessing the subject's T cell profile, comprising: (1) collecting PBMCs from subject (pre-vaccination sample), (2) preparing enzyme-linked immune absorbent spot (ELISPot) plates by coating with anti-IFN-γ antibody (incubate overnight), (3) incubating PBMCs with one of the pool of peptides of interest, i.e., the peptides expected to elicit a T cell response (incubate for 1-2 days), (4) washing the plates, adding a biotinylated secondary antibody (incubating for a few hours), (5) washing the plates, adding avidin conjugated horseradish peroxidase and incubating, (6) washing plates, adding aminoethyl carbazole (AEC) for a few minutes, (7) stopping the reaction (by adding water), and (8) visualizing on an ELISPot reader. The disclosed methods detect up to one in 100,000 to 300,000 cells. A two-fold increase in the frequency of antigen-specific T cells should be considered as a signal.

[0221] In various embodiments T cell proliferation is measured by 3H (tritiated)-thymidine. Such methods are sensitive and can be used for high throughput assays. Such techniques include, for instance, carboxyfluorescein succinimidyl ester (CFSE) and Ki64 intracellular staining.

[0222] Selecting Peptides based on Tropism. It is known in the art that some viruses display a tropism for particular type of tissue. For example: viruses that display a tropism for brain tissue include without limitation, JC virus, measles, LCM virus, arbovirus and rabies; viruses that display a tropism for eye tissue include without limitation herpes simplex virus, adenovirus, and cytomegalovirus; viruses that display a tropism for nasal tissue include without limitation, rhinoviruses, parainfluenza viruses, and respiratory syncytial virus; viruses that display a tropism for oral tissue, e.g., oral mucosa, gingiva, salivary glands, pharynx, include without limitation, herpes simplex virus type I and type II, mumps virus, Epstein Barr virus, and cytomegalovirus; viruses that display a tropism for lung tissue include without limitation, influenza virus type A and type B, parainfluenza virus, respiratory syncytial virus, adenovirus, and SARS coronavirus; viruses that display a tropism for nerve tissue, e.g., the spinal cord, include without limitation poliovirus and HTLV-1; viruses that display a tropism for heart

tissue, include without limitation, Coxsackie B virus; viruses that display a tropism for liver tissue, include without limitation, hepatitis viruses types A, B, and C; viruses that display a tropism for gastrointestinal tissue, e.g., stomach, and large and small intestine, include without limitation, adenovirus, rotavirus, norovirus, astrovirus, and coronavirus; viruses that display a tropism for pancreatic tissue, include without limitation, coxsackie B virus; viruses that display a tropism for skin tissue, include without limitation, varicella zoster virus, herpes simplex virus 6, smallpox virus, molluscum contagiosum, papilloma viruses, parvovirus B19, rubella, measles and coxsackie A virus; and viruses that display a tropism for genital tissue, include without limitation, herpes simplex type 2, papillomaviruses, human immunodeficiency virus (HIV).

[0223] In various embodiments, a method for treating a cancer in a subject in need thereof is provided by administering an IRC described herein to the subject wherein the peptide is a CD8+ epitope of a pathogen that has a tropism for the tissue that is the source of the cancer (the "source tissue"). In various embodiments, the appropriate IRC is selected by first determining the source tissue of the tumor cell and then selecting a peptide: (1) to which the patient already has existing CD8+ T cells, and (2) that has a tropism for the source tissue of the tumor. The selected IRC(s) are then administered to the subject in need thereof.

[0224] In various embodiments, provided are methods for treating a lung cancer comprising determining if a subject has been actively vaccinated against a pathogen that infects lung cells, e.g., an influenza virus, e.g., influenza virus type A or type B, then administering an effective amount of an IRC composition described herein, wherein the CD8+ T cell epitope of the peptide is of the antigenic determinants of the pathogen contained in the vaccine and which T cell epitope forms a complex with an MHC molecule class I of the subject. The methods and uses described herein for treating a lung cancer includes, in some embodiments, determining if a subject has been infected with pathogen that infects lung cells, e.g., an influenza virus, e.g., influenza virus type A or type B, then administering an effective amount of an IRC composition described herein wherein the CD8+ T cell epitope of the peptide is of that pathogen and which T cell epitope forms a complex with an MHC class I molecule of the subject.

[0225] Provided also are methods for treating an oral cancer, which are part of the group of cancers commonly referred to as head and neck cancers, by administering an IRC composition described herein, wherein the CD8+ epitope of the peptide is of a pathogen that has a tropism for oral tissue, e.g., a mumps virus, Epstein Barr virus, cytomegalovirus, or a herpes simplex virus type 1. The method comprises determining if a subject in need thereof has been actively vaccinated against, or infected with, e.g., a mumps virus, Epstein Barr virus,

cytomegalovirus, or a herpes simplex virus type 1, and if the subject has been vaccinated or infected previously then administering to the subject an IRC composition described herein wherein the CD8+ epitope of the peptide is of a mumps virus or a measles virus or of the antigenic component of the vaccine the subject had received, or of the pathogen, i.e., mumps, measles, Epstein Barr virus, cytomegalovirus, or a herpes simplex virus type 1, that had previously infected the subject.

Combination Therapy

[0226] In various embodiments, the IRC compositions described herein are co-administered with other cancer therapeutics. Furthermore, in some embodiments, the IRCs described herein are administered in conjunction with other cancer treatment therapies, e.g., radiotherapy, chemotherapy, surgery, and/or immunotherapy. In some aspects of methods and uses described herein, the IRC compositions described herein are administered in conjunction with checkpoint inhibitors. In various embodiments the capsid backbone is administered in conjunction with an immune agonist. In various embodiments, the IRC is administered in conjunction with treatment with a therapeutic vaccine. In various embodiments, the IRC is administered in conjunction with treatment with a conjugated antigen receptor expressing T cell (CAR-T cell). In various embodiments, the IRC is administered in conjunction with treatment with another immuno-oncology product. The IRCs of the present disclosure and other therapies or therapeutic agents are, in some embodiments, administered simultaneously or sequentially by the same or different routes of administration. The determination of the identity and amount of therapeutic agent(s) for use in the methods of the present disclosure is readily made by ordinarily skilled medical practitioners using standard techniques known in the art.

Combination Therapies with Immune Checkpoint Inhibitors (ICI) and Synergism

[0227] In some embodiments described herein, a ViP or an AIR-ViP, such as, but not limited to VERI-101, is administered for inhibiting the progression and/or metastasis of, a cancer in a subject (a patient) in need thereof, via intratumoral ("IT") injection to one accessible target tumor amenable to repeat administration, on a weekly (QW) basis. For example, the treated lesion receives 2 cycles of 3 intratumoral doses of VERI-101, unless the lesion disappears or becomes inaccessible for injection. If the treated lesion disappears or becomes inaccessible for injection, a new target lesion may be injected at subsequent visits. In some embodiments, the duration of therapy is 2 cycles (about 6 weeks), or more at the discretion of

skilled clinician, who may employ dose escalation in the range of 10 to 150 μg, or more, as therapeutically indicated and/or in consultation with a Safety Review Committee (SRC), or other relevant authorities. (See, e.g., Table 5 below and the Examples).

TABLE 5

Exemplary Clinical Dose Escalation Of AIR-ViP

Dose	Delivery Route	Days Administered	Cycle length
10 μg	IT	1, 8, 15	3 weeks
30 μg			
60 μg			
120 μg			
150 μg			

[0228] In accordance with various embodiments described herein, the AIR-ViP, e.g., VERI-101, is co-administered in a combination therapy regimen with one or more immune checkpoint inhibitor (ICI) substances (delivered to the subject/patient in separate pharmaceutical preparations, or in a single pharmaceutical combined preparation or composition). Some exemplary embodiments of useful immune checkpoint inhibitor(s) include, but are not limited to, pembrolizumab (KEYTRUDA®; Merck), nivolumab (OPDIVO®; Bristol-Myers Squibb), durvalumab (IMFINZI®; AstraZeneca), atezolizumab (TECENTRIQ®; Genentech), or a combination of any of these immune checkpoint inhibitors. Other examples of immune checkpoint inhibitors that are employed in certain embodiments described herein are ipilimumab (YERVOY®; Bristol-Myers Squibb) or a combination of nivolumab, a PD-1 blocking antibody, and relatlimab, a LAG-3 blocking antibody, indicated for unresectable or metastatic melanoma (OPDUALAG®; Bristol-Myers Squibb). Accordingly, the co-administration regimen followed in the inventive method is, in certain embodiments, optionally, a dosing schedule approved for the immune checkpoint inhibitor substance(s) that are employed, or a different dosing regimen as the skilled clinician may determine to be medically indicated.

[0229] As already noted above, in some embodiments, the one or more ICIs that are provided in combination with the IRCs function to inhibit the binding of one or more of (i) PD-1 to PD-L1; (ii) Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA-4) to B7-1 and/or to

B7-2, (iii) Lymphocyte Activation Gene-3 (LAG3) to its respective ligand(s), (iv) T-cell Immunoglobulin domain and Mucin domain 3 (TIM3) to its respective ligand(s), (v) TIGIT, to its respective ligand(s), and (vi) CD96 to its respective ligand(s). Other such ICIs include, but are not limited to, for instance, V-set domain-containing T-cell activation inhibitor 1 (VTVN1 or B7-H4), Cluster of Differentiation 276 (CD276 or B7-H3), B and T Lymphocyte Attenuator (BTLA), Galectin-9 (GAL9), Checkpoint kinase 1 (Chk1), Adenosine A2A receptor (A2AR), Indoleamine 2,3-dioxygenase (IDO), Killer-cell Immunoglobulin-like Receptor (KIR), and V-domain Ig suppressor of T cell activation (VISTA). In some embodiments, the ICI specifically binds to a cognate molecule selected from 4-1BB, GITR, CD40, CD40L, OX40, OX40L, CXCR2, B7-H3, B7-H4, BTLA, HVEM, and CD28.

[0230] In some embodiments, the one or more ICIs are antibodies or antigen-binding fragments thereof. In some embodiments, the one or more ICIs are one or more of pembrolizumab, nivolumab, durvalumab, atezolizumab, ipilimumab, and relatlimab.

[0231] That is, some of the noted ICIs require their cognate binding partners, or ligands, in order to functionally deliver their immune inhibitory activity. For example, A2AR is the receptor of adenosine A2A and binding of A2A to A2AR activates a negative immune feedback loop. As another example, PD-1 associates with its two ligands, PD-L1 and PD-L2, to down regulate the immune system by preventing the activation of T-cells. PD-1 promotes the programmed cell death of antigen specific T-cells in lymph nodes and simultaneously reduces programmed cell death of suppressor T cells, thus achieving its immune inhibitory function. As yet another example, CTLA4 is present on the surface of T cells, and when bound to its binding partner CD80 or CD86 on the surface of antigen-present cells (APCs), it transmits an inhibitory signal to T cells, thereby reduces the immune response.

[0232] Cancer cells are known to exploit the immune checkpoint proteins to escape being attacked by the immune system. Therefore, the use of immune checkpoint inhibitors to enhance an immune response against cancer, and thus treating cancer, have been described. In certain embodiments, the types of tumors that are treated and/or treatable via administration of the described compositions and methods of the present disclosure include, without limitation, premalignant neoplasms, malignant tumors, metastases, or any disease or disorder characterized by uncontrolled cell growth such that it would be considered cancerous or precancerous. The cancer is, in some embodiments, a primary or metastatic cancer. For further description and guidance, cancers contemplated as being treated utilizing the presently disclosed compositions and methods include, but are not limited to, ocular cancer, biliary tract cancer, bladder cancer, pleura cancer, stomach cancer, ovary cancer, meninges cancer, kidney

cancer, brain cancer including glioblastomas and medulloblastomas, breast cancer, cervical cancer, choriocarcinoma, colon cancer, endometrial cancer, esophageal cancer, gastric cancer, hematological neoplasms including acute lymphocytic and myelogenous leukemia, multiple myeloma, AIDS-associated leukemias and adult T-cell leukemia lymphoma, intraepithelial neoplasms including Bowen's disease and Paget's disease, liver cancer, lung cancer, lymphomas including Hodgkin's disease and lymphocytic lymphomas, neuroblastomas, oral cancer including squamous cell carcinoma, ovarian cancer including those arising from epithelial cells, stromal cells, germ cells and mesenchymal cells, pancreatic cancer, prostate cancer, rectal cancer, sarcomas including leiomyosarcoma, rhabdomyosarcoma, liposarcoma, fibrosarcoma, and osteosarcoma, skin cancer including melanoma, Kaposi's sarcoma, basocellular cancer, and squamous cell cancer, testicular cancer including germinal tumors such as seminoma, non-seminoma, teratomas, choriocarcinomas, stromal tumors and germ cell tumors, thyroid cancer including thyroid adenocarcinoma and medullar carcinoma, and renal cancer including adenocarcinoma and Wilms' tumor. Commonly encountered cancers include breast, prostate, lung, ovarian, colorectal, and brain cancer. In some embodiments, the tumor is a melanoma, carcinoma, sarcoma, or lymphoma.

[0233] "An effective amount" as used herein refers to the amount of each agent required to confer therapeutic effect on the subject, either alone or in combination with one or more other agents. Effective amounts vary, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, the individual subject parameters including age, physical condition, size, gender and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a subject may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

[0234] Empirical considerations, such as the half-life, generally will contribute to the determination of the dosage. For example, agents that are compatible with the human immune system, such as agents comprising regions from humanized antibodies or fully human antibodies, may be used to prolong half-life of the compound and to prevent the compound being attacked by the host's immune system. Frequency of administration may be determined

and adjusted over the course of therapy, and is generally, but not necessarily, based on treatment and/or suppression and/or amelioration and/or delay of a disease. Alternatively, sustained continuous release formulations of a compound may be appropriate. Various formulations and devices for achieving sustained release are known in the art.

[0235] The appropriate dosage of a therapeutic agent as described herein will depend on the specific agent (or compositions thereof) employed, the formulation and route of administration, the type and severity of the disease, whether the compound is administered for preventive or therapeutic purposes, previous therapy, the subject's clinical history and response to the antagonist, and the discretion of the attending physician, among other possible factors. Typically, the clinician, physician or doctor, will administer an agent until a dosage is reached *in vivo* that achieves the desired result. Administration of the described ICIs and IRCs are, in some embodiments, continuous or intermittent, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of an agent may be essentially continuous over a preselected period of time or may be in a series of spaced dose, e.g., either before, during, or after developing a disease.

[0236] In some embodiments, each therapy (each of the ICI and IRC) can result in an independent pharmaceutical effect, and together can result in an additive or synergistic pharmaceutical effect. As used herein, the term “synergistic” or exhibiting “synergism” means that the combined effect of the two molecules (ICI and IRC) when used in combination is greater than their additive effects when used individually. A way of calculating synergy is by means of the combination index. That is, the term “synergistic,” or “synergistic effect” or “synergism” as used herein, generally refers to an effect such that the one or more effects of the combination of compositions is greater than the one or more effects of each component alone, or they can be greater than the sum of the one or more effects of each component alone. The synergistic effect can be greater than about 10%, 20%, 30%, 50%, 75%, 100%, 110%, 120%, 150%, 200%, 250%, 350%, or 500% or more than the effect on a subject with one of the components alone, or the additive effects of each of the components when administered individually. The effect can be any of the measurable effects described herein.

[0237] Advantageously, such synergy between the agents when combined, may allow for the use of smaller doses of one or both agents, may provide greater efficacy at the same doses, and may prevent or delay the build-up of multi-drug resistance. The concept of the combination index (CI) has been described by Chou et al., *Adv. Enzyme Regul.*, 22:27-55, 1984. The synergistic effect may be attained by co-formulating the agents of the pharmaceutical

combination. The synergistic effect may be attained by administering two or more agents as separate formulations administered simultaneously or sequentially.

[0238] The compositions may, e.g., comprise one IRC molecule and one ICI molecule. In other embodiments, the composition comprises multiple different IRCs with different epitopes conjugated thereto and multiple different ICIs, to create the synergistic effect. In one embodiment the IRC is MPV.10.34.d. Any of the IRCs described herein are able to be combined with any of the ICIs described herein. That is, any of the IRCs conjugated to any of the epitopes or antigens described herein, using MPV.10.34.d as a backbone, for instance, are contemplated herein as being combinable with any of the known ICIs to create or generate the described synergistic effects in the treatment and amelioration of cancers or tumors.

[0239] All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties for all purposes.

[0240] While the methods, uses, and compositions described herein have been illustrated and described in detail in above, such illustration and description are to be considered illustrative or exemplary and not restrictive. It will be understood that changes and modifications may be made by those of ordinary skill within the scope and spirit of the following claims. In particular, the present disclosure covers further embodiments with any combination of features from different embodiments described above and below.

[0241] The present disclosure is additionally described by way of the following illustrative non-limiting examples that provide a better understanding of the present disclosure and of its many advantages. The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques used in the present disclosure to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the present disclosure.

EXAMPLES

Example 1

Production of Truncated Mouse papillomavirus (MPV1) L1 protein

[0242] The composition of the invention comprises, *inter alia*, an active agent, which is a virus-like particle (VLP) or capsid, or more particularly an IRC or ViP, comprised of sixty (60) copies of a truncated version of the mouse papillomavirus major capsid protein L1.

[0243] The truncated mouse papillomavirus L1 DNA sequence of 1138 base pairs was codon-optimized for *E. coli* expression and synthesized (SEQ ID NOS:135 and 136, two varieties of codon optimization) (GeneScript Biotech, Piscataway, NJ) and subsequently cloned into the T7 expression vector Pet-24a(+) (MilliporeSigma, Burlington, MA). The sequence was based on the wild type mouse (*Mus musculus*) papillomavirus L1 protein sequence except that it contains three deletion mutations at three specific regions: one deletion at the amino-terminus (10 amino acids removed), one at deletion the carboxy-terminus (34 amino acids deleted), and a third deletion in the helix four (H4) region close to the carboxy-terminal region (deletion of amino acids 411 to 436 of the MPV L1 sequence). This mutant MPV L1 protein is hereinafter referred to as "MPV.10.34.d." (See, Figure 1B).

[0244] The wild type mouse (*Mus musculus*) L1 wild type protein sequence is depicted in Figure 1A and has the following protein sequence (SEQ ID NO:132, NCBI Reference Sequence: YP_003778198.1, DNA: 9434943):

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

[0245] Likewise, the wild type nucleic acid sequence for MPV1 L1 protein (SEQ ID NO:133) is as follows:

ATGGCAATGTGGACACCCCAGACCGGGAAGCTTTACCTCCCACCTACAACTCCAGTGGCAAAAGTGCA
GAGCACAGACGAATATGTGTACCCTACGTCTCTCTTCTGTCATGCACACACGGACCGTTTGCTAACAG
TGGGCCACCCTTTTTTTTCTGTCATTGACAATGACAAGGTCACTGTGCCTAAAGTGTCTGGCAACCAA
TATAGGGTTTTCAGACTTAAATTCCCAGATCCAAATAAATTTGCATTGCCCCAAAAGGATTTCTATGA
TCCTGAGAAAGAACGGTTAGTGTGGAGGTTAAGGGGTCTGGAAATTGGAAGAGGTGGCCCATTAGGGA
TTGGCACTACCGGGCACCCCCTTTTTAACAAGCTTGGAGACACGGAAAATCCAAATAAATATCAGCAA
GGCTCTAAGGATAATAGGCAGAACACTTCCATGGACCCCAAACAAACACAGCTGTTTATTGTTGGCTG
TGAACCCCCTACAGGGGAACACTGGGATGTAGCTAAGCCCTGTGGAGCTCTGGAGAAGGGTGACTGCC
CTCCTATCCAACTTGTAAATAGTGTAATTGAGGATGGGGATATGTGTGACATTGGCTTTGGGAATATG
AACTTCAAAGAGCTGCAGCAGGATAGGAGTGGTGTGCCTCTTGATATTGTATCTACCCGGTGCAAATG
GCCCGACTTTCTGAAAATGACCAATGAGGCATATGGGGATAAGATGTTCTTCTTTGGAAGGAGAGAGC
AAGTGTATGCAAGACACTTTTTCACCAGGAATGGCTCTGTGGGGGAGCCCATACCAAACTCTGTGAGT
CCCAGTGACTTTTACTACGCACCCGACAGCACACAGGACCAGAAGACACTCGCACCCTCCGTGTACTT
TGGAACTCCTAGTGGGTCACTTGTGTCGAGTGATGGTCAGCTGTTTAACAGGCCATTTTGGCTTCAAA
GGGCTCAGGGAAACAATAATGGTGTGTGCTGGCACAATGAGCTCTTTGTTACTGTTGTCGACAACACA
AGGAATACAAACTTTACTATCTCCCAGCAAACCAACACACCAAACCCAGATACATATGACTCTACTAA
TTTTAAAAACTATTTAAGACATGTGGAACAATTTGAGCTGTCCCTTATTGCTCAACTGTGTAAGGTTC
CACTTGACCCGGGTGTGCTTGCCCATATAAACACTATGAACCCAACCATCTTGGAGAACTGGAACTTG
GGTTTTGTACCTCCCCCACAGCAGTCCATCTCTGATGACTATAGGTATATAACATCATCGGCAACTCG
CTGTCCAGATCAGAATCCGCCCAAGGAAAGAGAGGATCCTTACAAGGGTCTTATATTTTGGGAAGTTG
ATCTTACTGAGAGGTTTTCTCAGGACCTTGATCAGTTTGCTCTGGGACGAAAGTTTCTGTATCAAGCT
GGTATACGTACTGCTGTTACGGGCCGCGGGGTCAAAAGGGCAGCGTCTACAACCTCTGCGTCTTCTAG
ACGAGTTGTAAAACGGAAGAGGGGAAGCAAATAA

[0246] In contrast, the mutant MPV sequence selected for the following studies is depicted in Figure 1B and has the following amino acid sequence (SEQ ID NO:134):

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

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

[0247] Alignment of the wild type sequence with the triple truncation MPV.10.34.d sequence is shown in Figure 2. Additionally, the nucleic acid sequence (below) of MPV.10.34.d was optimized for expression. The sequence was optimized for codon usage within the target host as well as for expression level to maximize expression efficiency within the host. Below are provided two alternative optimized nucleic acid sequences for MPV.10.34.d used herein (SEQ ID NO:135):

ATGCTGTACCTGCCGCCGACCACCCCGGTGGCGAAAGTTCAGAGCACCGACGAATACGTTTATCCGAC
CAGCCTGTTCTGCCACGCGCACACCGATCGTCTGCTGACCGTGGGTCACCCGTTCTTTAGCGTTATCG
ACAACGATAAGGTGACCGTTCCGAAAGTGAGCGGCAACCAGTACCGTGTTTTTCGTCTGAAGTTCCCG
GACCCGAACAAATTTGCGCTGCCGCAAAAGGACTTCTATGATCCGGAGAAGGAACGTCTGGTGTGGCG
TCTGCGTGGTCTGGAAATTGGTCGTGGTGGCCCGCTGGGTATTGGTACCACCGGTCACCCGCTGTTCA
ACAAACTGGGCGATACCGAGAACCCGAACAAATATCAGCAAGGTAGCAAGGACAACCGTCAGAACACC
AGCATGGACCCGAAGCAGACCCAACTGTTTATTGTTGGTTGCGAGCCGCCGACCGGTGAACACTGGGA
TGTTGCGAAACCGTGCGGTGCGCTGGAAAAGGGCGATTGCCCGCCGATCCAACTGGTGAACAGCGTTA
TTGAGGACGGTGATATGTGCGACATCGGTTTTGGCAACATGAACTTCAAAGAACTGCAGCAAGACCGT
AGCGGCGTGCCGCTGGATATTGTTAGCACCCGTTGCAAATGGCCGGACTTCCTGAAGATGACCAACGA
AGCGTACGGTGATAAGATGTTCTTTTTCGGCCGTCGTGAGCAGGTTTATGCGCGTCACTTTTTCACCC
GTAACGGTAGCGTGGGCGAGCCGATCCCGAACAGCGTTAGCCCGAGCGACTTCTACTATGCGCCGGAC
AGCACCCAGGATCAAAAAACCCTGGCGCCGAGCGTGTACTTTGGTACCCCGAGCGGCAGCCTGGTTAG
CAGCGATGGTCAACTGTTTAACCGTCCGTTCTGGCTGCAGCGTGCGCAGGGTAACAACAACGGCGTGT
GCTGGCACAACGAACTGTTTGTTACCGTGGTTGACAACACCCGTAACACCAACTTCACCATCAGCCAG
CAAACCAACACCCCGAACCCGGACACCTACGATAGCACCAACTTTAAAAACTATCTGCGTCACGTGGA
GCAGTTCGAACTGAGCCTGATTGCGCAACTGTGCAAAGTGCCGCTGGACCCGGGTGTGCTGGCGCACA
TCAACACCATGAACCCGACCATTCTGGAGAACTGGAACCTGGGTTTCGTTCCGCCGAAAGAGCGTGAA
GACCCGTACAAGGGCCTGATCTTCTGGGAAGTGGATCTGACCGAACGTTTCAGCCAGGACCTGGATCA
ATTTGCGCTGGGCCGTAAATTCCTG TATCAGTAA

And (SEQ ID NO:136):

GAATTGGCGGAAGGCCGTCAAGGCCACGTGTCTTGTCCGCGGTACCCATATGCTGTATCTGCCTCCAA
CTACACCGGTTGCAAAAGTTCAGAGCACCGATGAATATGTTTATCCGACCAGCCTGTTTTGTCATGCA
CATACCGATCGTCTGCTGACCGTTGGTCATCCGTTTTTTAGCGTTATTGATAACGATAAAGTGACCGT
TCCGAAAGTTAGCGGTAATCAGTATCGTGTTTTTCGCCTGAAATTTCCGGATCCGAACAAATTTGCAC
TGCCGCAGAAAGATTTTTACGACCCGGAAAAAGAACGTCTGGTTTGGCGTCTGCGTGGTCTGGAAATT

GGTCGTGGTGGTCCGTTAGGTATTGGCACCACCGGTCATCCGCTGTTTAACAAACTGGGTGATACCGA
AAATCCGAATAAATACCAGCAGGGCAGCAAAGATAATCGTCAGAATACCAGTATGGATCCGAAACAGA
CCCAGCTGTTTATTGTTGGTTGTGAACCGCCTACCGGTGAACATTGGGATGTTGCAAAACCGTGTGGT
GCACTGGAAAAAGGTGATTGTCCGCCTATTCAGCTGGTTAATAGCGTGATTGAAGATGGTGATATGTG
CGATATTGGCTTTGGCAACATGAACTTTAAAGAACTGCAGCAGGATCGTAGCGGTGTTCCGCTGGATA
TTGTTAGCACCCGTTGTAAATGGCCTGATTTTCTGAAAATGACCAATGAAGCCTATGGCGACAAAATG
TTTTTTTTCGGTCGTCGTGAACAGGTTTATGCCCGTCACTTTTTTACCCGTAATGGTAGCGTTGGTGA
ACCGATTCCGAATAGCGTTAGCCCGAGCGATTTCTATTATGCACCGGATAGCACCCAGGATCAGAAAA
CCCTGGCACCGAGCGTTTATTTTGGCACCCCGAGCGGTAGCCTGGTTAGCAGTGATGGTCAGCTGTTC
AATCGTCCGTTTTGGCTGCAGCGTGCACAGGGTAATAACAATGGTGTTTGTTGGCATAACGAACTGTT
TGTTACCGTTGTTGATAATACCCGCAATACCAACTTTACCATTAGCCAGCAGACCAATACACCGAATC
CGGATACCTATGATAGCACCAACTTCAAAAACTATCTGCGTCATGTGGAACAGTTTGAACTGAGCCTG
ATTGCCCAGCTGTGTAAAGTGCCGCTGGATCCGGGTGTTCTGGCACATATTAACACCATGAATCCGAC
CATTCTGGAAAATTGGAATCTGGGTTTTGTTCCGCCTAAAGAACGTGAAGATCCGTATAAAGGTCTGA
TTTTTTGGGAAGTTGATCTGACCGAACGTTTTAGCCAGGATCTGGATCAGTTTGCACTGGGTCGCAAA
TTTCTGTATCAGTAACTCGAGGAGCTCGGAGCACAAGACTGGCCTCATGGGCCTTCCGCTCACTGCC

[0248] The general protocol for recombinant expression and purification of the mutant MPV.10.34.d is schematically depicted in Figure 3.

[0249] The MPV.10.34.d nucleic acid sequence was generated from wild type mouse papillomavirus sequence via site-mutagenesis (Genscript Biotech, Piscataway, NJ) using the following primer sequence (SEQ ID NO:137):

AAGCTTGTCGACGGAGCTCGAATTCGGATCCTTATTACTGATACAGGAAT
TTACGGCCCAGC//SEQ ID NO:137.

[0250] The MPV.10.34.d nucleic acid sequence was then cloned into the multicloning site of expression vector pet24a(+) (MilliporeSigma, Burlington, MA) using restriction endonucleases Ndel and BamH1 according to standard protocols. The correct cloning into the multiple cloning site and construct sequence was confirmed by both restriction endonuclease enzyme digestion using MIu1 and BamH1 as well as Sanger sequencing using both T7 forward and reverse primers.

[0251] Expression was achieved by transforming the pet24a(+) plasmid containing MPV.10.34.d into T7 expression competent *Escherichia coli* 2566 cells (New England Biolabs, Ipswich, MA, US), and colony selection on solid media. A single colony was grown according to standard protocols in Lurea broth (LB) media. Briefly, 5 mL sterile LB including 50 µg/mL kanamycin (Quality Biological, Gaithersburg, MD, US) was seeded with a single colony selected from the solid media and grown overnight at 37 °C with shaking. The seed culture was then diluted 1:25 and growth was continued at 37 °C until OD_{600} reached about 0.6 to 0.8. Then about 1 mM final concentration of isopropyl β- d-1-thiogalactopyranoside (IPTG, Invitrogen,

Carlsbad, CA, US) was added to the culture to induce expression from the plasmid. Induction was continued under these conditions for an additional four hours after which cell pellets are collected by centrifugation at 4000 x *g* for 15 minutes at 4 °C. The supernatant was discarded and the cell pellets were stored at -20 °C unless immediately used.

[0252] MPV.10.34.d was expressed as inclusion bodies (IBs). To recover IB MPV.10.34.d, pellets were first thawed (if frozen) and then resuspended in 20 mL per 1 L pellet lysis buffer (50 mM Tris, pH 8.0, 500 mM NaCl, 1 mM EDTA, 1 mM protease inhibitor phenylmethylsulfonyl fluoride (PMSF). Resuspended material was then homogenized using a high pressured homogenizer (Avestin Emulsiflex C3™, ATA Scientific, Taren Point, Australia) and cells were passed through the homogenizer and lysed 4 times at about 15,000 to 20,000 PSI. The lysed bacterial cells were then centrifuged at 25,000 x *g* at 4 °C for 20 min. Supernatant was then discarded and the inclusion body pellet was stored at -20 °C.

[0253] Next the IB were solubilized by resuspending the pellet (50 mL per 1 L pellet) in of 6 M urea buffer (8 M Urea, 50 mM Tris, pH 8.0, 500 mM NaCl, 1 mM EDTA, 1 mM PMSF, and 1 mM DTT). Resuspended contents were once more passaged three to four times through the homogenizer (Avestin Emulsiflex C3™, ATA Scientific, Taren Point, Australia) at about 15,000 to 20,000 PSI. The resolubilized samples were centrifuged at 25,000 x *g* at 4 °C for 20 min. The supernatant was collected into a container that is sufficiently large enough to hold the volume of a sample. The pellet was discarded. The supernatant was stored at 4 °C or -20 °C.

[0254] Following solubilization, the MPV.10.34.d was refolded by removal of the denaturant (6M Urea) in a step-gradient manner. The solubilized samples were inserted into dialysis tubing (snakeskin dialysis tubing, 10,000 Da molecular weight cut off, 35 mm. (ThermoFisher Scientific, Waltham, MA, US). In general, about 100 to about 150 mL of resolubilized sample solution was dispensed into a single dialysis tube. The samples were first dialyzed (sample to buffer ratio 1:12.5) against 4 M urea buffer (50 mM Tris, pH 8.0, 500 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mM DTT, and 0.05% Tween®-80) for 3 ± 1 hour in a cold room at about 4°C on a stir plate. Then, the samples were again dialyzed against a fresh 1 M urea buffer (50 mM Tris, pH 8.0, 500 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mM DTT, and 0.05% Tween-80) for 3 ± 1 hour in a cold room on a stir plate. Subsequently, the samples were dialyzed against 0 M urea buffer (50 mM Tris, pH 8.0, 500 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mM DTT, and 0.05% Tween-80) overnight (about 16 to 18 hours) in a cold room at about 4°C on a stir plate. The dialyzed / refolded sample solutions were aliquoted into 50 mL conical tubes and stored in a -20 °C freezer.

[0255] To obtain a MPV.10.34.d of greater than 95% purity for subsequent medicinal use, samples were subjected to a two-step chromatography purification which involves a capture step utilizing cation exchange chromatography (CEX) followed by a polishing step using a hydrophobic interaction column (HIC). For the capture step, the refolded MPV.10.34.d samples were removed from the -20 and thawed on ice. Next, the sample was dialyzed into capture buffer A (25 mM $NaPO_4$, 25 mM NaCl, pH 6.0). Following dialysis, samples were centrifuged 4000 x *g*, for about 10 min, at 4 °C and then filtered through a 0.22 µm polyethersulfone (PES) membrane. The refolded MPV.10.34.d protein was then captured by CEX (Fractogel® EMD S03- M, EMD Millipore, Burlington, MA, US) and then step eluted with 30% 25 mM $NaPO_4$, 1.5 M NaCl, pH 6.0. This resulted in purified refolded MPV.10.34.d of purity of at least 80%.

[0256] To further remove contaminants and increase purity of the MPV.10.34.d to above 95%, the CEX eluate was diluted with high-salt buffer to achieve loading conditions of 25 mM $NaPO_4$, 3 M NaCl, pH 6.0, and applied to HIC resin (butyl-S-Sepharose® Fast Flow, GE Healthcare Life Sciences/Fisher Scientific, Waltham, MA, US). The bound refolded MPV.10.34.d product was subjected to a pre-elution wash with 30% 25 mM $NaPO_4$, 25 mM NaCl, pH 6.0, and then eluted with a single step gradient of 70% 25 mM $NaPO_4$, 25 mM NaCl, pH 6.0. Greater than 95% purity MPV.10.34.d was stored in a -20 °C freezer in the elution buffer.

[0257] Greater than 95% purity MPV.10.34.d was confirmed via SDS-PAGE followed by Coomassie blue gel and silver staining. For Coomassie staining, gels were incubated in water to remove SDS-PAGE running buffer, then incubated for 5 minutes in SimplyBlue SafeStain (Novex, Carlsbad, CA). Gels were de-stained in water. (See photographs of gels in Figure 4). Silver staining was performed using a Pierce Silver stain kit (ThermoFisher Scientific, Rockford, IL) according to manufacturer's instructions. To estimate purity, the images of the gels were taken using the Bio-Rad Image Lab 6.01 software. Gel images were then uploaded into the software and the entire vertical lane containing the band of interest ("lane profile") was analyzed using the image analysis software. The specific total density of the band of the protein of interest was calculated by drawing a box or freehand shape around the band. Subsequently, the total density of the entire lane was measured in the same manner. After obtaining the measurements, the background density of a suitably matched area on the gel in each case was subtracted. This background-corrected density of the protein band by the background-corrected density of the whole lane was then multiplied by 100 to obtain the percent purity.

[0258] From this analysis and as seen in Figure 4, the main process steps described above provided incremental purification of the ~50 kDA MPV.10.34.d protein. Non-specific proteins above and below the 50 kDA band were significantly reduced across each of the purification steps, where lane 1 was a post-cell harvest sample, wash, and homogenization; lane 2 was a post-IB solubilization sample, lane 3 was a post-refolding sample, lane 4 was a post-capture chromatography via CEX sample, and lane 5 was a post-polishing step sample using HIC.

Example 2

Determination of MPV.10.34.d Structure and Size

[0259] DLS (dynamic light scattering) and TEM revealed that upon refolding MPV.10.34.d unexpectedly forms capsid backbones that are about 20 nm to 30 nm in diameter. To analyze the refolded MPV.10.34.d samples, the purified samples were first analyzed by DLS to obtain determine whether refolding of MPV.10.34.d occurred.

[0260] 60 µl of sample was placed in a 40 µL solvent-resistant micro-cuvette (ZEN0040, Malvern Panalytical, Waltham, MA) and the cell was subsequently placed into a Zetasizer Nano ZS Dynamic Light scattering instrument (Malvern Panalytical, Waltham, MA). This was a research-grade dynamic light scattering system for measurement of protein size, electrophoretic mobility of proteins, zeta potential of colloids and nanoparticles, and optionally the measurement of protein mobility, and microrheology of protein and polymer solutions. The high performance of the Zetasizer Nano ZS also enables the measurement of the molecular weight and second virial coefficient, A_2, of macromolecules and k_D, the DLS interaction parameter. The system can also be used in a flow configuration to operate as a size detector for SEC or FFF. Once in the machine, the sample was processed with the companion software (Zetasizer Nano Software, Malvern Panalytical). The program was set to read the sample for a total of 5 runs to generate two plots.

[0261] The two plots generated were determine capsid backbone size and structure, intensity (Figure 5A), and volume (Figure 5B). The intensity distribution provides the amount of light scattered by the particles in the different sized bins. The volume distribution demonstrates the total volume of particles in various sized bins. In other words, the intensity plot (Figure 5A) provides an assessment of the overall population sizes of MPV.10.34.d particles including host cell contaminants within the sample, whereas the volume plot (Figure 5B) determines the relative proportion of refolded protein with respect to other contaminants, i.e., host bacterial cell proteins.

[0262] Note that each individual plot line in the graphs of Figures 5A and 5B represent individual samples (five samples for each of A and B). If the volume curve was between about 10 to 15 nm (X-axis), the shell was determined to be a capsomer made up of 5 MPV.10.34.d units. For T=1 icosahedral capsid backbones made up of 60 MPV.10.34.d, the volume curve plot is about 20 to 30 nm. For T=7 icosahedral capsid backbones made up of 360 MPV.10.34.d, the volume curve plot was about 50 to 60 nm. As is readily apparent from the intensity graph (Figure 5A), there are two peaks, one lower at approximately 20 to 30 nm and one at approximately > 100 nm, whereas the volume graph (Figure 5B) shows a single size of about 20 nm to 30 nm. It was postulated that the two peaks on the intensity figure were attributable to the presence of distinct populations of capsid backbones that arose after refolding of the MPV.10.34.d into the capsid backbone since the samples at this stage had not undergone purification. However, the larger of the two peaks shown in the intensity plot constitute only a small portion of the sample, and a majority of the sample falls within the smaller peak. Hence, the DLS results show that a majority of the MPV.10.34.d are structures that are about 20 nm to 30 nm in size.

[0263] In DLS, information regarding the motion (diffusion) of submicron particles in a solution is extracted from the rate of scattering intensity fluctuations using a statistical technique called intensity autocorrelation. The mean particle size and distribution are calculated from the distribution of diffusion coefficients using the Stokes Einstein equation. Because the magnitude of the scattering intensity varies roughly with the 6^{th} power of the particle size, DLS is highly sensitive to the presence of small amounts of aggregates in a mixture of capsid backbones which is believed to be reflected in the intensity plot shown in Figure 5A.

[0264] TEM analysis was also employed to obtain further visual confirmation of the structure and size of the refolded proteins. Samples (10 µL) were adsorbed to glow discharged (EMS GloQube) carbon coated 400 mesh copper grids (EMS), by floatation for 2 min. Grids were quickly blotted and then rinsed in 3 drops (1 min each) of TBS. Grids were negatively stained in 2 consecutive drops of 1% uranyl acetate with tylose (1% UAT, double filtered, 0.22 µm filter), blotted then quickly aspirated to obtain a thin layer of stain covering the sample. Grids were imaged on a Phillips CM-120 TEM operating at 80 kV with an AMT XR80 CCD (8 megapixel).

[0265] TEM results revealed that MPV.10.34.d formed capsid backbones that had a markedly grooved appearance, with pentagonal/capsomer "towers." Measurements showed that these capsid backbones were approximate 30 nm is size.

[0266] These results were unexpected because deletion of residues in the helix four H4 region of L1 has been reported to not lead to T=1 geometry capsid backbone formation. Further, it has been shown that the same deletions result in capsomers of T=7 in HPV11 and HPV16 L1 proteins. (See, Chen et al., *Mol. Cell*, 5:557-567, 2000, WO 2000054730, Bishop et al., *Virol. J.*, 4:3, 2007, and Schädlich et al., *J. Virol.*, 83(15):7690-7705, 2009). In summary, these findings support the conclusion that the MPV.10.34.d constructs form icosahedral capsid backbones of T=1 lattice geometry comprised of 60 monomers, or 12 capsomers.

Example 3

Soluble and IB MPV.10.34.D Form T=1 Capsid Backbones

[0267] The MPV.A4 antibody is a conformational antibody that specifically binds to MPV L1 in the form of T=1 or T=7 capsid backbone structure. This antibody will not bind to denatured or monomeric MPV L1. (Hafenstein *et al.*, 2020, "Atomic Resolution CRYOEM structure of Mouse Papillomavirus," International Papillomavirus Conference, July 20-24, 2020). To determine whether MPV.10.34.d exhibits a T=1 capsid backbone, ELISA was performed on these samples with the MPV.A4 monoclonal antibody.

[0268] Samples of equal concentrations of MPV.10.34.d (starting concentration of 1000 ng/well) were subjected to ELISA. To ensure that both soluble and refolded MPV.10.34.d were equally bound to the ELISA plate (Nunc Maxisorp, ThermoFisher Scientific, Waltham, MA, US), both samples were first buffer exchanged into either 50 mM $NaPO_4$, 450 mM NaCl at pH 6 or pH 7. This resulted in two different pH conditions for both samples. Based on this, a total of four sample conditions were two-fold serially diluted and subjected to ELISA with the MPV.A4 monoclonal antibody.

[0269] Briefly, eight different amounts of protein (7.8 ng to 1 μg) for each sample under both pH conditions (into either 50 mM $NaPO_4$, 450 mM NaCl at pH 6 or pH 7) were first added to the ELISA plate and the plate was stored at 4 °C. Two days later, ELISA was performed by incubating each plate for one hour at room temperature on an orbital shaker (300 rpm) with MPV.A4 mAb diluted 1:1000 using blocking buffer (4% dry milk, 0.2% Tween-20) and the plates incubated for one hour at 4 °C. A wash step was then employed using wash buffer (0.35 M NaCl, 1.5 mM KH_2PO_4, 6.5 mM Na_2HPO_4, 0.05% Tween-20) at room temperature for a total of three washes (200 μL per sample per wash). Following the wash step, a goat anti-mouse IgG-HRP antibody (Millipore Sigma, St. Louis, MO, US) was added at 1:7000 dilution in blocking buffer (4% dry milk, 0.2% Tween-20) to a final concentration of 82.9 ng/mL and the plates incubated for one hour at room temperature on an orbital shaker (300 rpm). After the

incubation, the plate was washed and incubated with a peroxidase substrate (3, 3', 5, 5' tetramethyl benzidine, SeraCare Life Sciences, Inc., Milford, MA, US) for 30 minutes, followed by the addition and incubation of stop solution (0.36 N H_2SO_4) (J.T. Baker / Avantor, Allentown, PA, US) for 20 minutes. The absorbance of the sample plates were read at 450 nm and 620 nm with a plate reader (BioTek, Winooski, VT, US).

[0270] Results depicted in Figure 6 show that the undialyzed soluble MPV10.34.d capsid backbone, which are captured using buffer at pH 7 (solid circles), as well as the soluble form dialyzed against buffer at pH 7 (solid squares) and pH 6 (solid triangles) were recognized by the MPV.A4 monoclonal antibody.

[0271] In summary, MPV.10.34.d capsid backbones are recognized by the MPV.A4 conformational monoclonal antibody.

Example 4

IRC Formation: MPV.10.34.d Capsid Backbone Conjugation

[0272] To functionalize the MPV.10.34.d capsid backbones such that they are effective in recruiting preexisting immune system to attack cancer cells in the subject, the MPV.10.34.d capsid backbones were conjugated to various peptide epitopes including ovalbumin peptide SIINFEKL (OVA, SEQ ID NO:95), HPV16 E7 protein (SEQ ID NO: 96), and CMV peptide pp65 (SEQ ID NO:129) to form IRCs.

[0273] Design of Peptides: The peptides are epitopes having a general length of about 8 to 10 amino acids that are preceded upstream by a protease recognition site. (See, Figure 7). The following experiments incorporate an exemplary protease recognition site, the furin protease cleavage sequence R X R/K R (SEQ ID NO:89) which is designed to be located upstream of the epitope peptide. In addition, the epitope peptide is chemically modified at the N-terminus to contain maleimide. The incorporation of maleimide, a sulfhydryl reactive reagent, to the N-terminus of the peptide antigen allows for conjugation of the protease / peptide to the reduced sulfhydryl groups, i.e., cysteines, on the MPV.10.34.d capsid backbones. The end production of this reaction is a conjugated MPV.10.34.d capsid backbone.

[0274] To conjugate purified MPV.10.34.d capsid backbones of about > 95% purity, the MPV.10.34.d were further dialyzed in conjugation reaction buffer (50 mM $NaPO_4$, pH 6.5, 500 mM NaCl, 2 mM EDTA, and 0.05% Tween® 80), exchanging the buffer three times (3 ± 1 hours, 3 ± 1 hours, and overnight 16 ± 3 hours, at 2 °C to 8 °C). The next day, the MPV.10.34.d was adjusted to a final concentration of at least 0.6 µg/µL. The MPV.10.34.d were then treated with a mild reducing agent, tris(2-carboxyethyl)phosphine (TCEP), for 1 hour

without shaking at room temperature (21 °C) at a TCEP:MPV.10.34.d ratio of 10:1. Subsequently, the peptide was then added to the reaction at a molar ratio of X10 the amount of MPV.10.34.d. The reaction was shaken at room temperature (21 °C), 200 rpm, for 1 hour. Following conjugation, to remove excess free peptide, contents from the reaction were subjected to 10 rounds of Amicon spin filtration (molecular cut-off 100 kDa) at 1000 rcf for 10 mins each round. Following this purification step, samples were analyzed by SDS-PAGE stained with Coomassie Brilliant Blue R-250 dye (Bio-Rad, Hercules, CA, US) to determine percent conjugation (4 – 20% CRITERION™ TGX Stain-Free™ Precast Gels, 10 Well Comb, 30 µL, 1.0 mm, Bio-Rad, Hercules, CA, US). As seen in Figure 8, the upper band at about 50 kDa was determined to correspond to IRC. (See, Figure 8, lane 4). The lower bands represent the MPV.10.34.d lacking epitope peptide. These band identities were further confirmed via the conjugated control. (See, Figure 8, lane 3).

[0275] Importantly, the conjugation of MPV.10.34.d yielded a conjugation efficiency of about 50% as determined by densitometry. (See, Figure 8, lane 4). Percent conjugation was calculated by densitometry. The gel images were scanned into the computer using manufacturer-recommended software. Subsequently, the total density of the upper band and lower band was calculated by drawing a box or freehand shape around each band. The total density of these regions corresponds to the total amount of protein in each band. Next, only the upper lane was measured in the same way. Background density of a suitably matched area on each gel was collected and subtracted from the band signals. The background-corrected density of the upper protein band was then divided by the entire background-corrected density of the region of interest and then multiplied by 100 to obtain the percent conjugation.

Example 5

Binding of IRC to Tumor Cells via HSPG

[0276] To assess whether IRC bind to tumor cells, an *in vitro* cell binding assay was conducted. Specifically, both MPV.10.34.d capsid backbones (unconjugated) as well as different conjugated IRC (human CMV pp65, murine E7, and murine OVA peptide) were examined.

[0277] Briefly, 2 x 10^5 MC38 cells (murine colon adenocarcinoma, # ENH204-FP Kerafast, Inc., Boston MA) or pgsA-745 cells (Chinese hamster ovary cell mutant deficient in xylosyltransferase (UDP-D-xylose:serine-1,3-D-xylosyltransferase, ATCC CRL-2242) in which heparin sulfate proteoglycan (HSPG) expression is knocked out, were seeded overnight. The next day, the cells were treated with human CMV pp65, murine HPV16 E7, and murine

OVA peptide, as well as the MPV.10.34.d capsid backbone for one hour at 37 °C. Cells were then washed twice with 2 to 3 mL of a fluorescence activated cell sorting (FACS) buffer (1% bovine serum albumin in PBS) and then stained with 1 mL of rabbit anti-musPsV serum antibodies for 30 minutes at 4 °C. Following this, samples were washed once with 3 mL FACS buffer and stained with 0.5 mL of donkey anti-rabbit IgG-PE antibody (Biolegend, San Diego, CA) for 30 min at 4 °C in the dark. Finally, samples were washed once more with 3 mL FACS buffer and then resuspended in 250 mL of FACS buffer before being analyzed by a CytoFLEX flow cytometer (Beckman Coulter Life Sciences, Brea, CA, US).

[0278] As shown in Figure 9A and Figure 9B, all of the constructs, MPV.10.34.d – CMV pp65 IRC (solid line), MPV.10.34.d – E7 IRC (thick solid line), MPV.10.34.d – OVA IRC (thick dashed line), and MPV.10.34.d capsid backbones (dashed-line), exhibited specificity for tumor cells as evidences by the peak shifts to the right. The positive control in these experiments was MPV capsid backbone (wild type, dotted line). The negative control included no IRC or L1 (long-dashed line).

[0279] These experiments further show that the IRC exhibited HSPG-specific binding since no binding of MPV.10.34.d capsid backbones was observed in the cell line lacking HSPG expression (pgsA-745 cells, had no shift in the peaks). In summary, these results show that binding specificity of MPV.10.34.d capsid backbones for tumor cells is HSPG specific, and importantly, conjugation of epitope peptides to MPV.10.34.d capsid backbone does not reduce or otherwise negatively impact binding of MPV.10.34.d IRC to tumor cells *in vitro*.

Example 6

Loading of Peptide onto Tumor Cells by MPV.10.34.d IRC

[0280] The MPV.10.34.d IRC are designed such that upon entering the tumor microenvironment, the peptide will be cleaved from the IRC, thereby releasing the peptide in the near vicinity of a tumor cell surface. The cleavage event occurs, in some embodiments, upon contact with a tumor-specific protease, i.e., a protease present, in some embodiments at relatively higher concentrations than elsewhere in the subject's system, on or nearby a tumor cell. This cleavage event then is designed to result in the loading, or binding, of the peptide by MHC molecules expressed on the surface of tumor cells. The following experiments are designed to test this mode of operation and whether the designed IRC operate in the manner expected.

[0281] For this purpose, an MHC class I molecule loading assay was developed that directly detects peptide loading from IRC onto MHC class I molecules expressed on the surface

of tumor cells. This assay involves the use of an antibody that specifically recognizes an OVA peptide (SIINFEKL, SEQ ID NO:95) - MHC class I alloantigen H-2K^b molecule complex but not free peptide, empty MHC class 1 molecules, or peptides conjugated to the IRC. (See, Zhang et al., *Proc. Nat'l. Acad. Sci. USA*, 89:8403-84-7, 1992).

[0282] In this experiment, the OVA conjugated MPV.10.34.d IRC from Example 4 were examined side-by-side with OVA conjugated HPV16 IRC at equivalent molarity based on concentration of conjugated peptide. Briefly, 0.1 to 0.2 x 10^6 MC38 tumor cells (C57BL6 murine colon adenocarcinoma-derived cells, # ENH204-FP, Kerafast, Inc., Boston MA) were incubated with the IRC for one hour at 37 °C. A positive control including just free peptide and a negative control including no peptide or IRC were also tested. Cells were then washed twice with 2 to 3 mL FACS buffer and then stained with PE-conjugated-mouse anti-mouse MHC I bounded with OVA (SIINFEKL, SEQ ID NO:95) monoclonal antibody (Biolegend, San Diego, CA) for 30 minutes at 4 °C. Following this, samples were washed once with 3 mL FACS buffer then the cells were resuspended in 250 µL of FACS buffer before being analyzed by a CytoFLEX flow cytometer (Beckman Coulter Life Sciences, Brea, CA, US).

[0283] Results of these assays are provided in Figure 10. The results show that OVA-conjugated MPV.10.34.d IRC (1.4 µg/mL, thick solid line) and OVA-conjugated HPV16 IRC (2.5 µg/mL, solid line) demonstrated loading of epitopes on the surface of MC38 murine tumor cells, with the OVA-conjugated MPV.10.34.d IRC out-performing the OVA-conjugated HPV16 IRC. (See, Figure 10, negative control – long-dashed line, positive control – thin-dashed line). These results suggest that the MPV.10.34.d IRC is superior to the HPV16 IRC because a smaller amount of MPV.10.34.d IRC achieved the same, or better, "loading" potential of a larger amount of HPV16 IRC.

[0284] As OVA is a model antigen utilized for murine MHCs, this experiment was repeated substituting the CMV pp65 peptide for the OVA peptide. The HLA-A*0201 restricted epitope NLVPMVATV (NLV, SEQ ID NO:138) from the CMV pp65 was used for these studies and the pp65-conjugated MPV.10.34.d IRC were produced as described. As there was no commercially available monoclonal antibody that recognizes an MHC class I – NLV complex, a soluble T-cell receptor antibody (2S16) was employed that recognizes this HLA-A2 complex. (See, Wagner et al., *J. Biol. Chem.*, 295(15):5790-5804, 2019). The IRC constructs were analyzed in the same manner as above except that the cell lines HCT116 (human colorectal carcinoma cell line, HCT 116, ATCC, CCL-247) and MCF7 (human breast cancer cell line, MCF7, ATCC, HTB-22) were utilized in this study. These cell lines are HLA-

A*0201 restricted and thus are able to present the HLA-A*0201 restricted epitope NLVPMVATV (NLV, SEQ ID NO:138) from the CMV pp65 peptide.

[0285] Consistent with the OVA MHC class I loading results, loading of the NLV peptide onto human tumor cells was observed. Results are presented in Figure 11A (HCT116 cells) and Figure 11B (MCF7 cells). In Figure 11A and Figure 11B, an unrelated hepatitis B peptide was used as a negative control (thin dashed line, 10 μg/mL), unconjugated MPV.10.34.d capsid backbones were used as a further negative control (thin solid line, 100 μg/mL), CMV conjugated MPV.10.34.d IRC is represented as a thick solid line (100 μg/mL, at about 1.7 μg / mL of hCMV peptide conjugated), hCMV free peptide is represented as a thick dashed line (1 μg / mL).

[0286] Figures 10 and 11 show that incubation of the MPV.10.34.d IRC *in vitro* with the indicated cell lines leads to release of the peptide and binding to the tumor cell surface MHC Class 1 molecules. To further demonstrate that the mechanism of the IRC first involves the MPV.10.34.d IRC binding to the tumor cell followed by furin cleavage, competitive inhibition experiments were performed that either inhibited tumor cell binding or furin cleavage to show that ablation of either step results in an absence of peptide loading onto the tumor cells. These studies were performed with the OVA-conjugated MPV.10.34.d IRC and conducted under the same conditions as the binding assays described above.

[0287] To block tumor binding, soluble heparin (Sigma Aldrich, St. Louis, MO) at 1 mg/mL, 5 mg/mL, or 10 mg/mL was incubated with 2.5 μg/mL of OVA-conjugated MPV.10.34.d IRC for 1 hour at 37 °C, 5% CO_2 in the presence of 2 x 10^5 MC38 cells in a FACs tube. The final volume of the cells with the sample was 200 μL. A positive control sample was included which contained no soluble heparin as well as a negative control that contained no IRC or heparin. Cells were then washed twice with 2 to 3 mL FACS buffer and then stained with PE-conjugated-mouse anti-mouse MHC I bound to the OVA peptide monoclonal antibody (this monoclonal antibody is able to specifically detect OVA peptide, SIINFEKL, SEQ ID NO:95, in complex with MHC-I K^b) for 30 minutes at 4 °C. Following this, samples were washed once with 3 mL FACS buffer, then the cells were resuspended in 250 μL of FACS buffer before being analyzed by a CytoFLEX flow cytometer (Beckman Coulter Life Sciences, Brea, CA, US). As seen in Figures 12A, 12B, and 12C, no OVA peptide loading was observed in the negative control (thin black line in Figures 12A, 12B, and 12C). No OVA peptide loading was observed in samples including 10 mg/ml (dashed line, Figure 12A), 5 mg/ml (dashed line, Figure 12B), or 1 mg/ml (dashed line, Figure 12C) soluble heparin (these curves overlapped with the negative control data). Loading of OVA peptide was only

detected in the samples containing OVA-conjugated MPV.10.34.d IRC with no heparin (thick black line to the right, Figures 12A, 12B, and 12C). These results show that OVA-conjugated MPV.10.34.d IRC is HSPG-specific.

[0288] To show that loading of peptide from IRC onto tumor cells is dependent on protease cleavage of the epitope peptide form the MPV.10.34.d IRC, the experiments of Example 10 were repeated in the presence of a furin inhibitor, furin inhibitor I – Calbiochem, decanoyl-RVKR-CMKa, peptidyl chloromethylketone. (Millipore-Sigma, St. Louis, MO). This furin inhibitor binds irreversibly to the catalytic site of furin, blocking all furin protease activity.

[0289] Briefly, 2 x 10^5 MC38 cells (murine colon adenocarcinoma, # ENH204-FP, Kerafast, Inc., Boston MA) were seeded in a FACs tube and then incubated with either 0.5 μM, 5 μM, or 50 μM furin inhibitor dissolved in DMSO at total final sample volume of 200 μL. Control samples containing no inhibitor were prepared the same way with the same volume equivalent of DMSO. The samples were incubated for fifteen minutes in a tissue culture incubator at 37 °C, 5% CO_2. Then, 2.5 μg/mL of OVA-conjugated MPV.10.34.d IRC was added to all samples and the samples were incubated in a tissue culture incubator at 37 °C , 5% CO_2. Samples were then washed twice with 2 to 3 mL FACS buffer and then stained with PE-conjugated-mouse anti-mouse MHC I bounded with OVA (SIINFEKL, SEQ ID NO:95) monoclonal antibody (Biolegend, Cat# 141604, San Diego, CA) for 30 minutes at 4 °C. Following this, samples were washed once with 3 mL FACS buffer then the cells were resuspended in 250 μL of FACS buffer before being analyzed by a CytoFLEX flow cytometer. (Beckman Coulter Life Sciences, Brea, CA, US).

[0290] OVA-conjugated MPV.10.34.d IRC loaded OVA peptide onto tumor cells in the samples that had no inhibitor added, and samples treated only with DMSO and no furin inhibitor. In contrast, no OVA peptide loading was observed in the negative control (data not shown) as well as samples treated with furin inhibitor at 50 μM, 5 μM, and 0.5 μM. Therefore, inhibition of furin cleavage of the epitope peptide from the IRC prevented binding of OVA to the MHC molecules of the target cancer cell, thereby confirming the mechanism of action of the IRC. This mechanism is further confirmed by, and consistent with, the results shown in Figure 9, Figure 10, Figure 11, and Figure 12. To assess whether MHC-I loading occurs at the cell surface, cells deficient in intracellular MHC-I processing (RMA-S) were incubated with OVA-conjugated MPV.10.34.d. In RMA-S cells, any MHC that makes its way to the surface of the cell has no peptide, is unstable and is quickly endocytosed. After OVA-conjugated

MPV.10.34.d incubation, loading was still detected in a dose-dependent manner, indicating that loading occurs without the need for intracellular trafficking. (See, Figure 20).

Example 7

***In Vitro* Cytotoxic Killing Assays with MPV.10.34.d IRC**

[0291] Since it was shown in Example 6, that *in vitro* MPV.10.34.d IRC were able to deposit peptide epitopes onto murine and human MHC Class I molecules and that this mechanism was dependent on furin activity, additional experiments were designed to determine whether labelling these cancer cells would trigger activation and redirection of cellular immune system components against target tumor cells. Upon activation and redirection, the goal is delivery of a cytotoxic signal to the tumor cells and tumor cell death. For this purpose, three different *in vitro* cytotoxic T-cell-dependent tumor cell killing assays were designed involving the co-culture of tumor cells and viral antigen-specific $CD8^+$ T cells in the presence or absence of MPV.10.34.d IRC. The three CD8 T-cells were tested, including: (1) murine OVA-specific preclinical CD8+ T cells, (2) murine HPV16 E7-specific $CD8^+$ T cells, and (3) human HLA-A*0201-restricted CMV-specific T cells (Astarte Biologicals, cat#1049-4367JY19).

[0292] Murine B16 (melanoma/skin) (B16-F10 (ATCC® CRL-6475™), and murine ID8 (ovarian) tumor cells (Hung et al., *Gene Ther.*, 14(12):921-020, 2007) overexpressing luciferase gene (B16-luc and ID8-luc) were grown in culture. Under normal circumstances, murine tumor cell lines B16 and ID8 will not be killed by murine OVA-specific CD8+ T cells since these cell lines do not express the murine OVA (SIINFIKEL, SEQ ID NO: 95) antigen.

[0293] Approximately 0.01 x 10^6 B16-luciferase mutant (B16-luc) or 0.005 x 10^6 ID8-luciferase mutant (ID8-luc) tumor cells were seeded in 100 µL per well on a 96-well assay plate overnight. The cells were then treated with 100 µL of 2.5 µg/mL of MPV.10.34.d capsid backbones, OVA-conjugated MPV.10.34.d IRC, OVA-conjugated HPV16 IRC, and positive control containing 1 µg/mL of free OVA peptide (SIINFIKEL, SEQ ID NO:95), for one hour at 37 °C in a final volume of 200 µL per well. Cells not receiving any antigen were included as negative control (No Ag). The cells were then washed twice with 200 µL of Roswell Park Memorial Institute (RPMI) media and co-incubated with OVA-specific CD8+ T-cells (Jackson, stock no. 003831) at an effector (CD8+T-cell) to target cell (tumor cell) ratio ("E:T Ratio") of 10:1 (B16-luc) or 20:1 (ID8-luc) for 16 hours in a final volume of 200 µL per well in a cell incubator at 37 °C, 5% CO_2. An E:T ratio of 10:1 means that for every 1 tumor cell, ten CD8+ OT-1 T cells will be co-incubated with the tumor cell. These co-incubated cells were

then washed with 200 µL of PBS and lysed with 35 µL of 1X cell lysis buffer (Promega, Madison, WI, US) for 15 to 20 minutes before adding 50 µL of luciferase assay substrate and detected on a Promega GloMax Explorer Microplate Reader (Promega, Madison, WI, US).

[0294] The number of viable tumor cells after co-incubation with T cells were measured by quantification of concentration of luciferase released from lysed cells. This acts as a surrogate marker for cell viability since the target cells were incubated under conditions in which they over-express luciferase. Reduced luciferase activity indicated more cell death suggesting greater immune redirection and hence greater cytotoxicity.

[0295] As shown in the bar graphs of Figure 13A and Figure 13B, the OVA-conjugated MPV.10.34.d IRC, OVA-conjugated HPV16 IRC, and the peptide positive control showed much higher tumor cell cytotoxicity (> 70%) than the negative control samples in both B16-luc (Figure 13A) and ID8-luc (Figure 13B) tumor killing assays. At the same concentration (2.5 µg/mL), OVA-conjugated MPV.10.34.d IRC also showed similar high cytotoxicity on tumor cells as the OVA-conjugated HPV16 IRC.

[0296] Similar to the above experiment, a second experiment was performed in like manner, except with the substitution of the E7 peptide (RAHYNIVTF, SEQ ID NO:96) for the OVA peptide. The same tumor cells and samples were investigated in this experiment using the same protocol.

[0297] As shown in the bar graphs of Figure 14A and Figure 14B, the E7-conjugated MPV.10.34.d IRC, E7-conjugated HPV16 IRC, and the positive control showed much higher tumor cell cytotoxicity (>70%) than the negative control groups in both B16-luc (Figure 14A) and ID8-luc (Figure 14B) tumor killing assays. At the same concentration (2.5 µg/mL), OVA-conjugated MPV.10.34.d IRC also showed similar high cytotoxicity on tumor cells as the OVA-conjugated HPV16 IRC.

Example 8

MPV.10.34.d IRC Effectiveness in Human Assays

[0298] While the *in vitro* functional test results of the above experiments were promising, the next desired step in the analysis was to perform similar experiments in human-based assays. To this end, the response of mock human cellular immune system components to tumor cells exposed to MPV.10.34.d IRC was examined *in vitro*. Human CMV (HCMV or hCMV) was selected for this study since human CMV is highly prevalent (infecting 50-90% of the human population) and mostly asymptomatic in healthy individuals. (See, Longmate et al., *Immunogenetics*, 52(3-4):165-73, 2001; Pardieck et al., *F1000Res*, 7, 2018; and van den

Berg et al., *Med. Microbiol. Immunol.*, 208(3-4):365-373, 2019). Importantly, HCMV establishes a life-long persistent infection that requires long-lived cellular immunity to prevent disease. Hence, it is rational to hypothesize that a complex adaptive cell-mediated anti-viral immunity developed over many years to strongly control a viral infection in an aging person can be repurposed and harnessed to treat cancer.

[0299] In these experiments, CD8+ T cell responses to CMV peptides were tested in three different human tumor cell lines, including HCT116, OVCAR3, and MCF7. All three of these human tumor cell lines are HLA-A*0201 positive.

[0300] ***In vitro*** **cytotoxicity assays.** HTC112, human colon cancer cells, MCF7, human breast cancer cells, and OVCAR3, human ovarian cancer cells (all from ATCC, Manassas, VA, US) were seeded overnight at 0.01 to 0. 2 x 10^6 per well per 100 µL per 96 well plate. The next day (about 20 to 22 hrs later), each cell line was incubated for one hour at 37 °C under the following conditions: (1) CMV peptide at a final concentration of 1 µg/mL (positive control), (2) MPV.10.34.d at a final concentration of 2.5 µg/mL (negative control), (3) CMV-conjugated MPV.10.34.d IRC at a final concentration of 2.5 µg/mL, (4) CMV-conjugated HPV16 IRC at a final concentration of 2.5 µg/mL, and (5) no antigen (negative control). After 1 hour, the cells were washed vigorously with 200 µL of media for three times to remove non-specific binding. Human patient donor CMV T cells (ASTARTE Biologics, Seattle, WA, US) were added at the E:T (effector cell:target cell) ratio of 10:1 and incubated in a tissue culture incubator for 24 hrs at 37C, 5% CO_2. The total final volume of each sample after co-culture was 200 µL. Cell viability was measured after co-culturing. Cell viability was measured with CELLTITER-GLO® (Promega, Madison, WI, US). This assay provides a luciferase-expressing chemical probe that detects and binds to ATP, a marker of cell viability. The amount of ATP generated from tumor cells was quantified according to manufacturer protocols. In these assays, reduced luciferase activity indicates cell death and suggests greater immune redirection and greater cytotoxicity.

[0301] The results are provided in Figure 15. CMV-conjugated MPV.10.34.d IRC ("VERI-101" in Figures 15A, 15B, and 15C) was equally effective as CMV-conjugated HPV16 IRC ("CMV AIR-VLP" in Figures 15A, 15B, and 15C) in redirecting human healthy donor CMV pp65-specific CD8+ T-cells (Astarte Biologics, Inc., Bothell, WA, US) to kill immortalized HLA.A2 positive human colon cancer cells (HCT116), human ovarian cancer cells (OVCAR3), and human breast cancer cells (MCF7). The control samples ("No Ag" or "VERI-000" in Figures 15A, 15B, and 15C) showed no background tumor killing. Together,

these data demonstrate that MPV.10.34.d IRC redirects mouse and human immune responses against tumor cells *in vitro*.

Example 9

Sequential Mechanism of MPV.10.34.d IRC Binding and Peptide Cleavage

[0302] Example 6 demonstrates that MPV.10.34.d IRC binding must occur prior to furin-dependent cleavage of the peptide and peptide loading onto target tumor cells. A dose-response curve using different concentrations of OVA-conjugated MPV.10.34.d IRC to detect binding and loading in separate assays was generated. These assays were performed as described in Example 5. Based on the geometric MFIs from both assays, a correlation analysis was conducted.

[0303] The results shown in Figure 16 indicate that there is a highly statistically significant correlation between the number of OVA-conjugated MPV.10.34.d IRC binding to tumor cells with the level of the OVA peptide / K^b complex on the tumor cells (Spearman r = 0.92, P=0.0003; Pearson r = 0.98, $p < 0.001$). This statistical analysis further demonstrates the requirement for the sequential steps of OVA-conjugated MPV.10.34.d IRC to first bind or contact the tumor cell, followed by furin-dependent cleavage of the peptide from the IRC, and MHC loading of the peptide.

Example 10

Sequential Mechanism of MPV.10.34.d IRC Binding and Tumor Cell Death

[0304] Example 6 shows that inhibition of OVA-conjugated MPV.10.34.d IRC binding results in inhibition of furin-dependent cleavage of the peptide from the IRC and OVA peptide loading onto tumor cell surfaces. To further show that inhibition of this binding step also inhibits redirection of CD8+ T-cells and tumor cell death, cytotoxicity studies conducted as in Example 7 were performed in the presence and absence of soluble heparin, a competitor of HSPG binding.

[0305] A range of OVA-conjugated MPV.10.34.d IRC concentrations (0.156 μg/mL to 0.625 μg/mL) as well as E:T ratios (1:4.5, 1:9 and 1:18) were investigated in the presence and absence of 10 mg/mL of soluble heparin in the assays described earlier. This concentration of soluble heparin was previously shown to cause complete inhibition of OVA-conjugated MPV.10.34.d IRC binding, as well as inhibition of peptide loading onto tumor cells. In these assays, 15,000 TC-1 cells overexpressing luciferase were first seeded in a flat-bottom 96-well

plate overnight in a cell culture incubator at 37 °C, 5% CO_2. The next day, cells were washed 3 times with PBS before being incubated with AIM-V media (serum free) with 2% BSA for 1.5 hours in a cell culture incubator at 37 °C, 5% CO_2. In parallel, OVA-conjugated MPV.10.34.d IRC was diluted in the same AIM-V media + 2% BSA into 0.625, 0.3125, 0.156 µg/mL. (See, Figures 17A, 17B, and 17C). Each sample was incubated with (thick dash lines) or without (solid line) 10 mg/mL of soluble heparin for 1 hour at 2 °C to 8 °C. MPV.10.34.d capsid backbones (thin dash line, "ViP" only) was included as a negative control. After 1 hour, the samples were added to the TC-1 cells and co-incubated for a further 30 minutes in a cell culture incubator at 37 °C, 5% CO_2. Following this, treated cells were washed 3 times with just AIM-V media before being incubated with OT-1 T-cells at an effector to target (E:T) ratio of 18:1, 9:1, or 4.5:1. This co-culture was then incubated for a further 3 hours at 37 °C, 5% CO_2. After 3 hours, target cells were analyzed for cytotoxicity using the Promega Luciferase Assay system as per the manufacturer's protocol (Promega, Madison, WI, US). Cytotoxicity was determined by detection of loss of luciferase signal which is used as a surrogate marker of cell viability in this assay. All studies were performed in triplicate.

[0306] Results are shown in Figure 17. The presence of soluble heparin exhibited no OVA-conjugated MPV.10.34.d IRC-mediated cytotoxicity under all concentrations and E:T ratio conditions tested. These results further substantiate the sequential nature of the MPV.10.34.d IRC mechanism of action.

[0307] A correlation analysis was conducted on the binding and cytotoxicity activities of OVA-conjugated MPV.10.34.d IRC. Briefly, a dose-response curve using different concentrations of OVA-conjugated MPV.10.34.d IRC to detect binding and cytotoxicity in separate assays was generated. Cytotoxicity assays were conducted as previously described, above, with the following changes: a range of 6.25 x 10^{-5} µg/mL to 2.5 µg/mL of OVA-conjugated MPV.10.34.d IRC was tested at 3 different E:T ratios (18:1, 9:1, and 4.5:1).

[0308] Under all 3 E:T ratio conditions tested, a dose dependent killing was observed with OVA-conjugated MPV.10.34.d IRC concentrations below 0.04 µg/mL and higher, whereas concentrations of OVA-conjugated MPV.10.34.d IRC between 0.156 µg/mL to 2.5 µg/mL lead to a maximal level of cytotoxicity. Binding assays were conducted according to the protocols described previously with the following changes: a concentration range of 6.24 x 10^{-4} to 2.5µg/mL of OVA-conjugated MPV.10.34.d IRC was investigated.

[0309] Results show that a dose-dependent binding was observed and that the limit of binding detection was reached at 2.5 x 10^{-4} µg/mL. Both assays were repeated twice (with at

least 3 replicates). The mean values of geometric mean fluorescent intensity (MFI) was reported from the two experiments and is summarized in the Table provided in Figure 18.

[0310] Based on the MFIs from both assays (Figure 18), a graphical and correlation analysis was conducted using Spearman correlational analysis (Figure 19). Briefly, the mean of the percentages of two independent OVA-conjugated MPV.10.34.d IRC cytotoxicity assays performed on two different days and the mean of MFIs of OVA-conjugated MPV.10.34.d IRC binding experiments from two different days were calculated (Figure 18) and plotted (Figure 19). Spearman correlational analysis was performed on these results reveals a significant relationship (r = 0.83 to 0.9) between these two variables at all three E:T ratios. These results show that there is a highly statistically significant correlation (r value between 0.83 to 0.9, depending on E:T ratio) between the OVA-conjugated MPV.10.34.d IRC binding to tumor cells and the level of cytotoxicity that followed.

Example 11

Anti-Tumor Activity Of The AIR-VLP (Or "AIR-ViP") Approach In A MCMV Model

[0311] An *in vivo* efficacy study was conducted to demonstrate anti-tumor therapeutic activity of the present invention, e.g., using the CMV-conjugated MPV.10.34.d IRC described herein ("VERI-101"). Due to the species-specific nature of hCMV (human cytomegalovirus), the proposed *in vivo* model used in the study described above utilized a murine surrogate model of CMV (mCMV, murine cytomegalovirus) that accurately recapitulates the hallmarks of latent hCMV infection and associated long-term, functional T-cell responses. (See, Munks et al., *J. Immunol.*, 177(1):450-8, 2006; Brune et al., *Curr. Protoc. Immunol.*, 2001; Chapter 19:Unit 19.7; Smith et al., *PLoS Pathog.*, 10(7):e1004233, 2014).

[0312] This model specifically evaluated whether anti-CMV T-cell responses could be harnessed by AIR-VLPs (or AIR-ViPs) and can be redirected to kill tumors resulting in tumor regression, activation of a pro-inflammatory tumor immune microenvironment, and potentially, elicit adaptive antitumor immunity to epitopes not included in the treatment, i.e., antigen spreading.

[0313] To evaluate the anti-tumor activity of the AIR-VLP approach in the mCMV model, we decided to utilize a surrogate mouse-specific CMV product that contains the most relevant mCMV antigenic peptide (M38). (See, e.g., Jurak et al., *J. Virol.*, 82(10):4812–4822, 2008). The surrogate AIR-VLP product we used in the study was designated VERI-003 and is identical to the hCMV-AIR VERI-101 human-specific product except for the conjugated viral

epitope only, which for VERI-003 is MCMV m38 epitope, amino acids 316-323, i.e., SSPPMFRV (SEQ ID NO:97). The choice of the M38 epitope for VERI-003 over other mCMV antigens is due to findings that $CD8^+$ T-cell memory responses to this epitope become more prominent during chronic mCMV infection. (See, Munks et al. and Smith et al.). This phenomenon is known as memory inflation and, importantly, is also seen with the NLV antigen in hCMV infections. (*Id.*).

[0314] Therefore, the use of the M38-conjugated surrogate product VERI-003 closely mimics the expected immune response to the NLV antigen in the VERI-101 clinical product. To ensure consistency with the use of the *in vivo* model as described in Munks et al. (2006) and Smith et al. (2014), we further conducted a mCMV model development study to generate pre-existing mCMV immunity in mice with MC38 and TC-1 tumors. For this study, C57/Bl6 mice were infected with a sub-lethal dose of mCMV (1×10^4 PFU) and their $CD8^+$ T-cell response to the inflationary M38 and another mCMV epitope which is non-inflationary, M45, was measured in the blood over 60 days. The mice were then implanted with MC38 and TC-1 tumors which were harvested upon reaching an average of about 100 to 200 mm^3 in size (Figure 21B) and analyzed for the presence of tumor infiltrating M38 $CD8^+$ T cells. A mCMV dose of 1×10^4 PFU led to a high and sustained M38 $CD8^+$ T-cell memory response in the blood even after 60 days post-infection (Figure 21A) and memory M38 $CD8^+$ T cells were found in both the blood and tumors at the time of harvesting (Figure 21C and Figure 21D). These data are consistent with published literature and support our use of the mCMV model of immune memory. (See, Munks et al. and Smith et al.; Çuburu et al., *Proc. Natl. Acad. Sci. USA*, 119(26):e2116738119, 2022).

[0315] VERI-003 anti-tumor efficacy was tested against MC38 and TC-1 tumor types for our *in vivo* efficacy studies. A brief description of these tumor types follows:

[0316] MC38 murine colon adenocarcinoma: MC38 was derived from a Grade-III adenocarcinoma that was chemically induced in a female C57BL/6 mouse strain and used as a transplantable mouse tumor model. Several studies have shown that this murine tumor is immunogenic and can be used as a model to investigate anticancer immunity and immunotherapies. (See, e.g., Efremova et al., *Nat. Commun.*, 9(1):32, 2018). Importantly, further characterization of the genomic and transcriptomic landscape revealed that it is hypermutated and serves as a valid murine model for hypermutated or micro-satellite instability colorectal cancer. MC38 briefly responds to checkpoint immunotherapy and thus will serve as a tumor model for VERI-003 to test against checkpoint inhibitor-treated tumors that subsequently experience de novo resistance. (See, Efremova *et al.* (2018), *Ibid.*).

[0317] TC-1 immortalized and transformed primary lung epithelial cell line: Primary lung epithelial cells from C57B1J6 mice were immortalized by HPV-16 E6 and E7 and then transformed with an activated *ras* oncogene. The co-transformation produced a tumorigenic cell line expressing E6 and E7. This cell line mimics the natural sequence of tumor progression of cervical cancer in which HPV-l6 E6 and E7 immortalizes cells and additional mutations transform the cells into advanced tumor cells with metastatic potential. This tumor model is a well understood model for HPV-associated tumors, including cervical cancer which is checkpoint inhibitor refractory (as opposed to colorectal carcinomas) and will serve as a "ICI-resistant" model for AIR-ViP efficacy where checkpoint inhibitors fail.

[0318] Based on the rationale and data outlined above, the infectious mCMV model, surrogate product VERI-003 targeting the M38 inflationary response, and the selected murine tumor types are all appropriate models for our surrogate efficacy studies for the VERI-101 clinical product.

[0319] The goal of the pilot efficacy study was to demonstrate anti-tumor activity using VERI-003 whereby the treatment with VERI-003 provides statistically significant prolonged survival of treated mice bearing TC-1 or MC38 tumors when compared to control groups.

[0320] In this study, an initial dose escalation of 3 dose levels (0.5 µg, 5 µg, and 50 µg) of the murine surrogate product, VERI-003, was tested in tumor bearing mice possessing pre-existing mCMV immunity. This initial dose range was chosen to assess the range effect of 10 ng to 1000 ng of peptide being injected into the tumors.

[0321] Our calculations showed that 0.5 µg of VERI-003 contains approximately 9 ng of conjugated peptide and corresponds to the lower limit of the study. Similarly, 50 µg of VERI-003 contains approximately 900 ng of peptide which corresponds to the higher end of this dose range. This dosing frequency was intended to mimic the dosing frequency in the clinic, and the number of administrations represents a single cycle of treatment in the clinical trial (3 weekly administrations) with one additional administration to support the potential inclusion of a 4th dose in a cycle, as part of the adaptive elements in the clinical study. A recognized limitation of murine tumor models is that tumors are very aggressive and grow quickly, as such, the frequency here is every 48 hours as opposed to weekly administrations in the clinic.

[0322] Combination treatment of VERI-003 and an anti-mouse PD-1 inhibitor antibody (200 µg of InVivoMAb anti-mouse PD-1 CD279, Clone: RMP1-14; Bio X Cell) were also tested to assess for synergistic effects.

[0323] The overall study design for the pilot study disclosed herein is detailed in the synopsis below (Table 6).

TABLE 6

VERI-003 Pilot Efficacy Study Design Synopsis

Study Title:	***In vivo* pilot efficacy study to demonstrate anti-tumor efficacy with VERI-003 via intra-tumoral administration.**
Objective(s):	Demonstrate *in vivo* efficacy of VERI-003 in the surrogate CMV immunity tumor model.
Animals:	C57/BL6 mice Number of animals= 200 mice (all females)
Administration:	For mCMV infection (smith strain) – Route: intra-peritoneal injection – Frequency: once – Amount: 1 x 10^4 PFU For TC-1 tumor inoculation – Route: hindflank, subcutaneous – Frequency: once – Amount: 0.5 x 10^6 cells For MC38 tumor inoculation – Route: hindflank, subcutaneous – Frequency: once – Amount: 3 x 10^5 cells
Study Design:	Detailed description of the study All mice will be infected with 1 x 10^4 PFU of mCMV. Mice will be monitored for their immunity to mCMV CD8+ T cells on days 21 and 60 post-infection. Mice will also be bled once on either Day -1, -2 or 0 prior to mCMV infection to assess non-infection status (i.e., control/baseline immunity). Immunity to M38 mCMV CD8+ specific T cells will be monitored via flow cytometry using tetramer staining. Briefly, mice will be bled and blood lymphocytes will be subjected to tetramer staining for immune analysis over the 3 time points. After day 60, mice that possess detectable MC38 mCMV T cells consistent with the literature and our previous studies will be selected into tumor implantation and efficacy assessment phase. To assess efficacy, mice will first be divided equally into 2 groups of n = 70. Both groups will receive subcutaneous inoculation into the hindflank with 0.5 x 10^6 of TC-1 or 3 x 10^5 of MC38 murine tumor cells. Tumor growth will be monitored following the tumor cell injection every two to three days starting on Day 3 post-injection. Two orthogonal measurements (width [W]; length [L]) will be recorded in millimeters, and the tumor size will be expressed as volume (mm^3; formula [(W2 x L) / 2.]).

When the tumor size reaches average size of 75-150 mm^3, mice will subsequently be randomized into the following groups of at least n = 10 for treatment as seen in the table below:

Group	Treatment Arm	Administration route	Number of doses/ interval (hrs)	Number of mice
Dose Escalation of CMV AIR ViP via IT route in MC38 tumor bearing mice which has an immune response emulating mCMV chronic infection.				
1	Buffer injection control	N/A	6/48	10
2	Unconjugated ViP (VERI-000)	IT (5 µg)	6/48	10
3	mCMV AIR-ViP (VERI-003)	IT (0.5 µg)	6/48	10
4	mCMV AIR-ViP (VERI-003)	IT (5 µg)	6/48	10
5	mCMV AIR-ViP (VERI-003)	IT (50 µg)	6/48	10
6	Anti-mouse PD-1	IP (200 µg)	3/72	10
7	Anti-mouse PD-1 + MCMV AIR-ViP (VERI-003)	PD-1: IP (200 µg) ViP: IT (5 µg)	PD-1: 3/72 ViP: 6/48	10
Dose Escalation of CMV AIR ViP via IT route in TC-1 tumor bearing mice which has an immune response emulating mCMV chronic infection.				
8	Buffer injection control	N/A	6/48	10
9	Unconjugated ViP (VERI-000)	IT (5 µg)	6/48	10
10	MCMV AIR-ViP (VERI-003)	IT (0.5 µg)	6/48	10
11	MCMV AIR-ViP (VERI-003)	IT (5 µg)	6/48	10
12	MCMV AIR-ViP (VERI-003)	IT (50 µg)	6/48	10
13	Anti-mouse PD-1	IP (200 µg)	3/72	10
14	Anti-mouse PD-1 + MCMV AIR-ViP (VERI-003)	PD-1: IP (200 µg) ViP: IT (5 µg)	PD-1: 3/72 ViP: 6/48	10

	Tumor size will be measured three times per week for the duration of the experiment until the volume becomes 2000 mm^3 which will be the study endpoint. Survival curves will be plotted based on 2000 mm^3 as the endpoint. Thereafter, the animal will be humanely euthanized. If mice remain tumor free, tumor rechallenge studies in which the same tumor will be injected on the contra-lateral flank will be discussed with vendor to assess for systemic anti-tumor immunity.
Measurements:	1) Observations: Clinical observations will be conducted once daily including weekends and holidays. These observations will confirm the general health and viability of the animal. Any evidence of morbidity, hunched posture, ruffled fur, lethargy, diarrhea, will be documented. Loss of > 20% body weight will result in the sponsor being notified and animals being removed from study per sponsors instructions. 2) Pathology: Gross pathology will be performed on all animals that are found dead or are euthanized. 3) Statistical Analysis: The number of animals needed is estimated using a two-way ANOVA program with the following cutoff parameters: a) power of 0.80 b) alpha of 0.05 c) intragroup standard deviation of 15% d) detection threshold of 15% difference between groups. As applied to all the proposed experiments, these parameters are fulfilled with at least 9 animals per experimental group [Alpha: Level of significance set at 5%].

[0324] MC38 efficacy results. Results of the experiments conducted according to the experimental design shown in Table 6, using MC38 tumors in the model are shown in Figure 22 and Figure 23. Treatment of MC38 tumors started at day 10 post-implantation into the mice, with tumor sizes ranging from 25 to 80 mm^3 in diameter. Collectively, the results in the MC38 tumor model showed that there was a dose-dependent effect for VERI-003 from 0.5 µg to 50 µg (Figure 22A). VERI-003 treatment at the 50-µg dose showed the best efficacy as a monotherapy in the MC38 tumor model, although all 3 doses resulted in a statistically significant decrease in tumor volume compared to the control group in at least one time point (Two-way ANOVA adjusting for multiple comparisons). (See, Table 7). The combination of 5 µg of VERI-003 and the anti-PD-1 antibody also showed comparable efficacy to the 50-µg mono-therapy despite having 10 times less VERI-003. Treatment using an anti-mouse PD-1 antagonist antibody alone showed no efficacy in the MC38 tumor model and was similar to control treatments (both vehicle and unconjugated ViP, VERI-000, Figure 22B). VERI-003

monotherapy, or in combination with anti-PD-1 antibody, was able to induce complete responses against tumors in this recognized model.

TABLE 7

	Adjusted p Value		
Group	**Day 23**	**Day 25**	**Day 28**
Grp 1 Vehicle control vs. Grp 2 VERI-000	0.8606	0.5794	0.5493
Grp 1 Vehicle control vs. Grp 3 VERI-003 (0.5µg)	0.1011	0.0263	0.667
Grp 1 Vehicle control vs. Grp 4 VERI-003 (5µg)	0.066	0.0062	0.0426
Grp 1 Vehicle control vs. Grp 5 VERI-003 (50µg)	0.001	0.0009	0.0207
Grp 1 Vehicle control vs. Grp 6 Anti PD-1 only	0.9594	0.3767	0.8956
Grp 1 Vehicle control vs. VERI-003 (5 µg) + Anti PD-1	0.021	0.0069	0.0238

[0325] MC38 survival results and demonstration that AIR results in the induction of a systemic cancer-specific immunity: To assess for long term survival, all groups in the MC38 efficacy study were followed until 90 days post tumor implantation. By day 45, there were only 7 remaining tumor free mice: 1/7 from the PD-1 alone treatment, 4/7 from the combination therapy and 2/7 from the VERI-003 monotherapy groups (group 3 and group 5). These mice continued to remain tumor free with no recurrence of tumors even at day 90. (See, Figure 23A and Figure 23B). The median Time To Endpoint (TTE) was calculated for each group, and the difference in the TTE between the vehicle control group and each treated group (T-C) was used to calculate the Tumor Growth Delay (TGD). The TGD for the 5 µg VERI-003 treated group was 5.9%, the TGD for the PD-1 alone group was -5.9%, and the TGD for the combination group was 19.1%, demonstrating synergy of the VERI-003 and anti PD-1 in increasing survival. This is also reflected in the number of complete responders per group as the 5 µg VERI-003 treated group had no complete responders, the PD-1 alone group had 1, and the combination group had 4. (See, Table 8).

TABLE 8

Group	Median TTE	T-C (days)	TGD (%)	CR
Vehicle Control	34			0
VERI-000	40.5	6.5	19.1	0
VERI-003 0.5 μg	36	2	5.9	1
VERI-003 5 μg	36	2	5.9	0
VERI-003 50 μg	42	8	23.5	1
anti-mouse PD-1	32	-2	-5.9%	1
VERI-003 5 μg + anti-mouse PD-1	40.5	6.5	19.1	4

[0326] To further demonstrate the promise of the AIR approach, all 7 tumor-free mice underwent a tumor rechallenge study whereby the same amount of MC38 tumor cells were injected in the contralateral flank of these mice. N = 7 age-matched control mice were also injected with the same number of tumor cells to serve as a control. No additional treatments were provided to either group. Results as shown in Figure 25A and Figure 25B show 7/7 (100%) control mice grew tumors whereas 4/7 (57%) of the tumor-free mice (former complete responders in first study) remain tumor free even after re-challenge with the same amount of tumor cells in the opposite flank. Within these 4 mice, 3 were from the combination group and 1 was from the monotherapy group. The PD-1-treated mouse grew tumors. Based on these initial results, our AIR-ViP approach with VERI-003 and ultimately VERI-101 potentially leads to durable complete responses and improved survival. In addition, there appears to be a synergistic effect with respect to survival with checkpoint inhibitors. Importantly, rechallenge studies in the MC38 model also showed the induction of a systemic cancer-specific immunity. Finally, companion safety assessments/clinical observations showed no significant issues; no losses in body weight for the MC38 model during treatment suggest the safety of the treatment.

[0327] TC-1 efficacy results. Results of the experiments conducted according to the experimental design shown in Table 6, using TC-1 tumors in the model are shown in Figure 24A and Figure 24B. Treatment of TC-1 tumors started at day 9 post-implantation into the mice, with tumor sizes ranging from 30-90 mm^3 in diameter. Collectively, the results set forth in Figure 23A and Figure 23B show that in the TC-1 tumor model yielded a dose dependent effect for VERI-003 from 0.5 μg to 50 μg. VERI-003 treatment at the 50-μg dose showed the best efficacy as a monotherapy in the TC-1 tumor model. The combination of 5 μg of VERI-003 and the anti-PD-1 antibody also showed comparable efficacy to the 50-μg mono-therapy despite having 10 times less VERI-003, and both groups demonstrated statistically significant reductions in tumor volume compared to the vehicle control group in at least one time point (2-way ANOVA adjusting for multiple comparisons, see Table 9).

[0328] Treatment using an anti-mouse PD-1 antagonist antibody alone showed no efficacy in the TC-1 tumor model and was similar to control treatments (both vehicle and unconjugated ViP, VERI-000). Although no complete responses were observed in the TC-1 tumor model, results and statistics on antitumor activity were consistent with observations in the MC38 tumor model.

TABLE 9

	Adjusted p Value			
Group	**Day 23**	**Day 25**	**Day 28**	**Day 30**
Grp 1 Vehicle control vs. Grp 2 VERI-000	>0.9999	0.8342	0.9981	0.8684
Grp 1 Vehicle control vs. Grp 3 VERI-003 (0.5μg)	0.5812	0.9994	0.8807	0.9984
Grp 1 Vehicle control vs. Grp 4 VERI-003 (5μg)	0.1275	0.6923	0.169	0.1979
Grp 1 Vehicle control vs. Grp 5 VERI-003 (50μg)	0.0266	0.0663	0.0293	0.2424
Grp 1 Vehicle control vs. Grp 6 Anti PD-1 only	0.9983	>0.9999	0.4727	>0.9999
Grp 1 Vehicle control vs. VERI-003 (5μg) + Anti PD-1	0.0435	0.0243	0.0113	0.1583

[0329] TC-1 survival results: Consistent with our results in the MC38 model, for TC-1, a dose-dependent therapeutic effect was observed across the treated groups during treatment. However, the observed growth rate of the tumors in this study, especially for the untreated controls, was significantly slower than expected compared to our previous study as well as observed TC-1 growth rates in the literature (Çuburu et al., 2022) where most mice reached the TC-1 2000 mm^3 endpoint by Day 21. By Day 21, control mice tumors remain less than 500 mm^3 on average indicating the tumors were growing much slower than expected. Because of this analysis of overall survival was not possible to assess as these mice also experienced severe body weight (BW) loss. Briefly, the BW for all groups slowly decreased over time reaching -10% by Day 20 and -20% by Day 36. By Day 29, some animals started to be humanely euthanized as their BW loss was more than 25% of their initial BW. By Day 30, 17 animals were euthanized, 22 more by Day 36 and an additional 11 mice by Day 42. Euthanizing these many mice due to body weight loss and not tumor volume increase is unusual for this model, so on Day 36, 5 animals were necropsied. No sign of metastasis was observed, and all tissues were within the normality. As a result of these findings, caution must be taken in terms of interpreting the presence or lack of efficacy in this particular model for this study.

[0330] Clinical dosing. The VERI-101 clinical dose range is determined by the skilled practitioner, based on the therapeutic range determined from the above VERI-003 efficacy studies scaled by tumor volume as well as future studies. Direct peptide intratumoral injection studies have demonstrated a therapeutic effect between 10 ng to 1000 ng of a mix of free peptide injection (3 peptides) into tumors with sizes around 100 mm^3. (See, e.g., Schiller et al., US Pat. App. Pub. No. 20200330582A1). This effect began to plateau around 100 ng for mCMV peptide administration making a useful therapeutic range to be about 10-100 ng of peptide. This corresponds to 100 ng/cm^3 to 1000 ng/cm^3 of peptide. In contrast, VERI-003 treatment demonstrated a therapeutic effect between 10 ng to 1000 ng of free peptide injection into tumors with sizes around 50 mm^3. No plateau was observed across two tumor models therefore showing that the conjugation of the CMV peptide to the ViP results in an augmented response; this result is in contrast to the free peptide and work done by Cuburu et al. (See, Çuburu et al., 2022).

[0331] The demonstration of 5 μg of VERI-003 with anti-PD-1 antibody showing an equivalent effect to 50 μg of VERI-003 and both being better than PD-1 alone, across two different tumor models further substantiates the anti-tumor efficacy of the AIR-ViP composition.

[0332] In the experiments disclosed in this Example, VERI-003 treatment demonstrated a therapeutic effect between 10 ng to 1000 ng of free peptide injection into tumors with sizes around 50 mm^3. In contrast to free peptide and work done by Cuburu et al. (2022), no plateau was observed across two tumor models, which shows that the conjugation of the CMV peptide to the ViP results in an augmented response. The demonstration of 5 μg of VERI-003 with anti-PD-1 antibody showing an equivalent effect to 50 μg of VERI-003 and both being better than anti-PD-1 antibody alone, across two different tumor models, further substantiates the anti-tumor efficacy of the inventive AIR-ViP composition.

[0333] In 1000 ng of VERI-003, there is approximately 18 ng of conjugated mCMV peptide; therefore 5.5 $\mu g/cm^3$ to 55 $\mu g/cm^3$ of VERI-003 would contain approximately 100 ng/cm^3 to 1000 ng/cm^3 of peptide. This would also hold true for VERI-101 since this surrogate AIR-ViP product is identical to the hCMV-AIR VERI-101 human-specific product except for the conjugated viral epitope only.

[0334] In the clinic, the average tumor size could be 2.5 cm^3. Hence, the dose escalation is calculated, therefore, to be in the range of 13.75 μg ViP to 137.5 μg ViP (corresponding to 247.5 ng to 2475 ng of CMV peptide). The clinical dose range of VERI-101 for human subjects/patients is calculated to span 10 μg - 150 μg, or more, perhaps even as high as 1000 to

2000 μg, because VERI-003 did not show a therapeutic upper limit in the experiments disclosed in this Example, unlike free peptide.

Example 12

Confirmatory Study of *in vivo* Anti-Tumor Activity of AIR-VLP in mCMV with PD-1

[0335] A second efficacy study (termed 'confirmatory study') was conducted. This was designed similar to the study design above. The overall study design is detailed in the synopsis below (Table 10).

TABLE 10

VERI-003 Confirmatory Efficacy Study Design

N=190 pre-infected C57BL/6 female mice from Part 1 (remaining 10 as infected controls)

N=108 included in the study for treatment

Readouts: body weight and tumor volume 3 times a week, survival curve

G	N	Challenge (~Day 60 post-mCMV infection)	Treatment (~75-150 mm^3)	Dose	Route, frequency
1	12	MC38 tumor cells S.C. $3x10^5$ cells/animal	**untreated**	n/a	IT, Q2D x 6
2	12		ViP control (VERI-000)	100ug	IT, Q2D x 6
3	12		mCMV AIR-ViP (VERI-003)	5ug	IT, Q2D x 6
4	12		mCMV AIR-ViP (VERI-003)	50ug	IT, Q2D x 6
5	12		mCMV AIR-ViP (VERI-003)	100ug	IT, Q2D x 6
6	12		**anti-mouse PD-1 (RMP1-14)**	**200ug**	**IP, days 0, 3, 6, 9**
7	12		**anti-mouse PD-1** + mCMV AIR-ViP (VERI-003)	**200ug** + 5ug	**IP, days 0, 3, 6, 9** and IT, Q2D x 6
8	12		**anti-mouse PD-1** + mCMV AIR-ViP (VERI-003)	**200ug** + 50ug	**IP, days 0, 3, 6, 9** and IT, Q2D x 6
9	12		**anti-mouse PD-1** + mCMV AIR-ViP (VERI-003)	**200ug** + 100ug	**IP, days 0, 3, 6, 9** and IT, Q2D x 6

[0336] Briefly, similar to Example 11, n = 200 C57BL/6 mice were pre-infected with a sub-lethal dose of mCMV, 1 x 10^4 PFU (ATCC Smith Strain), then housed for 60 days. Blood

was be collected at two timepoints and analysed for peripheral mCMV CD8+ T cells via tetramer staining. After confirmation of immunity, based on their body weight, 190 animals will be selected for tumor implantation. The remaining 10 animals will be kept as infected control aged matched mice for future rechallenge studies.

[0337] To assess efficacy, mice first received a subcutaneous inoculation into the hind flank with 3 x 10^5 of MC38 murine tumor cells. Tumor growth was monitored following the tumor cell injection every two to three days starting on Day 3 post-injection. Two orthogonal measurements (width [W]; length [L]) was recorded in millimeters, and the tumor size will be expressed as volume (mm^3; formula [(W2 x L) / 2.]).

[0338] When the tumor sizes reached an average size of about 100 mm^3, mice were subsequently be randomized into 9 total groups of at least n = 12 for treatment as seen in table 7. At the end of randomization, each group had n = 12 mice with an average tumor size of about 100 mm^3. Figure 26 shows the overall tumor size of each mouse per group on Day 10 post tumor inoculation prior to treatment.

[0339] MC38 efficacy results. Results of the experiments conducted according to the experimental design shown in Table 10, using MC38 tumors in the model are shown in Figure 27, Figure 28, Table 11, and Table 12. Treatment of MC38 tumors started at day 10 post-implantation into the mice. Collectively, the results in the MC38 tumor model showed once more that there was a dose-dependent effect for VERI-003 from 5 µg to 50 µg to 100µg. (See, Figure 27A). VERI-003 treatment at the 100-µg dose showed the best efficacy as a monotherapy in the MC38 tumor model.

[0340] More combinations with anti-PD-1 antibody were tested and results show that there was no significant difference between combining anti-PD-1 antibody with either 5, 50, or 100 µg of VERI-003 as all three groups showed comparable efficacy. (See, Figure 27B). Combination treatment was superior than monotherapy treatment or anti-PD-1 antibody and resulted in the smallest p values when comparing tumor volume reductions to the non-treatment control group. In fact, treatment with anti-mouse PD-1 antibody alone showed no efficacy in the MC38 tumor model which was consistent with previous findings in Example 11 and was similar to no treatment control.

[0341] Unlike our previous observation in Example 11 where 5 µg of VERI-000 treatment (unconjugated ViP) showed no efficacy, unexpectedly, 100 µg of VERI-000 treatment (unconjugated ViP) showed some efficacy in this study with 4/12 mice having complete responses at some point in the experiment (total tumour clearance). However, the tumours in most of these mice eventually grew back after a period of time. Further analysis

showed that 100 µg VERI-000 contained 8 times more endotoxin compared to VERI-003 at the same amount, indicating that the therapeutic effect observed for VERI-000 was non-specific unlike the therapeutic effects observed for VERI-003 monotherapy and combination groups.

TABLE 11

	Adjusted p Value		
Group	**Day 22**	**Day 25**	**Day 27**
G1 - No Treatment vs. G2 – VERI-000	0.0009	0.0047	0.0016
G1 - No Treatment vs. G3 – VERI-003 5µg	0.0066	0.0632	0.1059
G1 - No Treatment vs. G4 – VERI-003 50 µg	0.0012	0.0029	0.0053
G1 - No Treatment vs. G5 – VERI-003 100µg	0.0008	0.0006	0.0003
G1 - No Treatment vs. G6 - anti-mouse PD-1	0.4726	>0.9999	0.5382
G1 - No Treatment vs. G7- VERI-003 5 µg + anti-mouse PD-1	0.0003	0.0002	<0.0001
G1 - No Treatment vs. G8 – VERI-003 50 µg + anti mouse PD-1	0.0011	0.0003	<0.0001
G1 - No Treatment vs. G9 – VERI-003 100 µg + anti mouse PD-1	0.0028	0.0004	0.0003

[0342] Confirmatory study MC38 survival results: To assess for long term survival, all groups in the MC38 efficacy study were followed until day 60 post tumor implantation. (See, Figure 28A and Figure 28B). Figure 28B shows the results on day 60 where > 50% of the mice in the combination groups had not met the endpoint. Within these groups, there were between 3 to 6 mice which had completely cleared their tumours and remained tumour free at day 60. In the monotherapy groups, there were less complete responders but all groups with the exception of non-treatment sample and 5 µg VERI-003 monotherapy still had mice not reaching the endpoint at Day 60. The median Time To Endpoint (TTE) was calculated for each group, and the difference in the TTE between the vehicle control group and each treated group (T-C) was used to calculate the Tumor Growth Delay (TGD). The TGD for the monotherapy groups was 17% to 24%, the TGD for the PD-1 alone group was 0%, and the TGD for the combination groups was 82% to 101%, demonstrating synergy of the VERI-003 and anti PD-1 in increasing survival. (Table 12).

TABLE 12

Group	Median TTE	T-C (days)	TGD (%)	CR
No treatment	29			0
VERI-000	39	10	34.5	1
VERI-003 5 µg	34	5	17.2	0
VERI-003 50 µg	34	5	17.2	1
VERI-003 100 µg	36	7	24.1	1
anti-mouse PD-1	29	0	0	2
VERI-003 5 µg + anti-mouse PD-1	53	24	82.8	6
VERI-003 50 µg + anti-mouse PD-1	55	26	89.7	3
VERI-003 100 µg+ anti-mouse PD-1	58.5	29.5	101.7	6

[0343] In summary, the confirmatory experiment in this Example further demonstrates significant reductions in tumor volume after VERI-003 treatment and that Combination treatment with anti PD-1 is more effective than VERI-003 with this current regiment of 6 treatment doses every 48 hours.

Example 13

***In Vivo* Anti-Tumor Activity of AIR-VLP Against B16.F10 Tumor With Mcmv Immunity**

[0344] To further demonstrate the potential utility of the AIR-ViP approach in targeting more diffused and difficult to treat tumors, a pilot intravenous efficacy study (termed "pilot systemic efficacy study") was conducted. In this study, the ability of intravenously administered VERI-003 was assessed against a highly aggressive metastatic lung tumor model.

[0345] The study design is shown in Figure 29. Briefly, similar to Example 11 and 12, C57BL/6 mice were pre-infected with a sub-lethal dose of mCMV, $1x10^4$ PFU (ATCC Smith Strain), then housed for 60 days. Blood was collected at two timepoints and analysed for peripheral mCMV CD8+ T cells via tetramer staining. After confirmation of immunity, the mice were divided into 5 groups (n=10 each) per the treatment groups as seen in Figure 30 and were implanted intravenously with B16F10 cells (2×10^5 cells/animal). Per previous experiments by Day 8 or 9, small metastatic lung nodules are visibly present on the lungs and by day 21 onwards, these tumors will spread and manifest as a black mass resulting in the need to humanely euthanize these mice. Based on this, mice were treated on day 8 for a total of 6 treatments every 48 hours per previous treatment cycles as seen in Example 11 and 12. Treatment type was as per the table in Figure 29. Following the final treatment on Day 18, on Day 22, all lung tumors will then be collected, weighed and lung metastases counted. Overall tumor burden on the individual mice lungs was also assessed qualitatively. As seen in Figure

30, AIR-ViPs alone or in combo showed strong reduction of metastatic tumor growth and spread. These results collectively demonstrate the ability for AIR-ViPs to be delivered via the blood stream and upon reaching tumors, it was able to significantly control tumor growth as well as prevent/delay metastatic spread compared to VERI-000 and no-treatment controls.

WHAT IS CLAIMED IS:

1. A composition, comprising:

a plurality of virus proteins, wherein each of said plurality of virus proteins comprises a mutated amino acid sequence of a Papillomaviridae L1 protein;

one or more peptides each comprising one or more epitopes from one or more pathogens other than a Papillomaviridae antigenic peptide; and

one or more immune checkpoint inhibitor (ICI) molecules,

wherein the mutated amino acid sequence of the Papillomaviridae L1 protein comprises at least the following mutations with respect to the wild type L1 protein sequence: (a) a deletion of at least five amino acid residues from an amino-terminus, and (b) a deletion of at least ten amino acid residues from the helix four region, and (c) a deletion of at least thirty amino acid residues from a carboxy-terminus,

wherein the one or more peptides are attached to the plurality of virus proteins.

2. The composition of claim 1, wherein the one or more ICI molecule inhibits the binding of one or more of:

(i) PD-1 to PD-L1;

(ii) CTLA-4 to B7-1 and/or to B7-2,

(iii) LAG-3 to its respective ligand(s),

(iv) Tim-3 to its respective ligand(s),

(v) TIGIT, to its respective ligand(s), and

(vi) CD96 to its respective ligand(s).

3. The composition of claim 1, wherein the one or more ICI molecules is an antibody.

4. The composition of claim 3, wherein the one or more ICI molecules is one or more of pembrolizumab, nivolumab, durvalumab, atezolizumab, ipilimumab, and relatlimab.

5. The composition of claim 1, wherein the one or more peptides are conjugated to the plurality of mutant Papillomaviridae L1 proteins.

6. The composition of claim 5, wherein the one or more peptides are conjugated to the mutant Papillomaviridae L1 proteins via disulphide, maleimide, or amide bond between the mutant Papillomaviridae L1 protein and a residue of the peptide.

7. The composition of claim 1, wherein each of the one or more peptides further comprise one or more protease cleavage sequences.

8. The composition of claim 7, wherein the one or more protease cleavage sequences comprise a furin cleavage sequence, a matrix metalloprotease cleavage sequence, or a disintegrin and metalloprotease (ADAM) cleavage sequence.

9. The composition of claim 1, wherein the one or more epitopes are viral, bacterial, parasitic, or fungal epitopes, and

wherein:

the viral epitopes are one or more of cytomegalovirus, respiratory syncytial virus coronavirus, vaccinia, varicella zoster, Herpes zoster, rubella, hepatitis, influenza, measles, mumps, poliovirus, variola, rabies, dengue, Ebola, West Nile, yellow fever, or zika epitopes;

the bacterial epitopes are one or more of *Bordetella pertussis*, *Clostridium tetani*, *Chlamydia trachomatis*, *Corynebacterium diphtheriae*, *Hemophilus influenza*, *Neisseria meningitidis*, *Streptococcus*, *Vibrio cholera*, *Mycobacterium tuberculosis*, Bacillus Calmette-Guérin, *Salmonella*, *Escherichia coli*, *Legionella pneumophila*, *Rickettsia*, *Treponema pallidum pallidum*, *Bacillus anthracis*, *Clostridium botulinum*, or *Yersinia* epitopes; or

the parasitic epitopes are one or more of *Entamoeba histolytica*, *Toxoplasma gondii*, *Trichinella*, *Trichomonas*, *Trypanosoma*, or *Plasmodium* epitopes.

10. The composition of claim 1, wherein at least one of the one or more epitopes is a childhood vaccine antigenic epitope.

11. The composition of claim 1, wherein the one or more peptides comprises at least two epitopes from one or more pathogens other than a Papillomaviridae antigenic peptides.

12. The composition of claim 1, wherein the proteoglycan expressed on tumor cells is heparin sulfate proteoglycan (HSPG), perlecan, hyalectan versican, glypican-3, small leucine-rich proteoglycans (SLRP), and/or biglycan.

13. The composition of claim 1, wherein said plurality of mutant Papillomaviridae L1 proteins are mouse mutant Papillomaviridae L1 proteins.

14. The composition of claim 1, wherein an amino acid sequence of each of said plurality of mutant Papillomaviridae L1 proteins is SEQ ID NO:134, and is encoded by nucleic acid sequence SEQ ID NO:135 or 136.

15. A method of treating, reducing the occurrence of, inhibiting the progression and/or metastasis of, a cancer in a subject in need thereof, which comprises administering to the subject a pharmaceutically effective amount of a composition comprising:

a plurality of virus proteins, wherein each of said plurality of virus proteins comprises a mutated amino acid sequence of a Papillomaviridae L1 protein;

one or more peptides each comprising one or more epitopes from one or more pathogens other than a Papillomaviridae antigenic peptide; and

one or more immune checkpoint inhibitor (ICI) molecules,

wherein the mutated amino acid sequence of the Papillomaviridae L1 protein comprises at least the following mutations with respect to the wild type L1 protein sequence: (a) a deletion of at least five amino acid residues from an amino-terminus, and (b) a deletion of at least ten amino acid residues from the helix four region, and (c) a deletion of at least thirty amino acid residues from a carboxy-terminus,

wherein the one or more peptides are attached to the plurality of virus proteins.

16. The method of claim 15, further comprising:

obtaining from the subject a tumor tissue sample; and

identifying in the tumor tissue a sequence of one or more MHC molecules expressed by one or more tumor cells in the tumor tissue sample.

17. The method of claim 16, wherein the subject was previously infected or vaccinated against a pathogen, and wherein the one or more epitopes is an antigenic epitope of the pathogen, and/or wherein the one or more epitopes are capable of complexing with one or more MHC molecules expressed by a tumor cell in a tumor tissue sample obtained from the subject.

18. The method of claim 15, wherein the one or more ICI molecules is an antibody.

19. The method of claim 18, wherein the one or more ICI molecules is one or more of pembrolizumab, nivolumab, durvalumab, atezolizumab, ipilimumab, or relatlimab, or a combination of any of these.

20. The method of claim 15, wherein the one or more ICI molecules inhibits the binding of one or more of:

(i) PD-1 to PD-L1;

(ii)CTLA-4 to B7-1 and/or to B7-2;

(iii) LAG-3 to its respective ligand(s),

(iv) Tim-3 to its respective ligand(s),

(v) TIGIT, to its respective ligand(s), and

(vi) CD96 to its respective ligand(s).

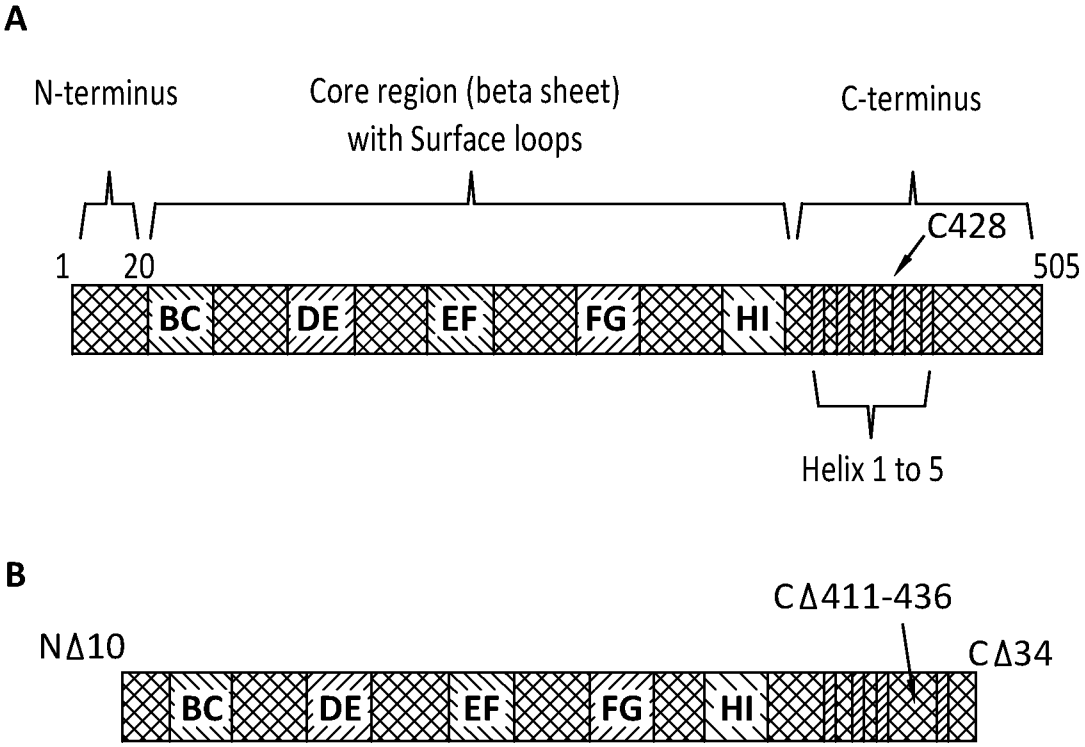
A
N-terminus
Core region (beta sheet)
with Surface loops
C-terminus
C428
1
20
505
BC
DE
EF
FG
HI
Helix 1 to 5
B
CΔ411-436
NΔ10
CΔ34
BC
DE
EF
FG
HI

FIG. 1

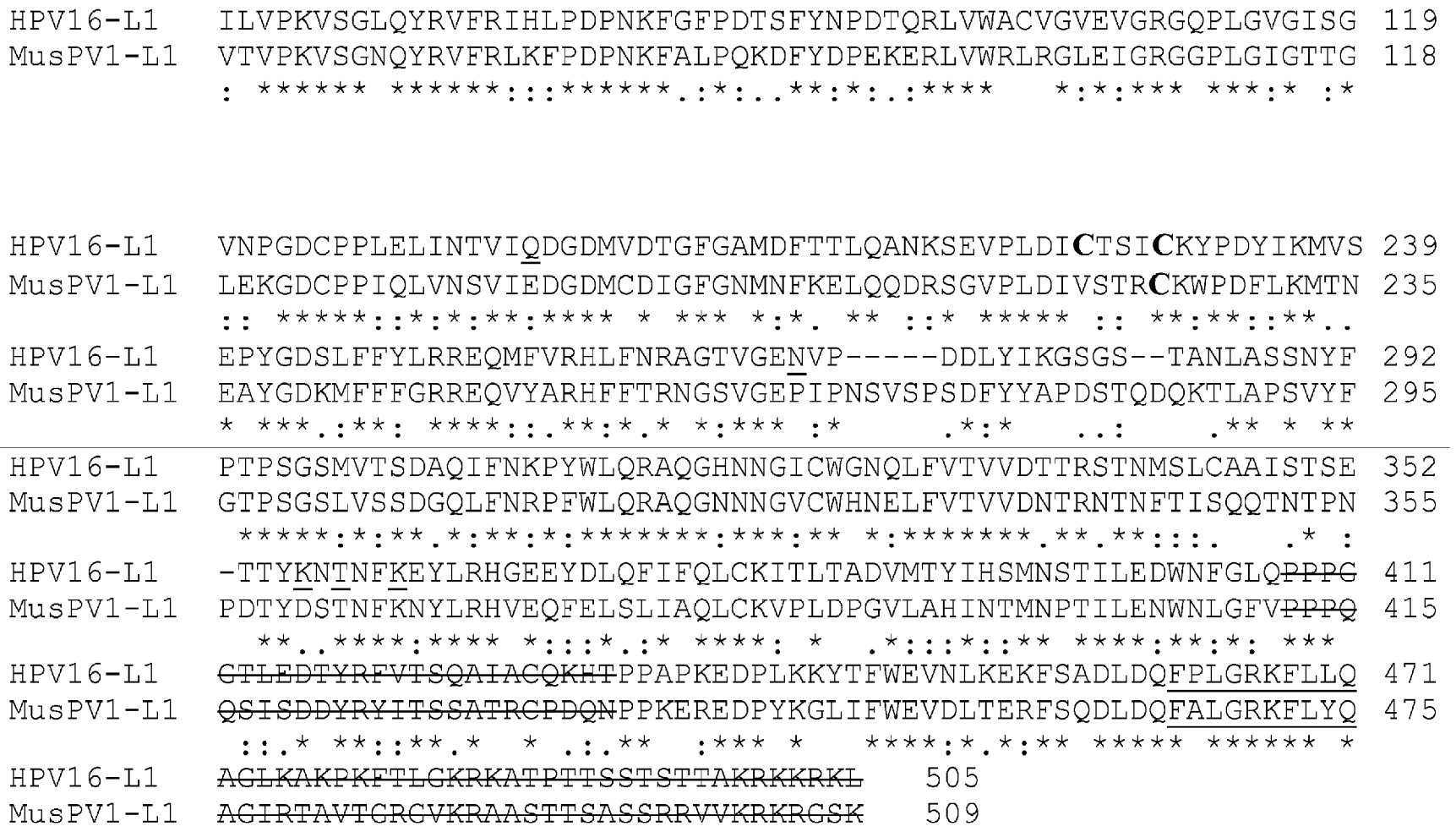

FIG. 2

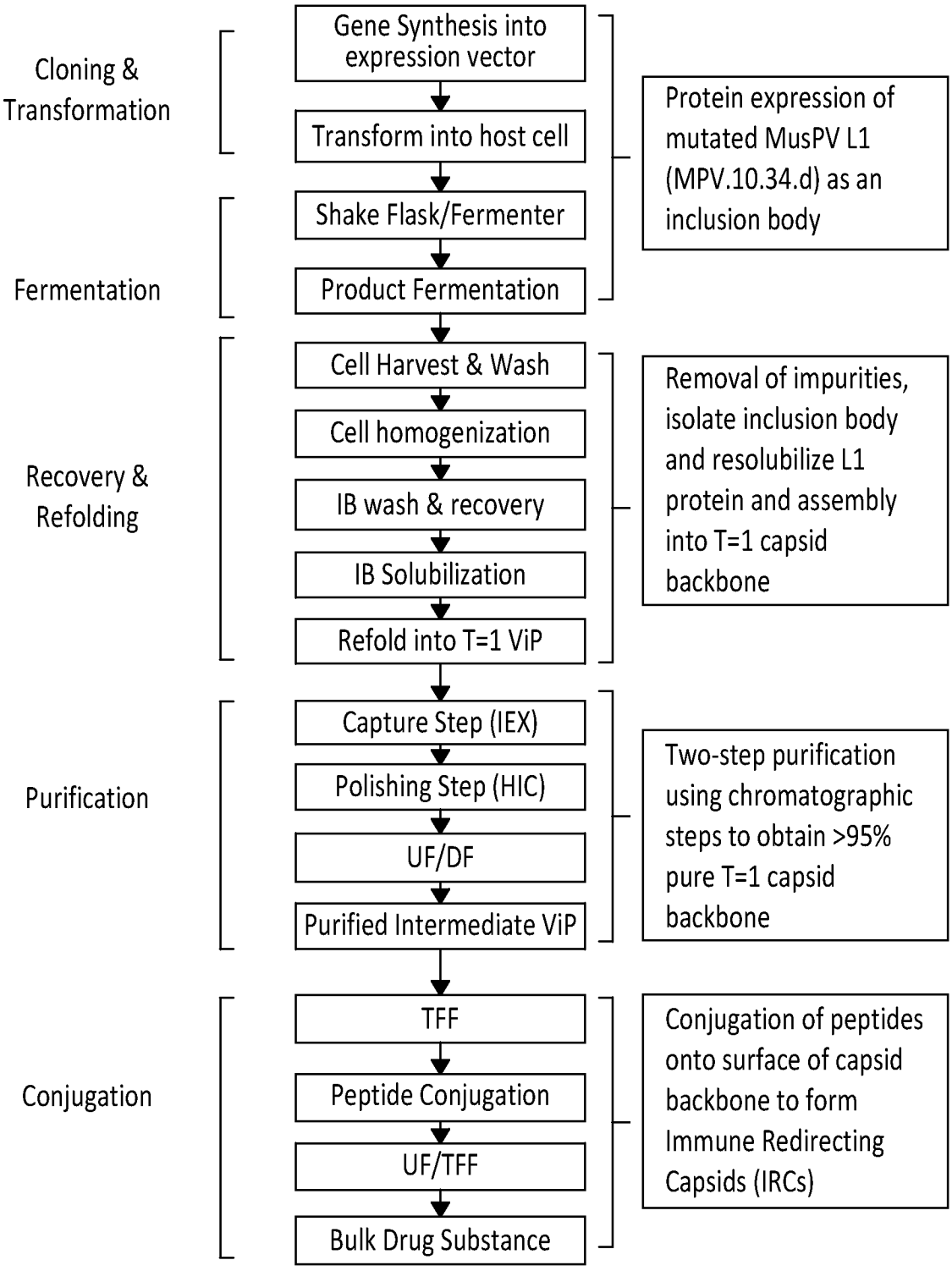
Cloning & Transformation
Fermentation
Recovery & Refolding
Purification
Conjugation
Gene Synthesis into expression vector
Transform into host cell
Shake Flask/Fermenter
Product Fermentation
Cell Harvest & Wash
Cell homogenization
IB wash & recovery
IB Solubilization
Refold into T=1 ViP
Capture Step (IEX)
Polishing Step (HIC)
UF/DF
Purified Intermediate ViP
TFF
Peptide Conjugation
UF/TFF
Bulk Drug Substance
Protein expression of mutated MusPV L1 (MPV.10.34.d) as an inclusion body
Removal of impurities, isolate inclusion body and resolubilize L1 protein and assembly into T=1 capsid backbone
Two-step purification using chromatographic steps to obtain >95% pure T=1 capsid backbone
Conjugation of peptides onto surface of capsid backbone to form Immune Redirecting Capsids (IRCs)

FIG. 3

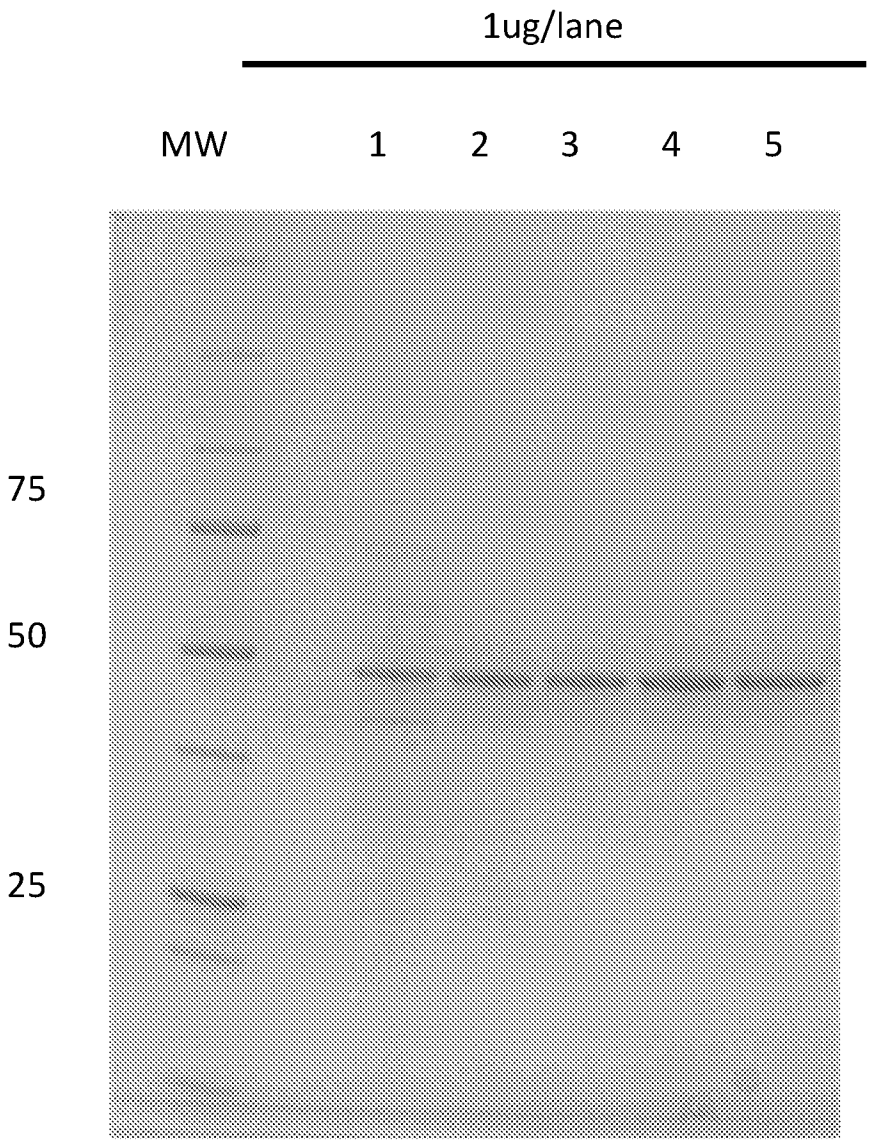

FIG. 4

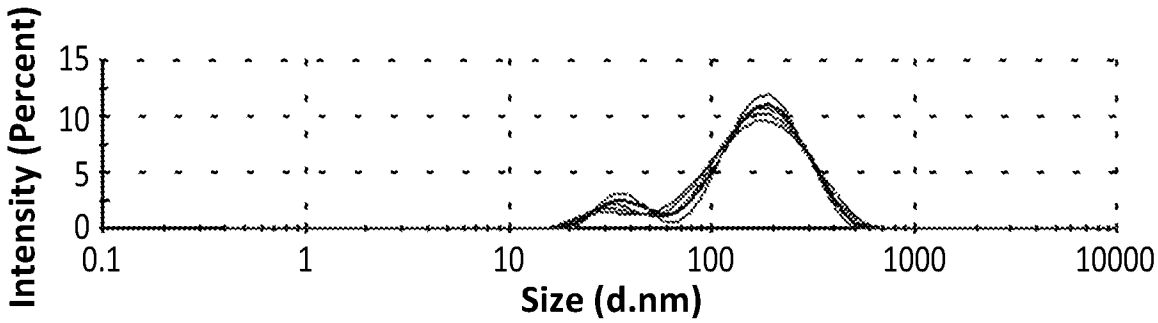
Intensity (Percent)
15
10
5
0
0.1
1
10
100
1000
10000
Size (d.nm)

FIG. 5A

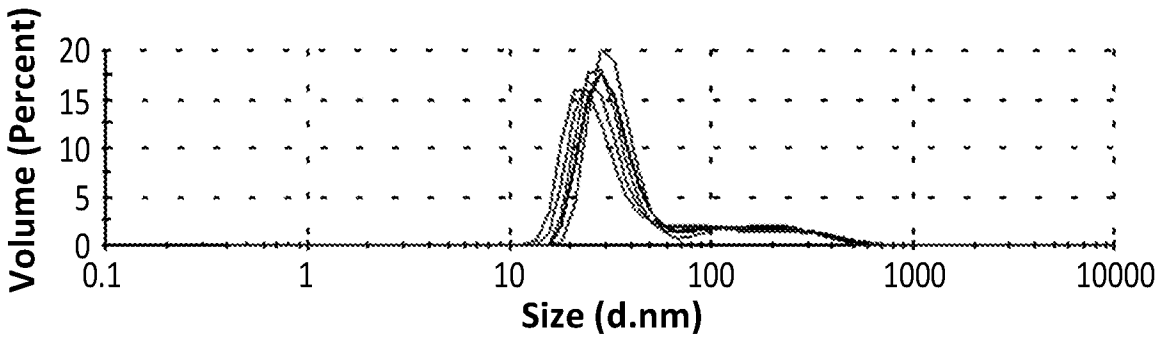
Volume (Percent)
20
15
10
5
0
0.1
1
10
100
1000
10000
Size (d.nm)

FIG. 5B

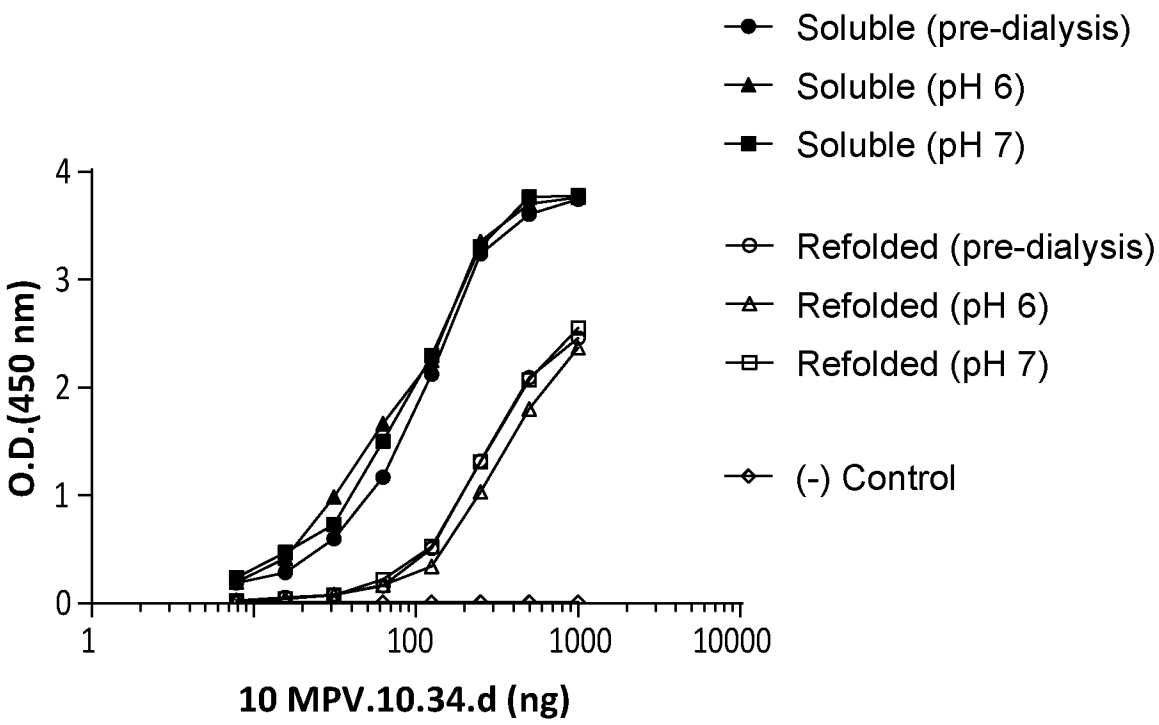
Soluble (pre-dialysis)
Soluble (pH 6)
Soluble (pH 7)
Refolded (pre-dialysis)
Refolded (pH 6)
Refolded (pH 7)
(-) Control
O.D.(450 nm)
0
1
2
3
4
1
100
1000
10000
10 MPV.10.34.d (ng)

FIG. 6

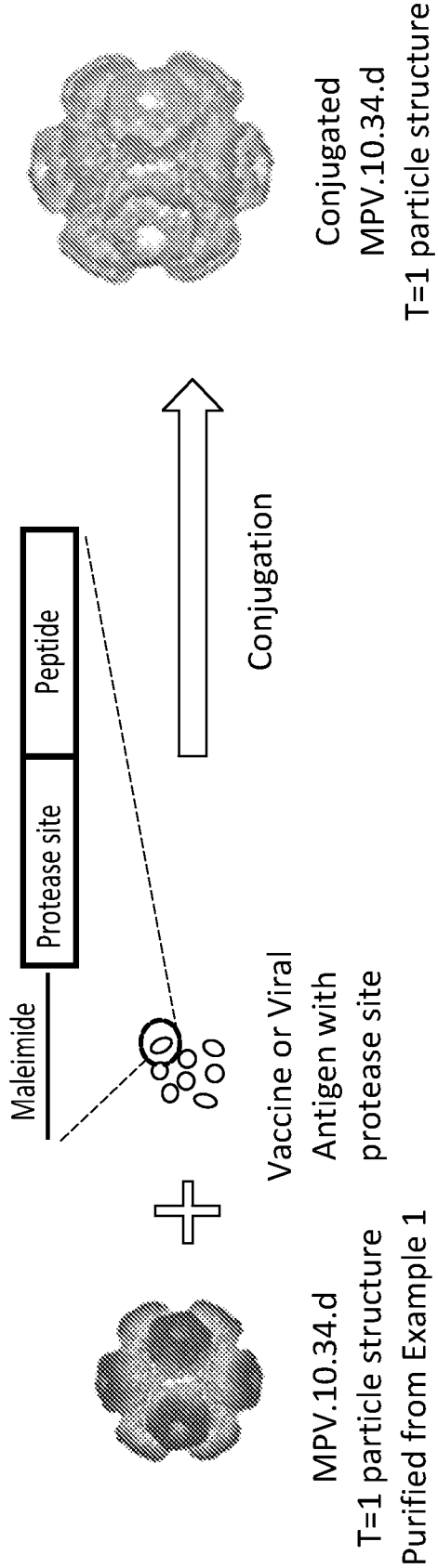
Maleimide
Protease site
Peptide
Vaccine or Viral Antigen with protease site
MPV.10.34.d T=1 particle structure Purified from Example 1
Conjugation
Conjugated MPV.10.34.d T=1 particle structure

FIG. 7

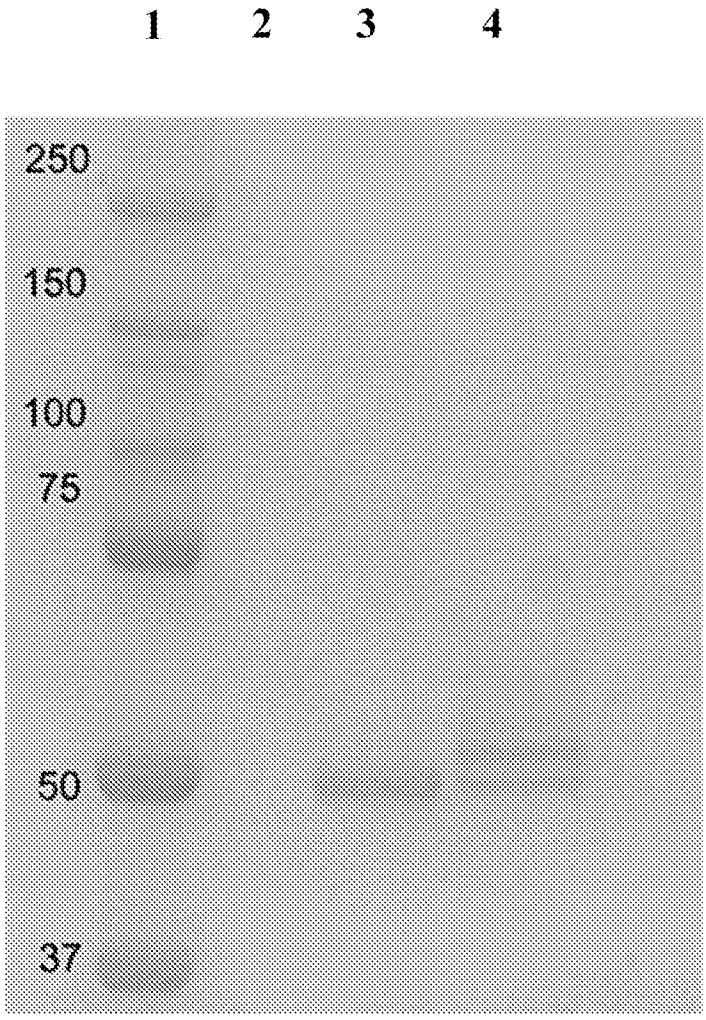

FIG. 8

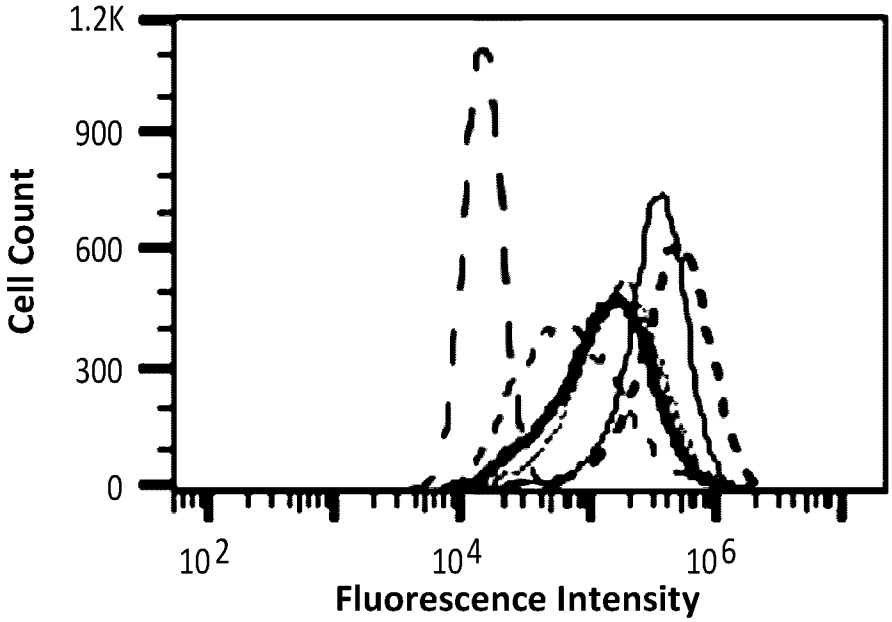
1.2K
900
600
300
0
Cell Count
10^2
10^4
10^6
Fluorescence Intensity

FIG. 9A

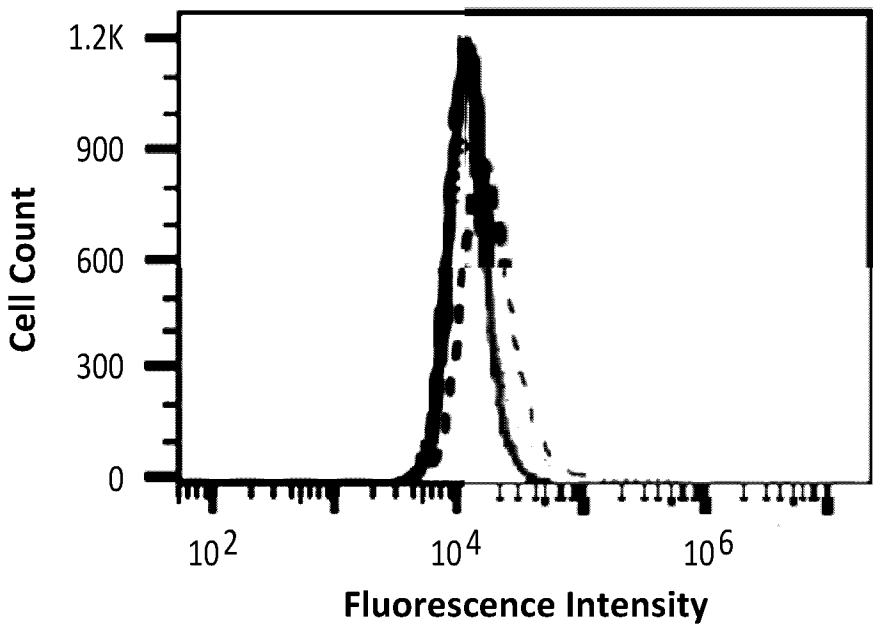
1.2K
900
600
300
0
Cell Count
10^2
10^4
10^6
Fluorescence Intensity

FIG. 9B

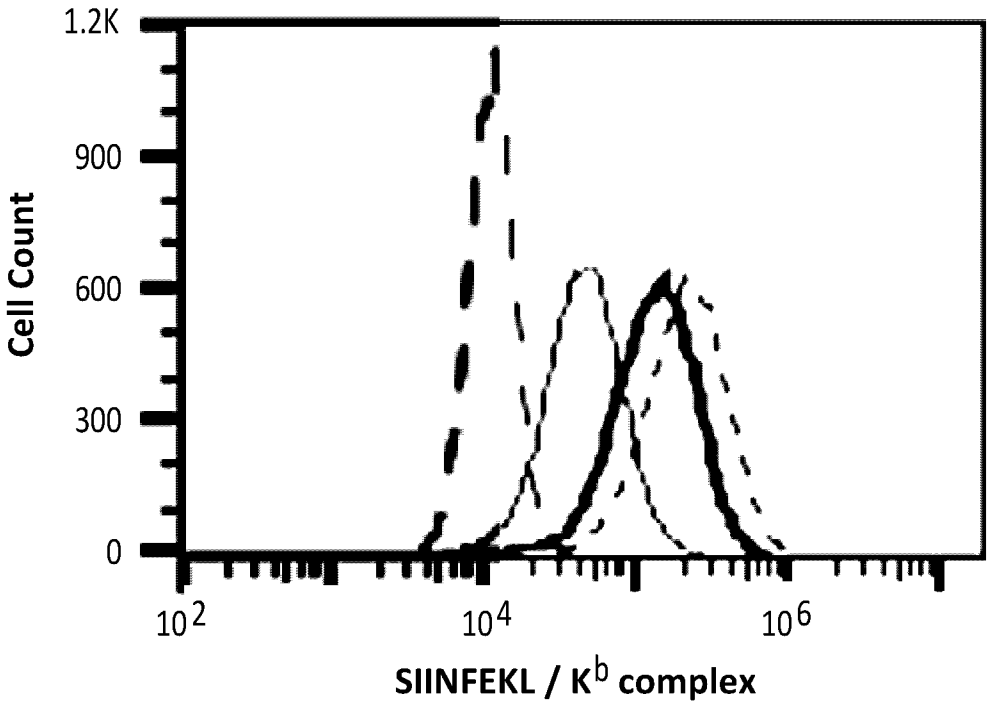

FIG. 10

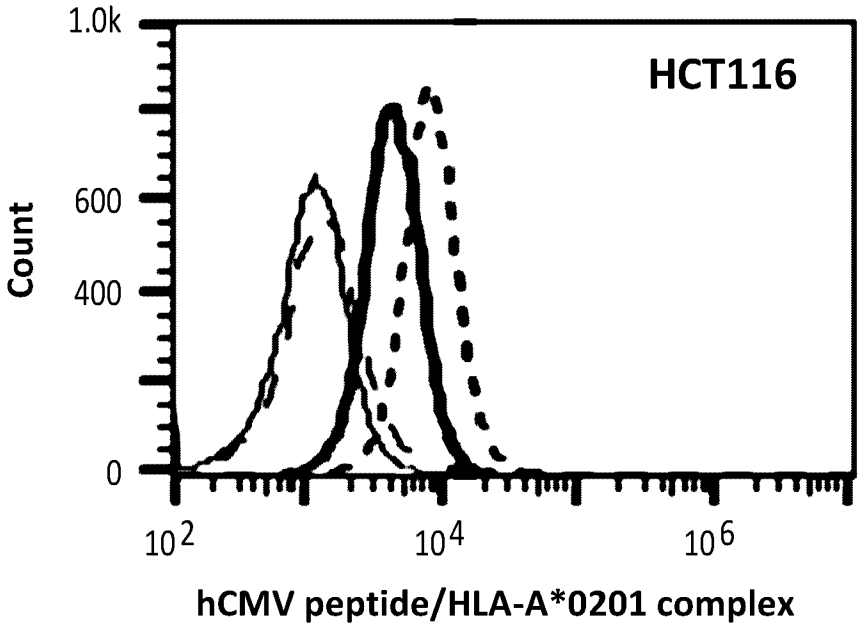

FIG. 11A

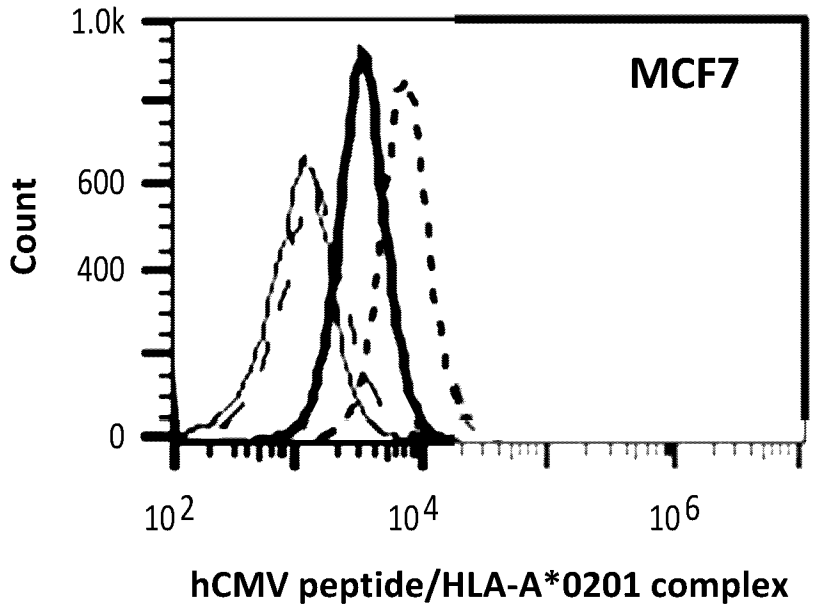

FIG. 11B

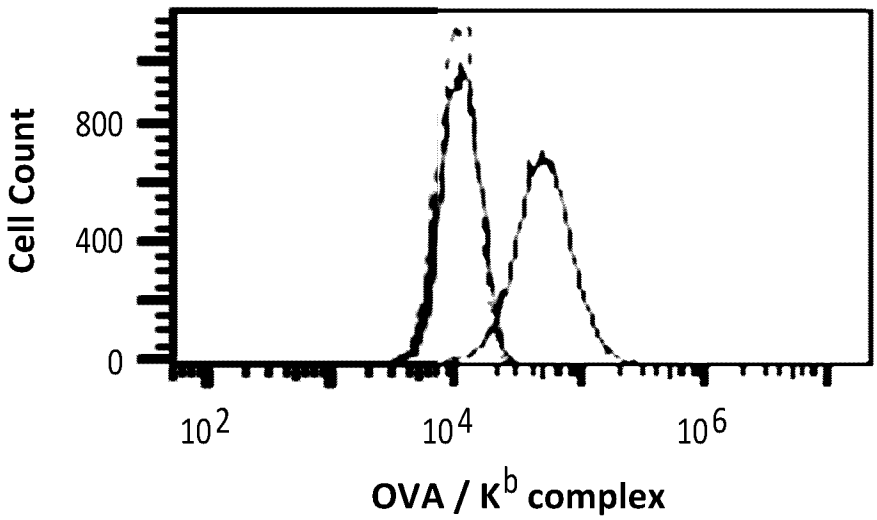

FIG. 12A

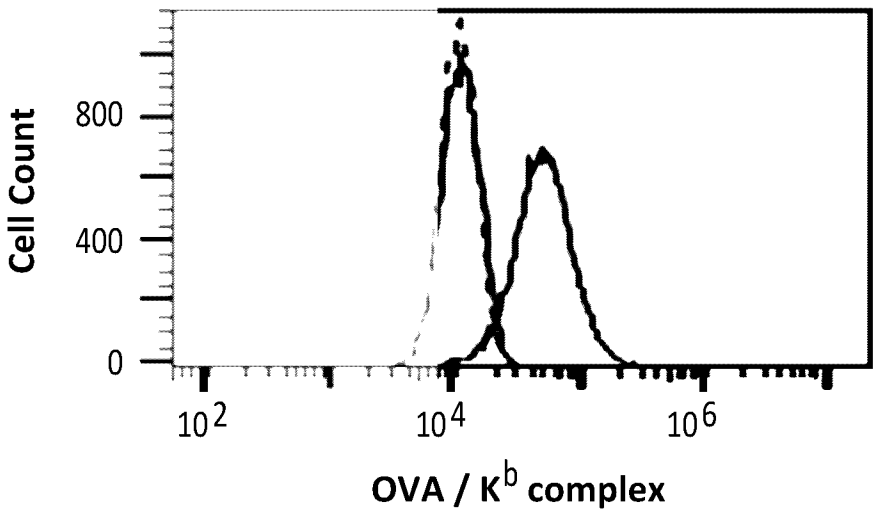

FIG. 12B

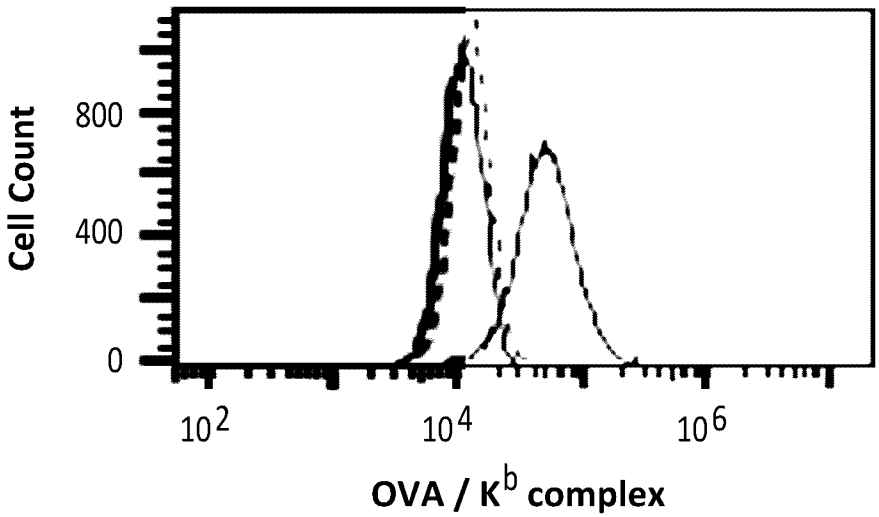

FIG. 12C

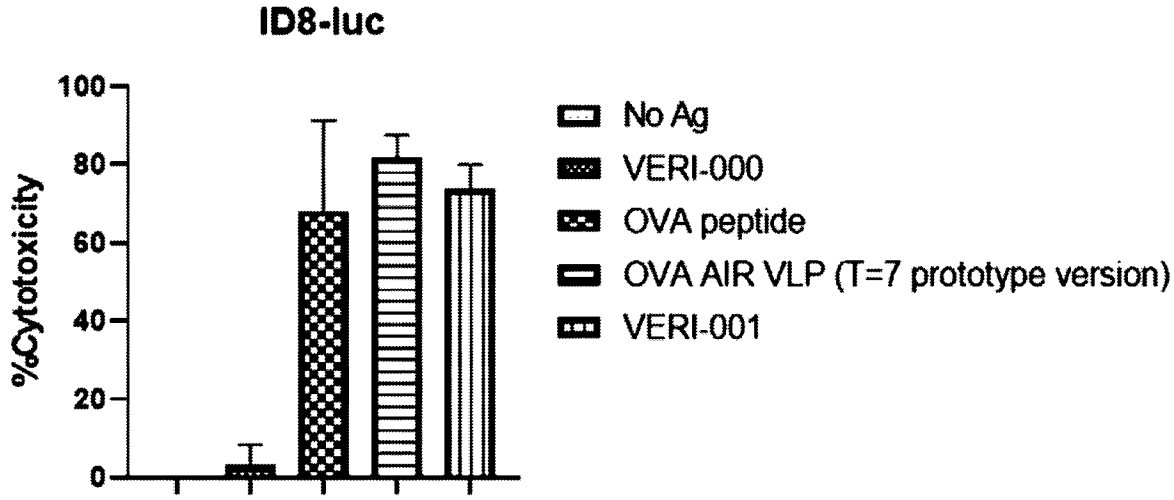

FIG. 13A

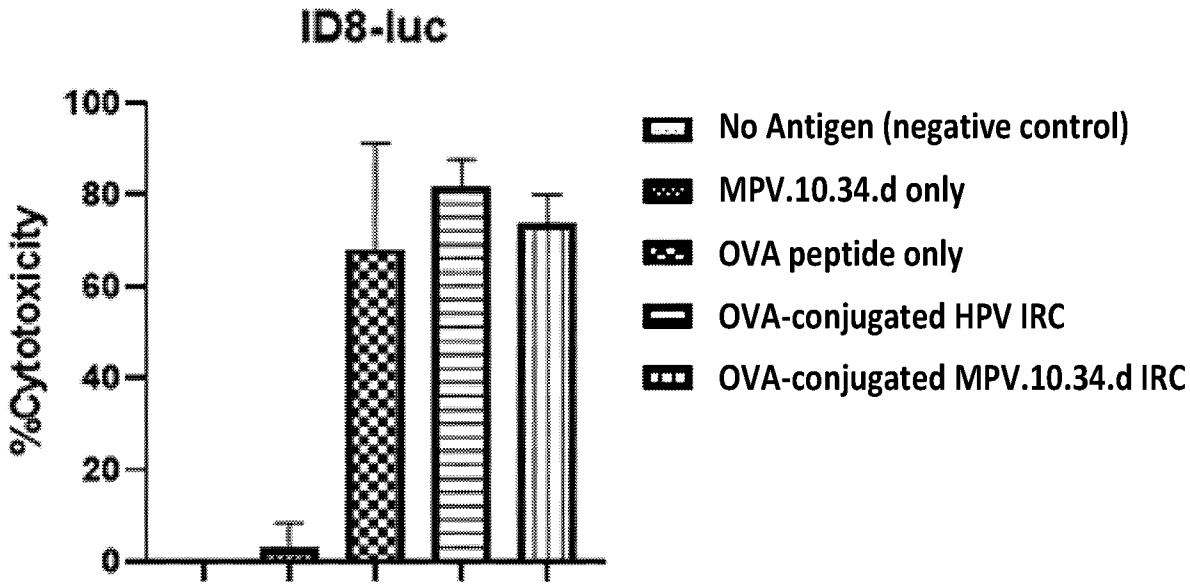

FIG. 13B

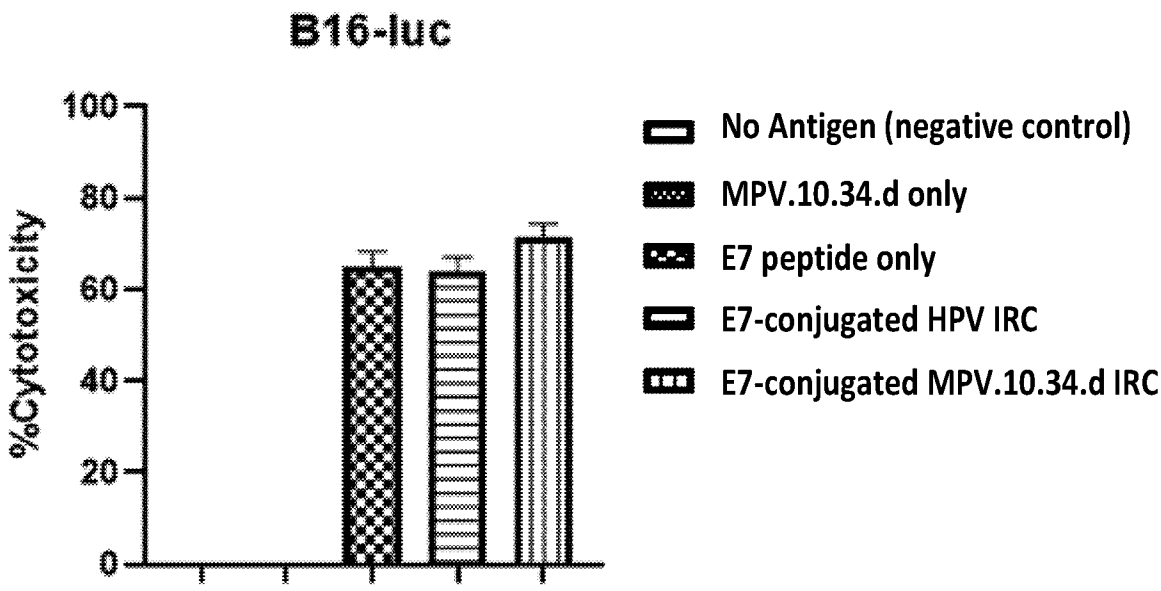

FIG. 14A

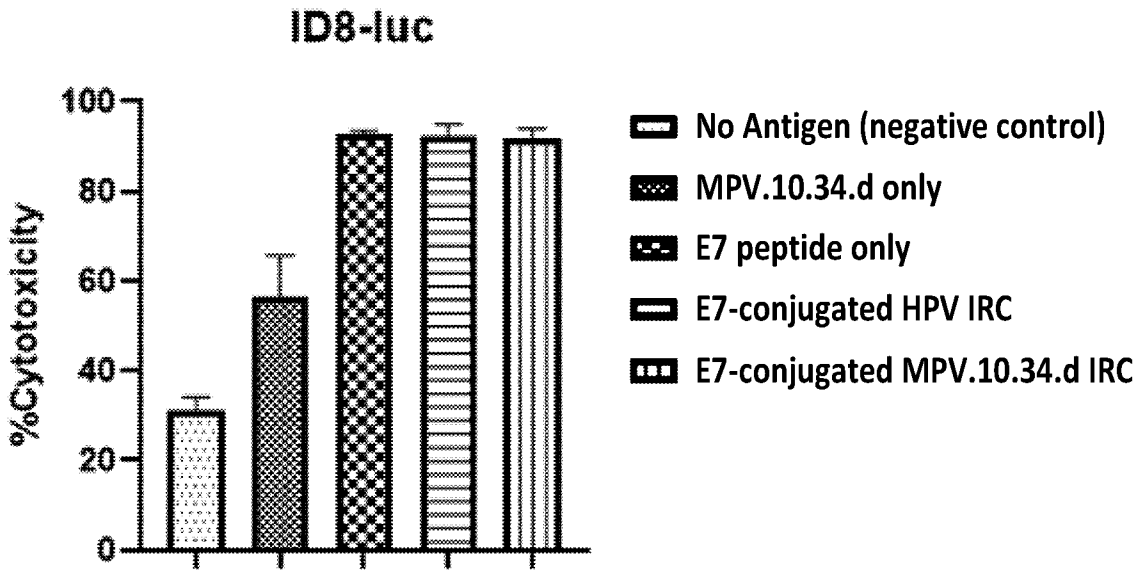

FIG. 14B

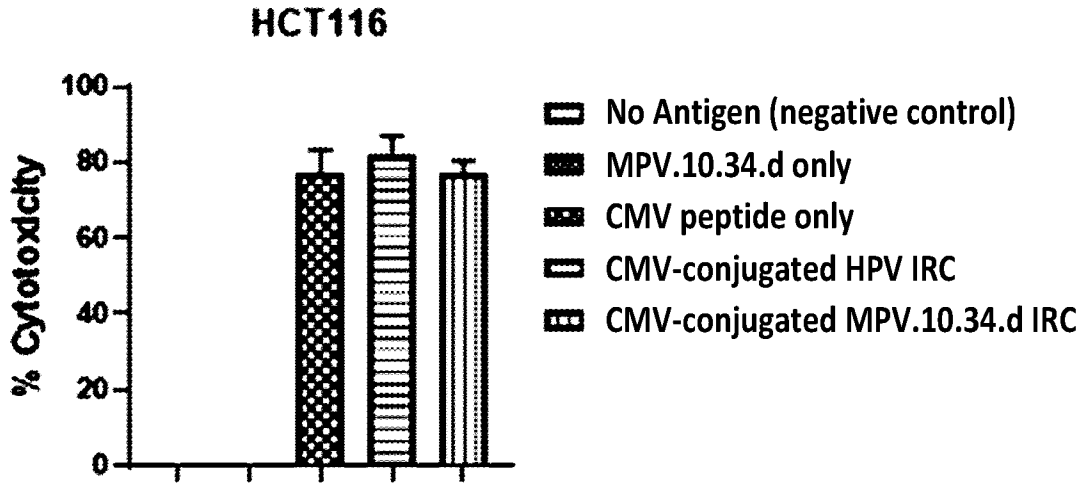

FIG. 15A

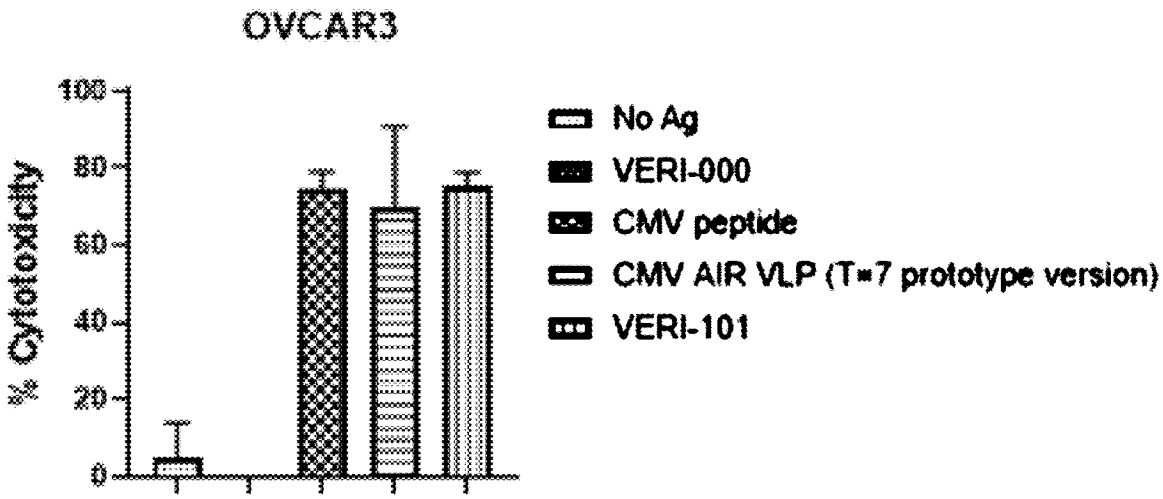

FIG. 15B

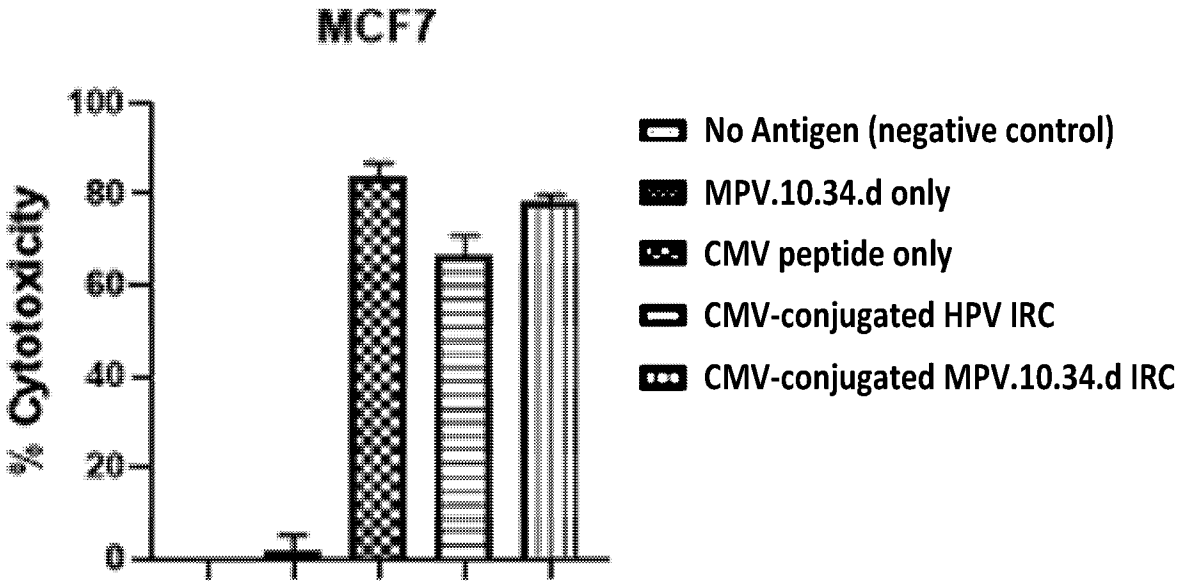
MCF7
% Cytotoxicity
100
80
60
40
20
0
No Antigen (negative control)
MPV.10.34.d only
CMV peptide only
CMV-conjugated HPV IRC
CMV-conjugated MPV.10.34.d IRC

FIG. 15C

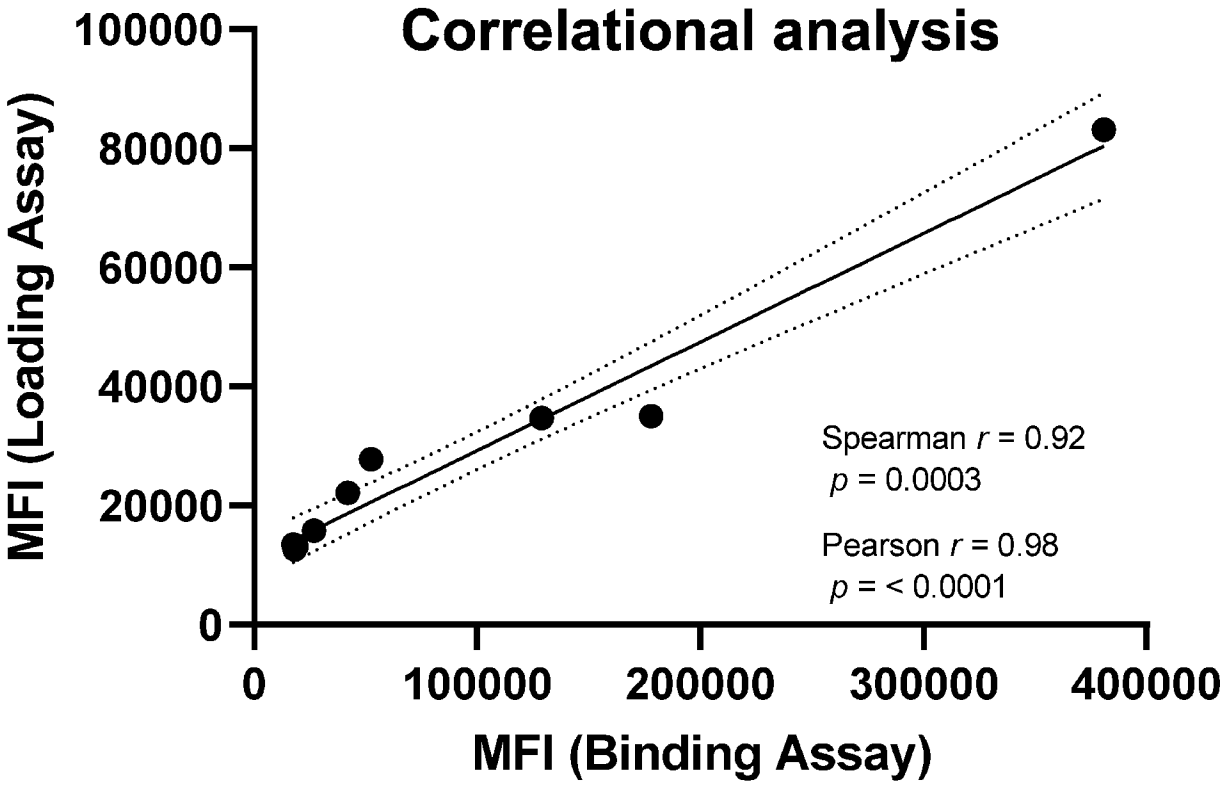
Correlational analysis
MFI (Loading Assay)
100000
80000
60000
40000
20000
0
0
100000
200000
300000
400000
MFI (Binding Assay)
Spearman r = 0.92
p = 0.0003
Pearson r = 0.98
p = < 0.0001

FIG. 16

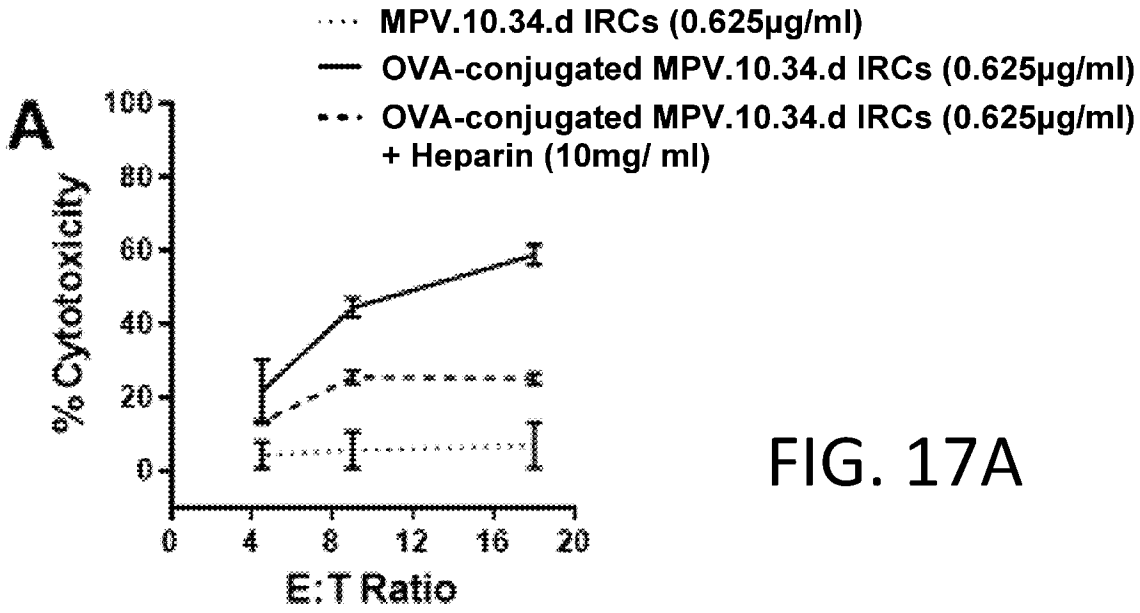

FIG. 17A

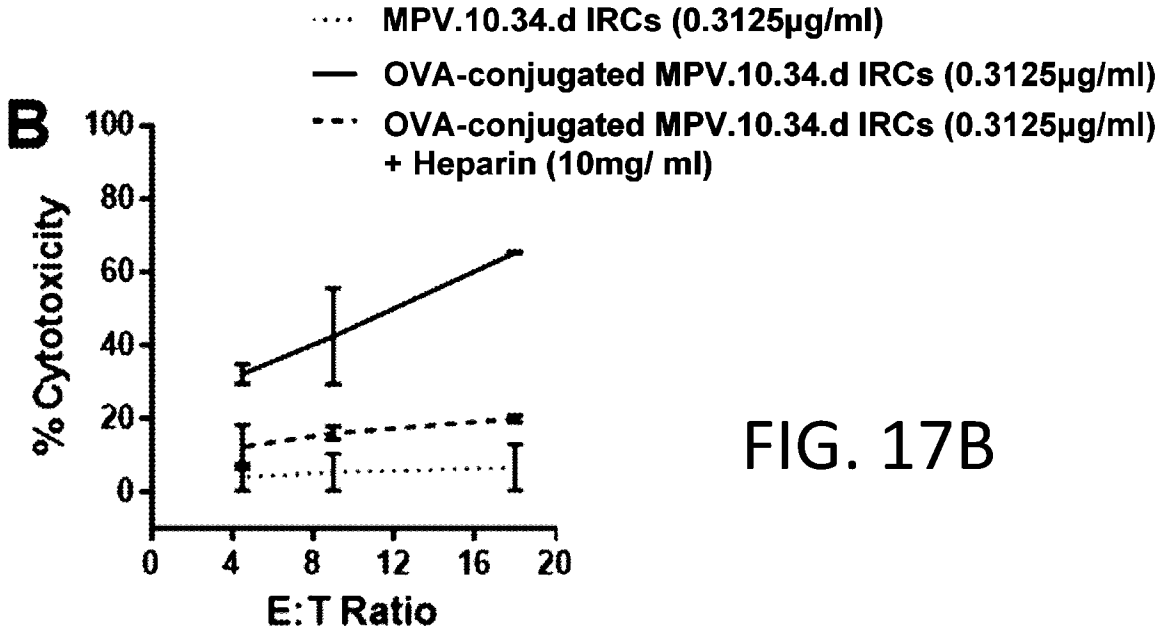

FIG. 17B

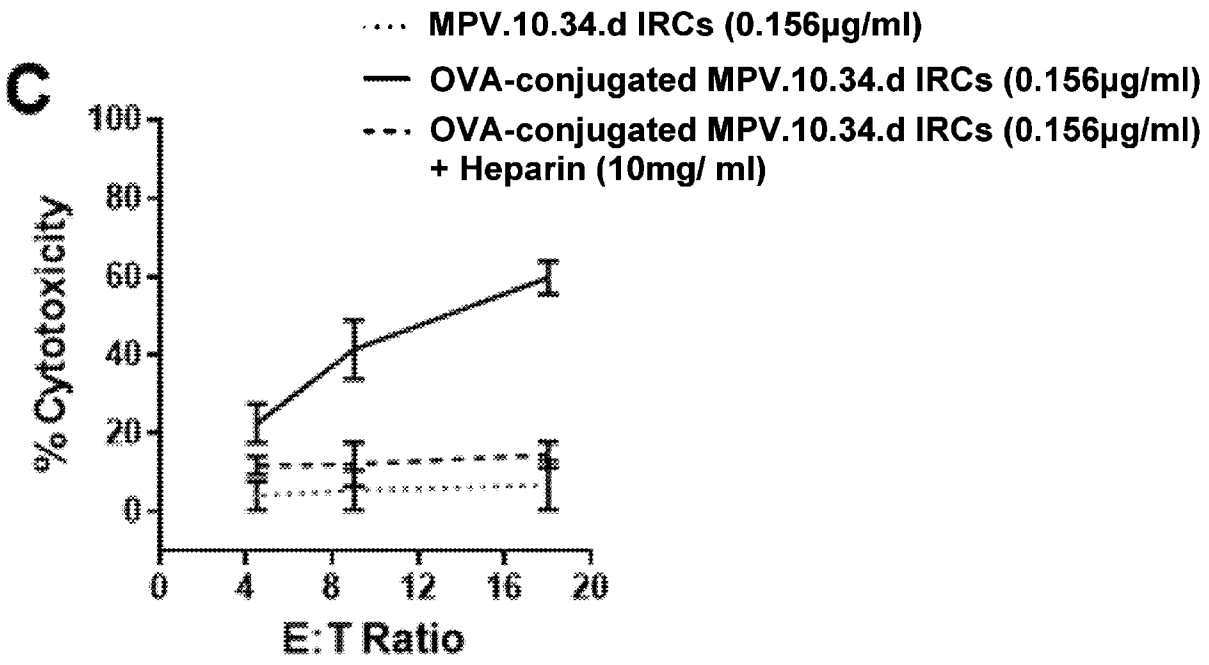
C
MPV.10.34.d IRCs (0.156µg/ml)
OVA-conjugated MPV.10.34.d IRCs (0.156µg/ml)
OVA-conjugated MPV.10.34.d IRCs (0.156µg/ml) + Heparin (10mg/ ml)
% Cytotoxicity
100
80
60
40
20
0
0
4
8
12
16
20
E:T Ratio

FIG. 17C

		Mean % Cytotoxicity (6 replicates)		
Sample (Amount of OVA-conjugated MPV.10.34.d IRC, μg/mL)	Geometric MFI (OVA-conjugated MPV.10.34.d IRC binding level, mean of three replicates)	E:T Ratio 18:1	E:T Ratio 9:1	E:T Ratio 4.5:1
No sample added	1964	13.7	9.3	1.7
0.000625	2127	25.7	19.9	11.0
0.0025	2743	47.4	38.7	22.3
0.01	4437	68.7	56.5	34.0
0.039	9892	79.0	66.9	44.1
0.156	19600	82.0	68.3	48.2
0.625	42281	81.0	68.5	46.2
2.500	139024	80.2	64.4	37.6

FIG. 18

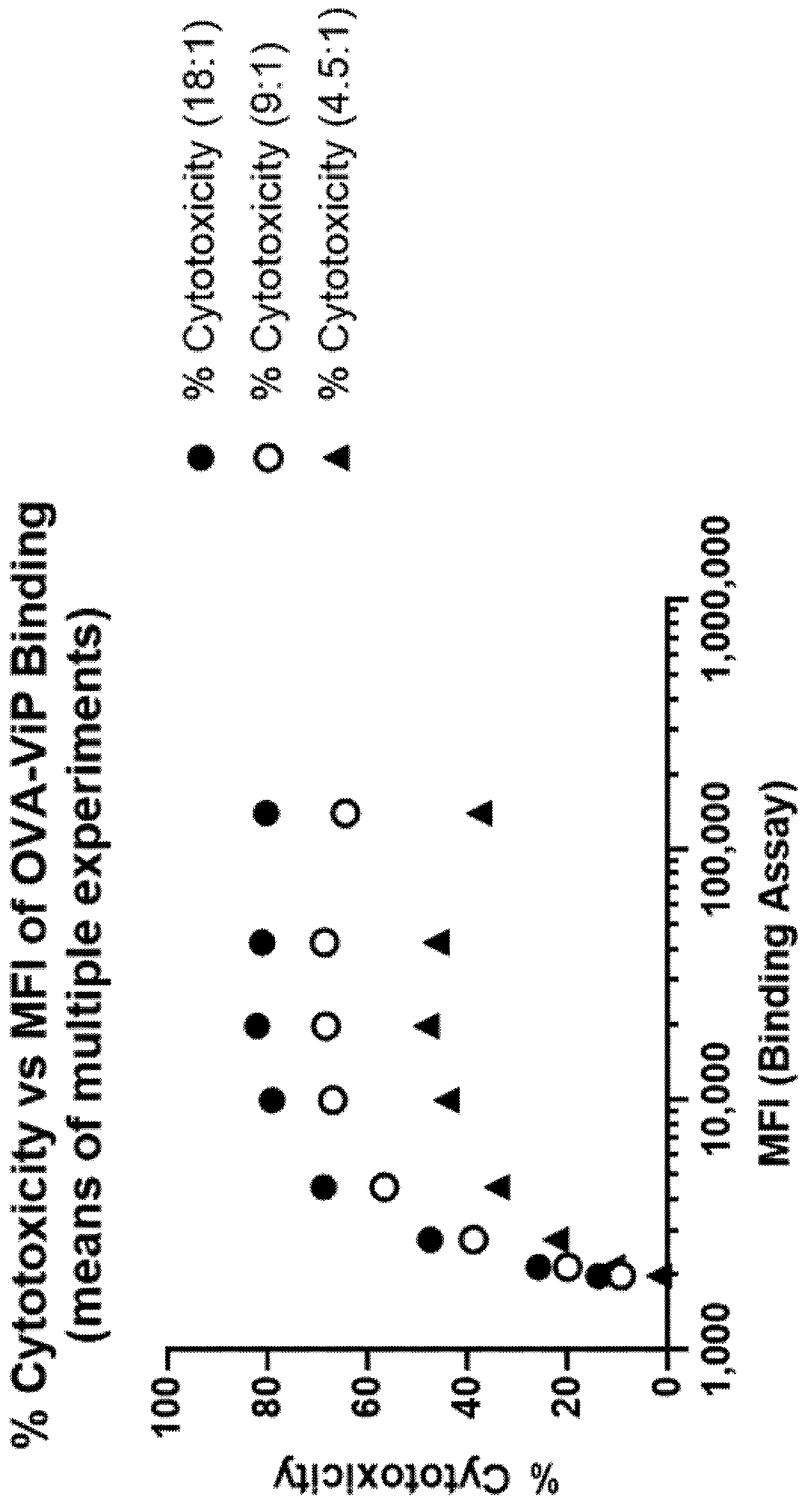
% Cytotoxicity vs MFI of OVA-ViP Binding
(means of multiple experiments)
% Cytotoxicity (18:1)
% Cytotoxicity (9:1)
% Cytotoxicity (4.5:1)
% Cytotoxicity
100
80
60
40
20
0
1,000
10,000
100,000
1,000,000
MFI (Binding Assay)

FIG. 19

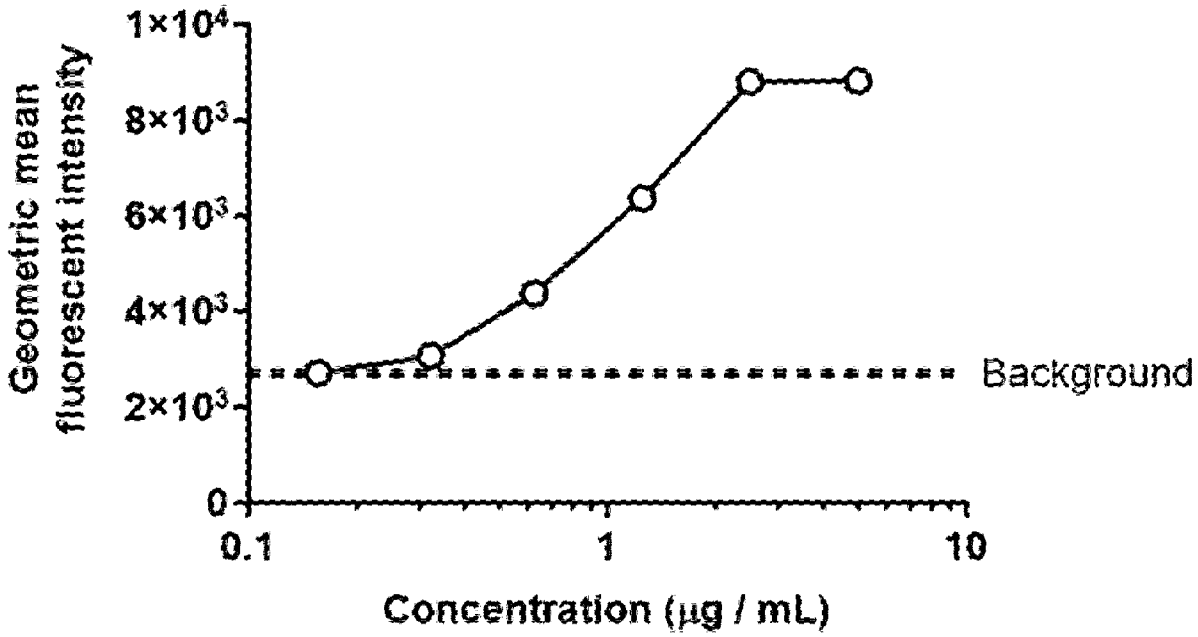
Geometric mean fluorescent intensity
1×10^4
8×10^3
6×10^3
4×10^3
2×10^3
0
0.1
1
10
Concentration (μg / mL)
Background

FIG. 20

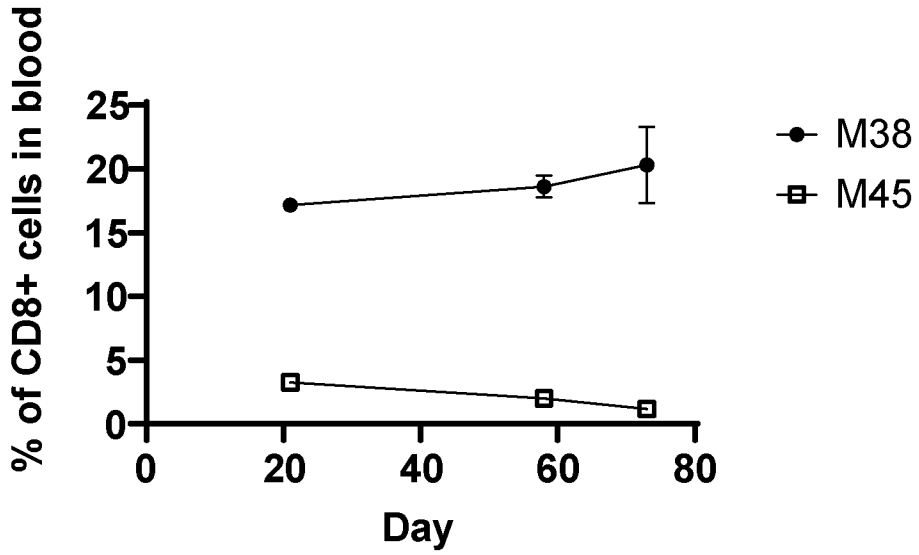

FIG. 21A

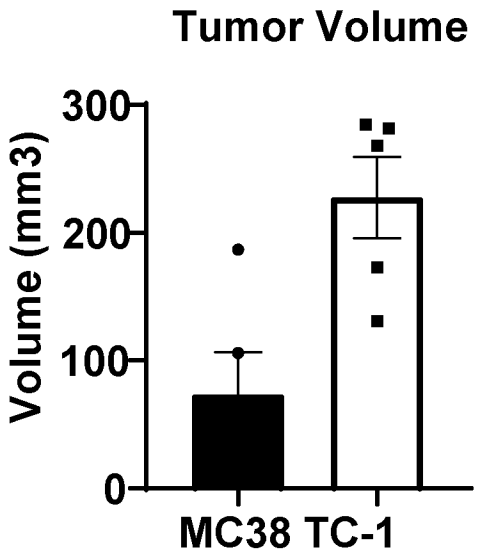

FIG. 21B

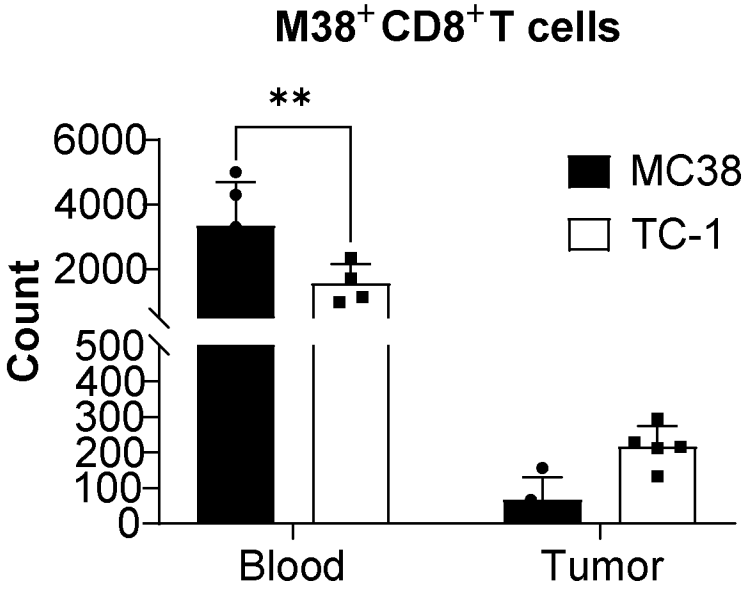

FIG. 21C

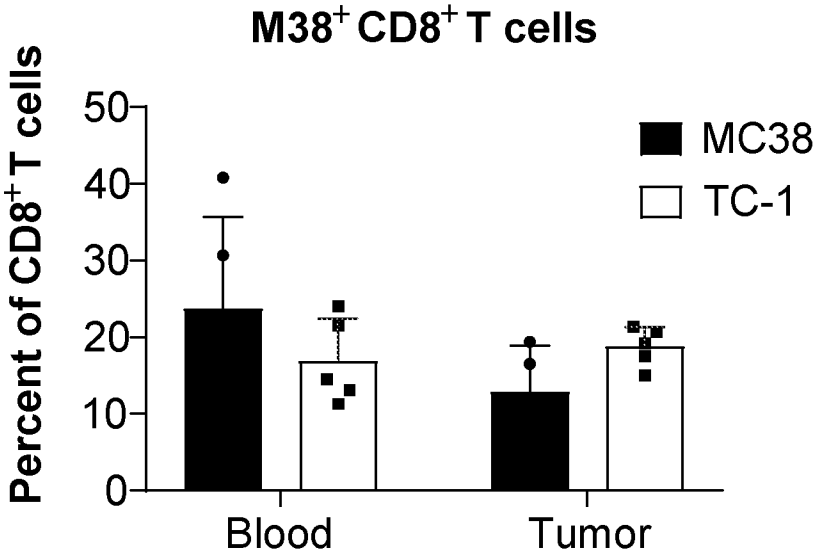

FIG. 21D

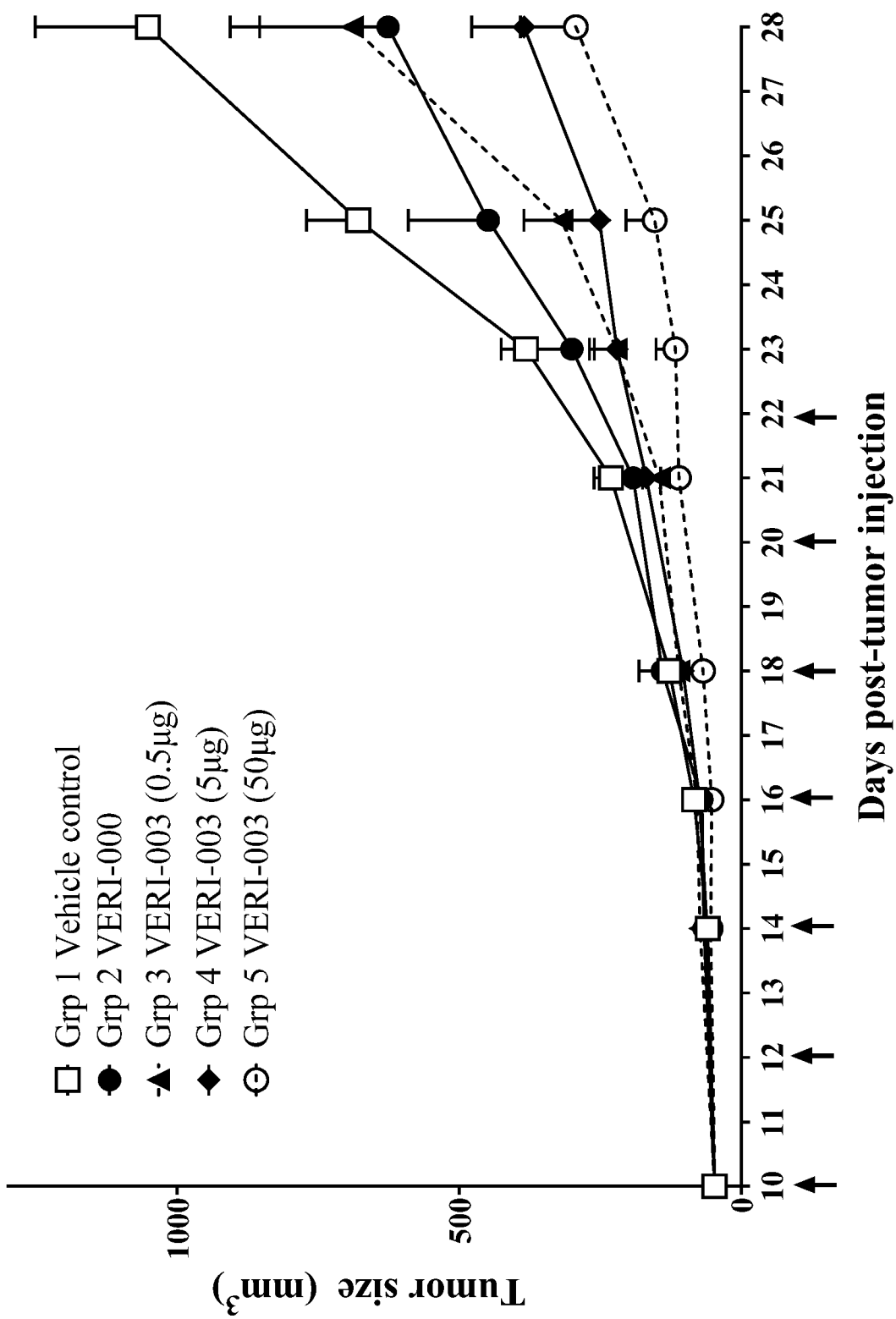
Tumor size (mm³)
1000
500
0
10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Days post-tumor injection
Grp 1 Vehicle control
Grp 2 VERI-000
Grp 3 VERI-003 (0.5µg)
Grp 4 VERI-003 (5µg)
Grp 5 VERI-003 (50µg)

FIG. 22A

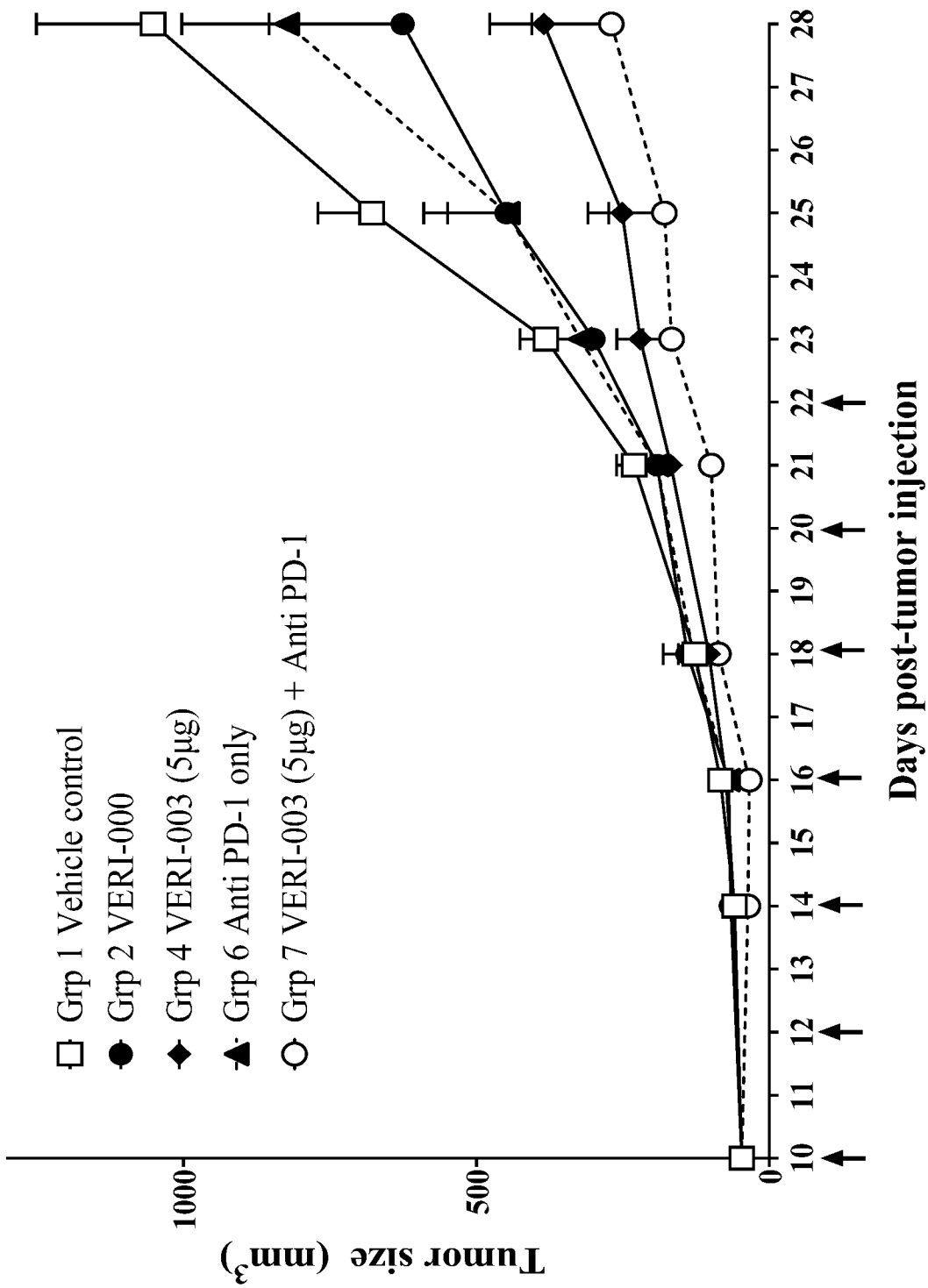
Grp 1 Vehicle control
Grp 2 VERI-000
Grp 4 VERI-003 (5µg)
Grp 6 Anti PD-1 only
Grp 7 VERI-003 (5µg) + Anti PD-1
Tumor size (mm³)
1000
500
0
10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Days post-tumor injection

FIG. 22B

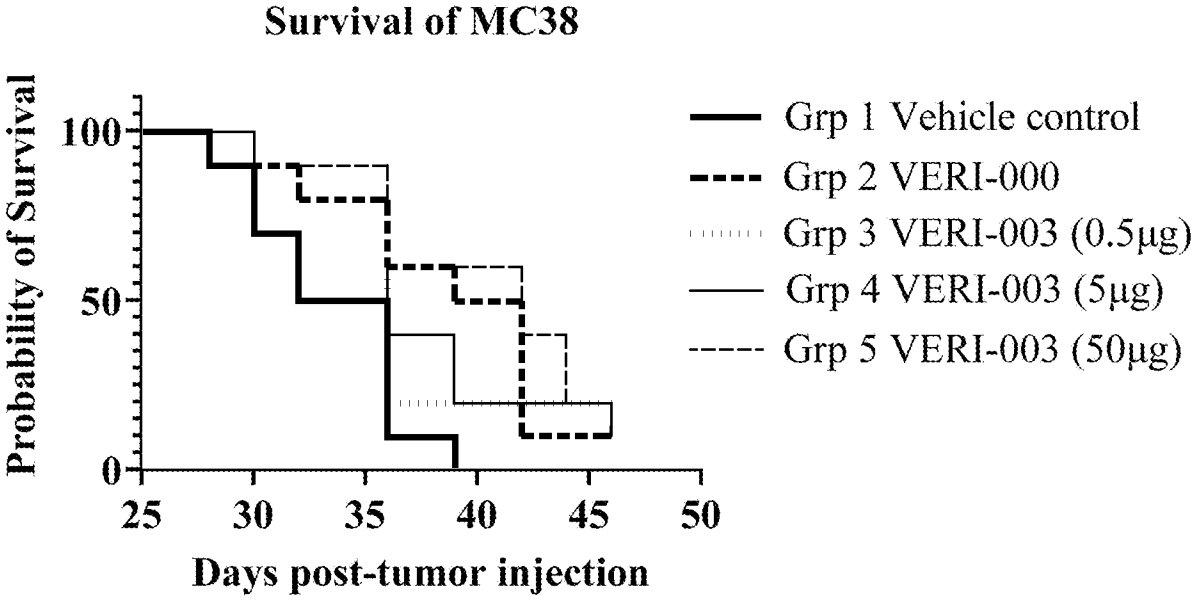

FIG. 23A

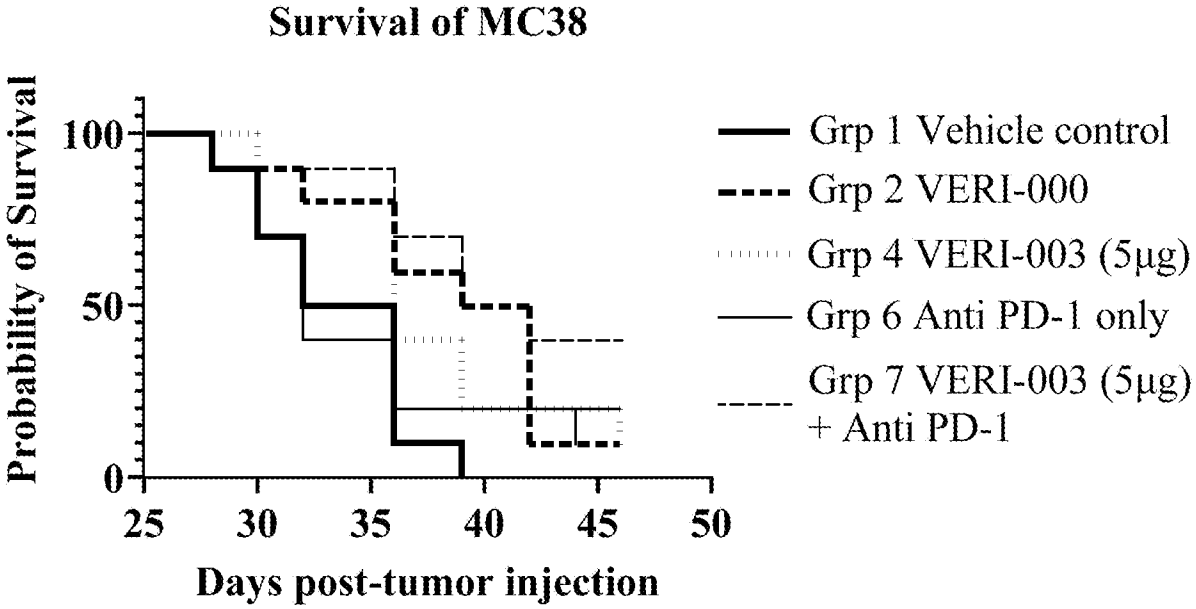

FIG. 23B

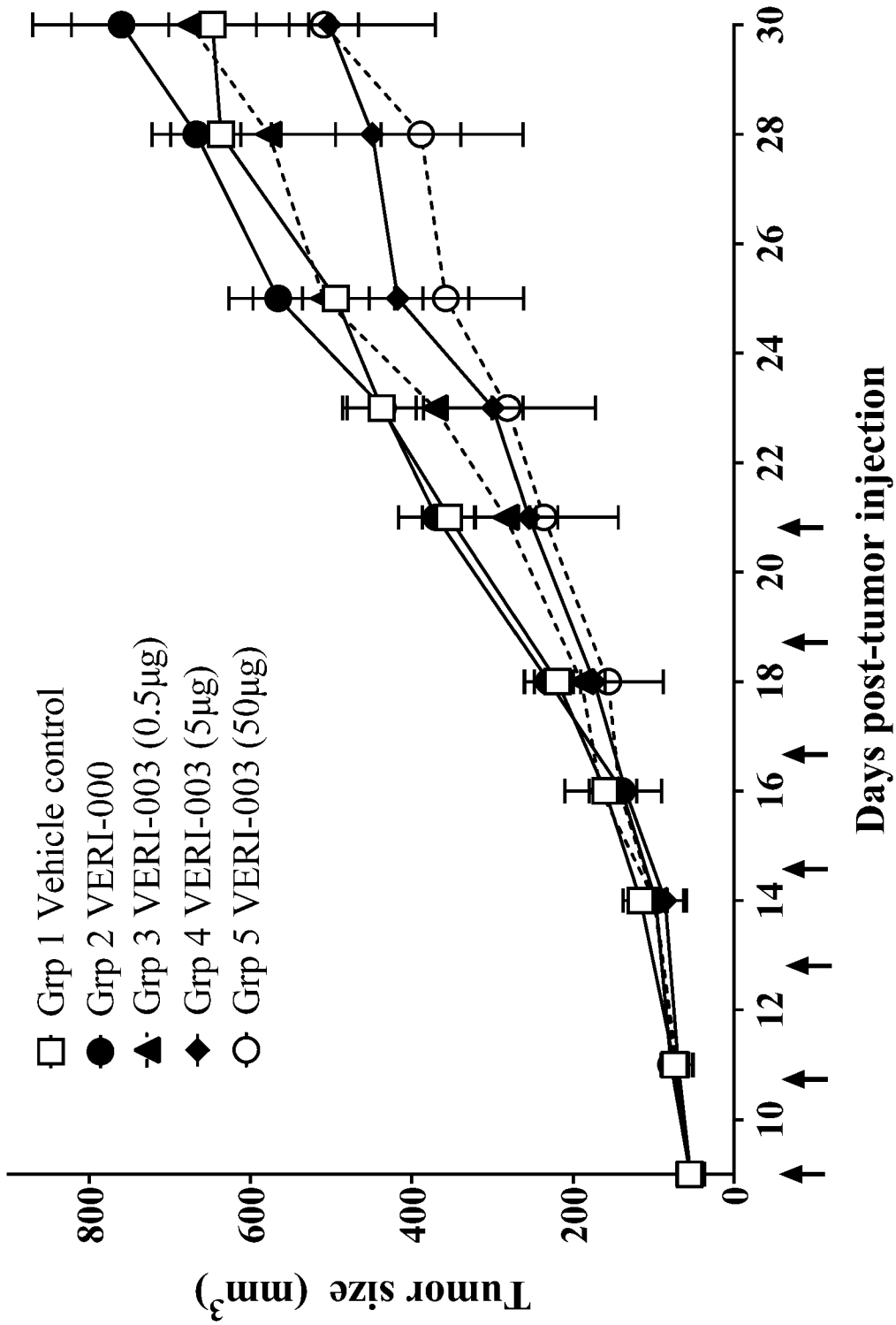
Tumor size (mm³)
800
600
400
200
0
10
12
14
16
18
20
22
24
26
28
30
Days post-tumor injection
Grp 1 Vehicle control
Grp 2 VERI-000
Grp 3 VERI-003 (0.5µg)
Grp 4 VERI-003 (5µg)
Grp 5 VERI-003 (50µg)

FIG. 24A

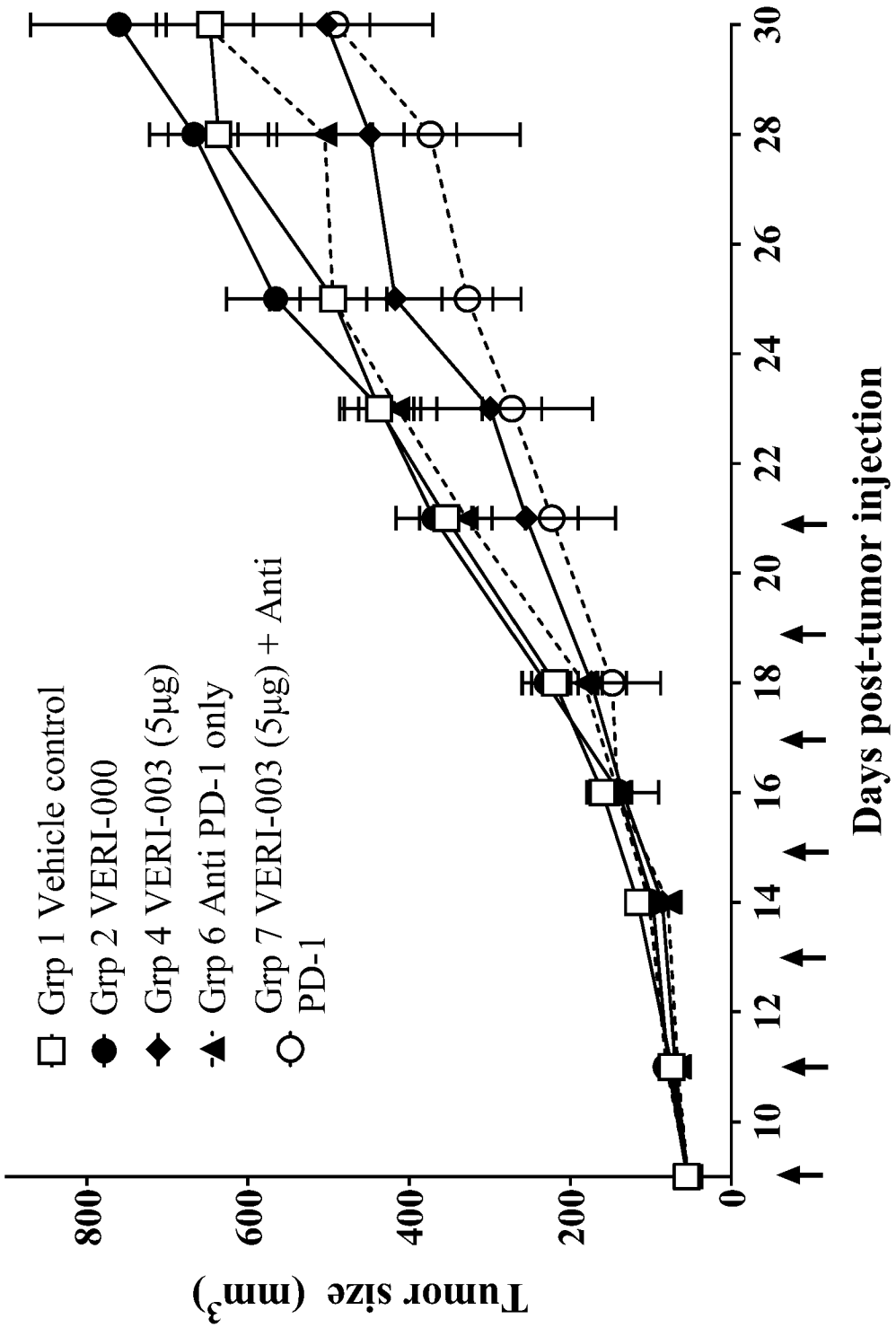
Tumor size (mm³)
800
600
400
200
0
10
12
14
16
18
20
22
24
26
28
30
Days post-tumor injection
Grp 1 Vehicle control
Grp 2 VERI-000
Grp 4 VERI-003 (5µg)
Grp 6 Anti PD-1 only
Grp 7 VERI-003 (5µg) + Anti PD-1

FIG. 24B

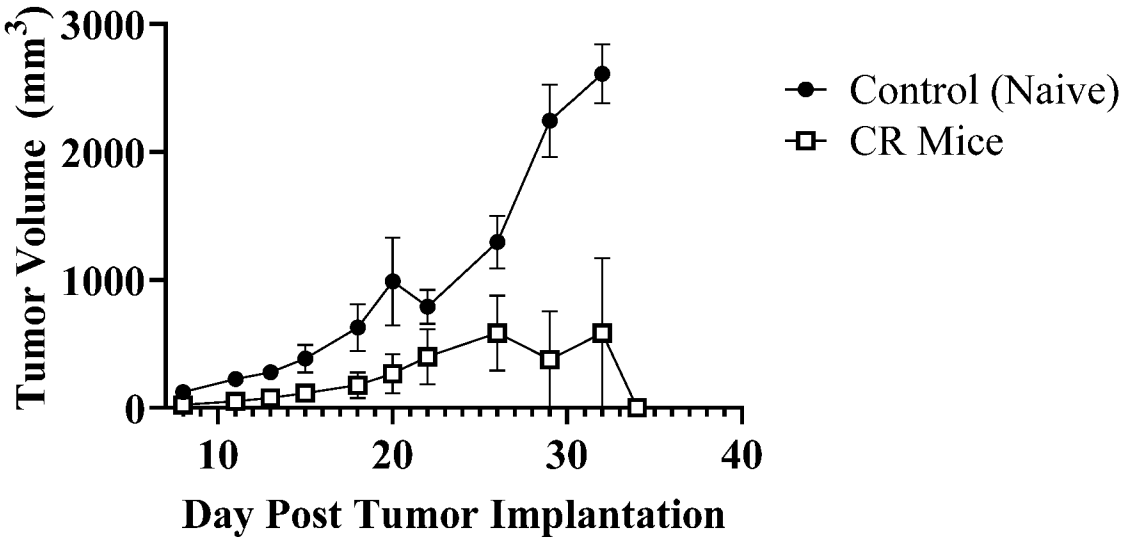

FIG. 25A

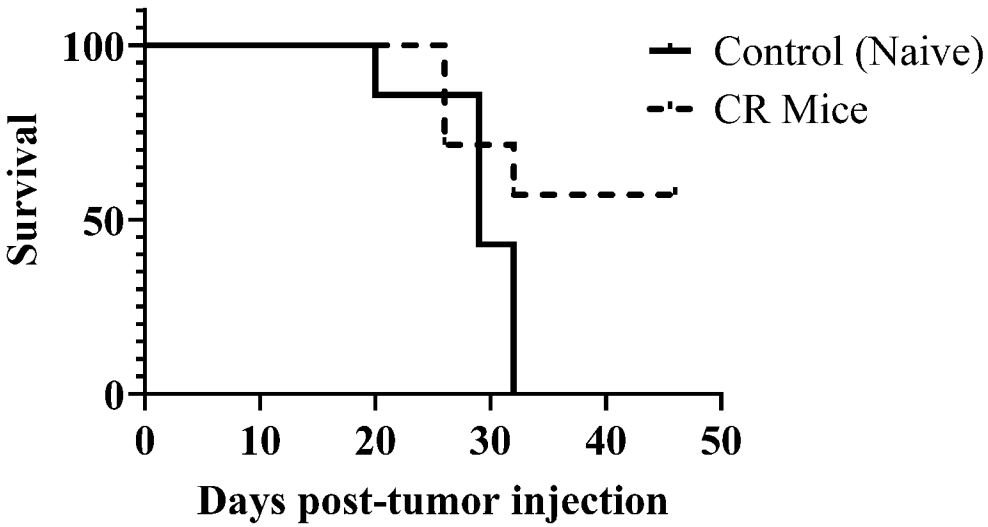

FIG. 25B

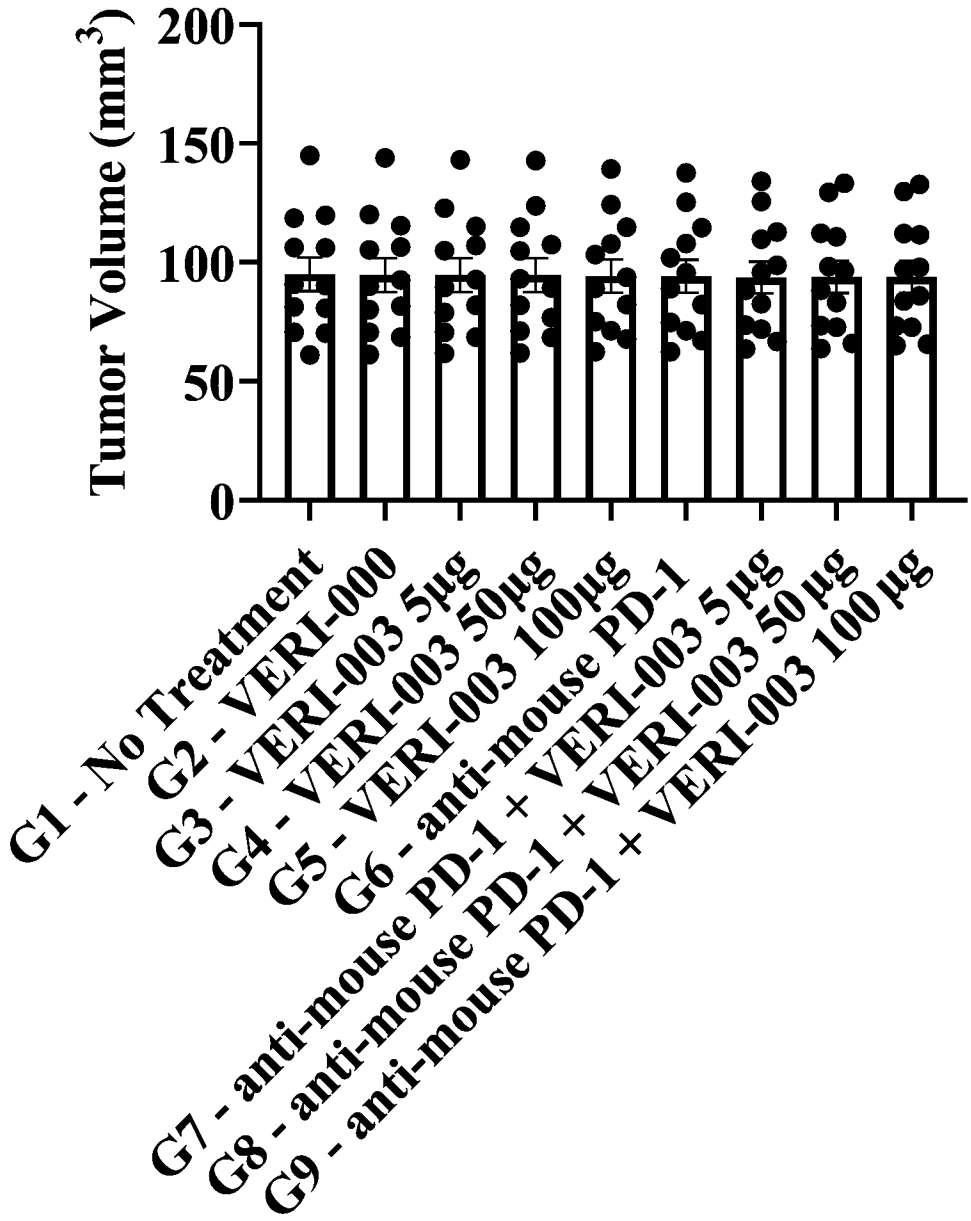
Tumor Volume (mm3)
200
150
100
50
0
G1 - No Treatment
G2 - VERI-000
G3 - VERI-003 5µg
G4 - VERI-003 50µg
G5 - VERI-003 100µg
G6 - anti-mouse PD-1
G7 - anti-mouse PD-1 + VERI-003 5 µg
G8 - anti-mouse PD-1 + VERI-003 50 µg
G9 - anti-mouse PD-1 + VERI-003 100 µg

FIG. 26

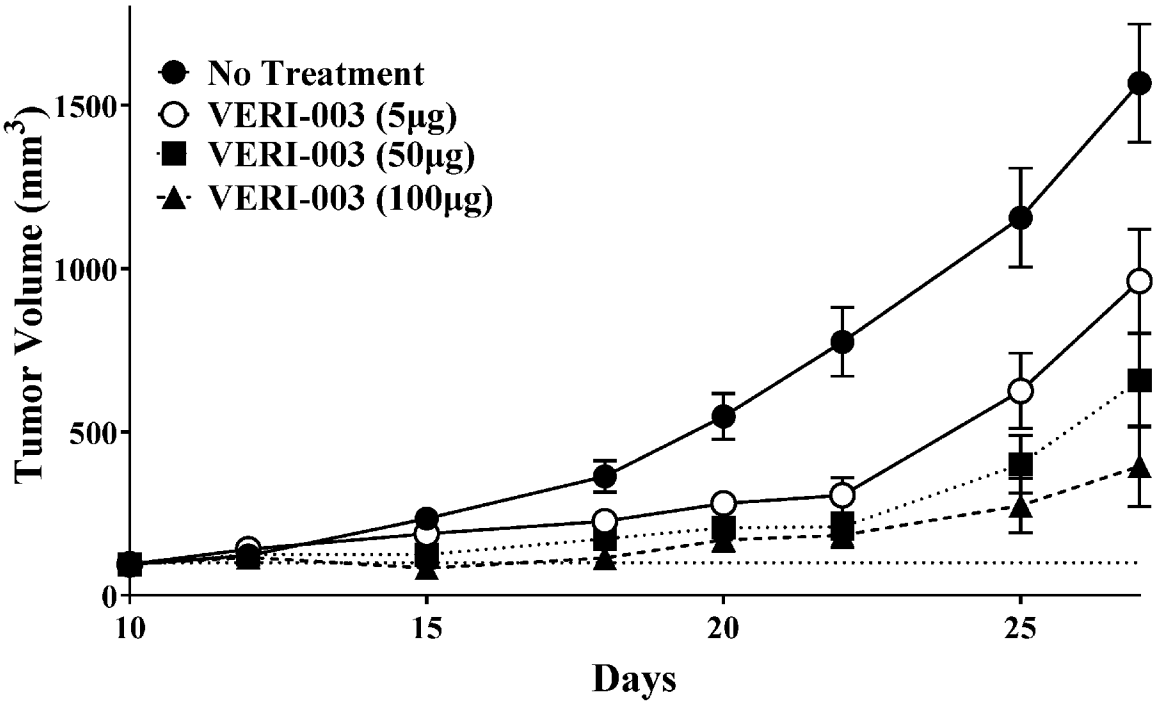

FIG. 27A

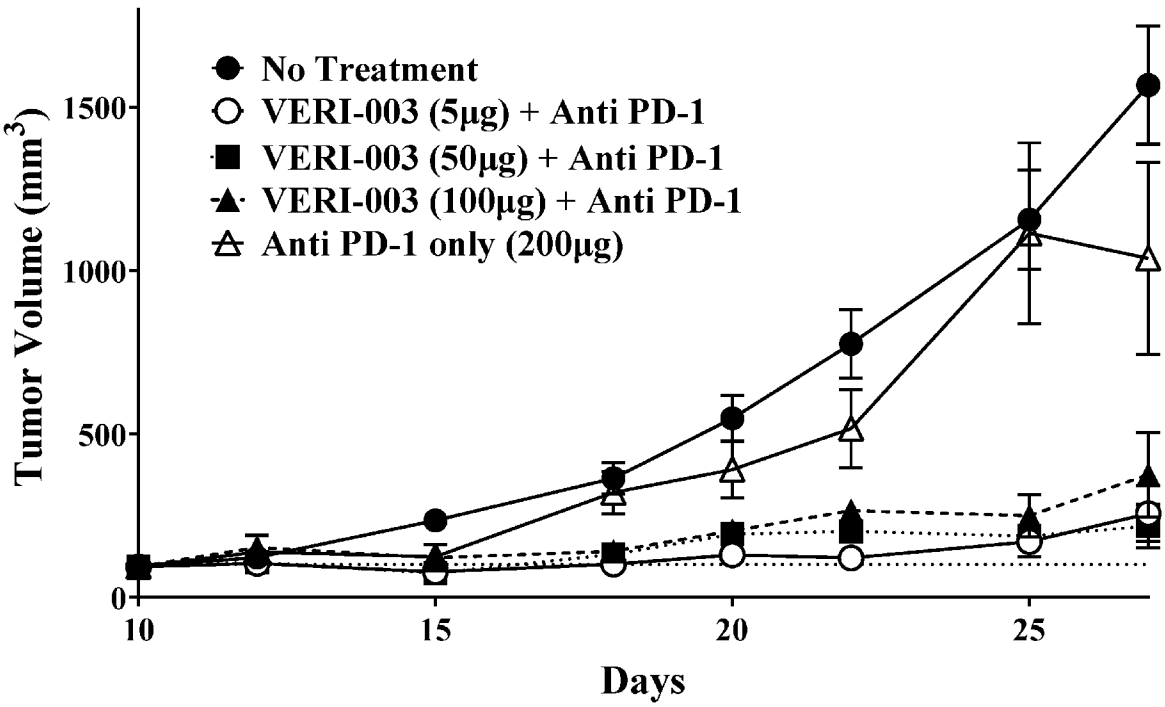

FIG. 27B

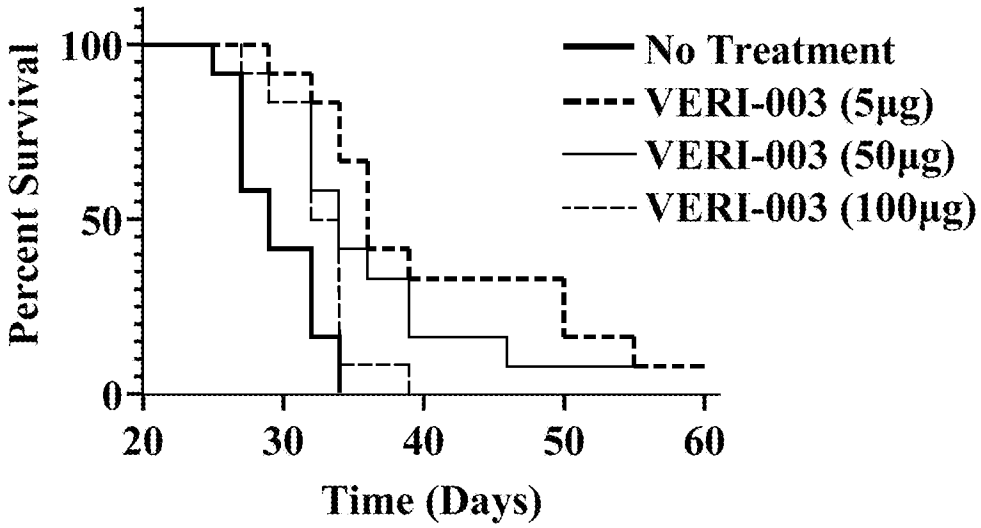

FIG. 28A

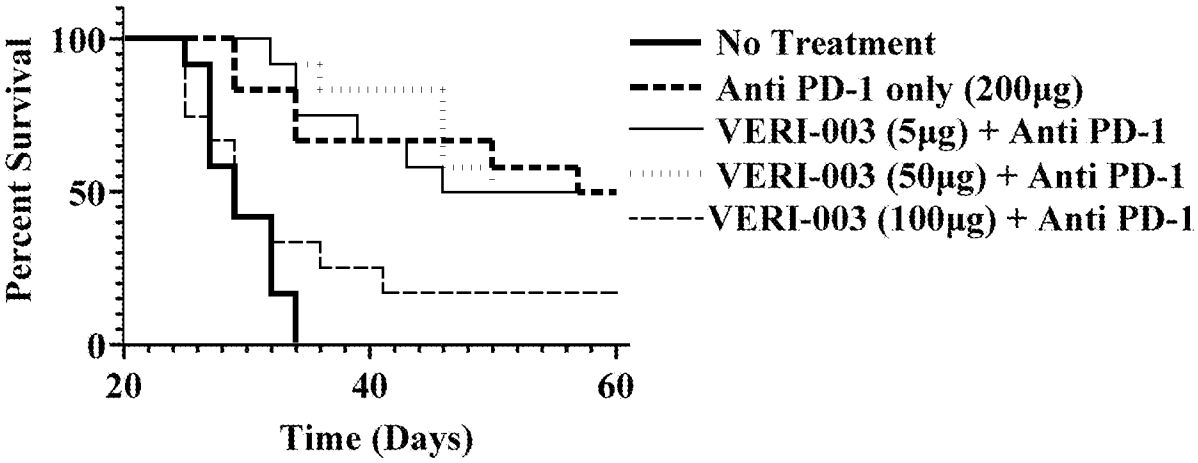

FIG. 28B

Grp	N	Challenge	Treatment Day 8	Dose	Route Frequency	Readouts - Day 22
1	10	B16-F10 tumor cells I.V., 2e5 cells/ animal	No treatment	n/a	n/a	In-life: • Body weights 3x/week Harvest at Day 22: • Count visible lung metastases, weigh lung, prep lung or IHC • Collect terminal blood and run FACS on n=5 per group for M38-specific T cell responses (plus naive mice)
2	10		VERI-000	200μg	IV, Q2D x 6	
3	10		mCMV AIR-ViP (VERI-003)	200μg	IV, Q2D x 6	
4	10		**anti-mouse PD-1 (RMP1-14)**	200μg	**IP, days 8,11,14**	
5	10		**anti-mouse PD-1** + mCMV AIR-ViP (VERI-003)	200μg + 200μg	**IP, days 8,11,14** IV,Q2D x 6	

FIG. 29

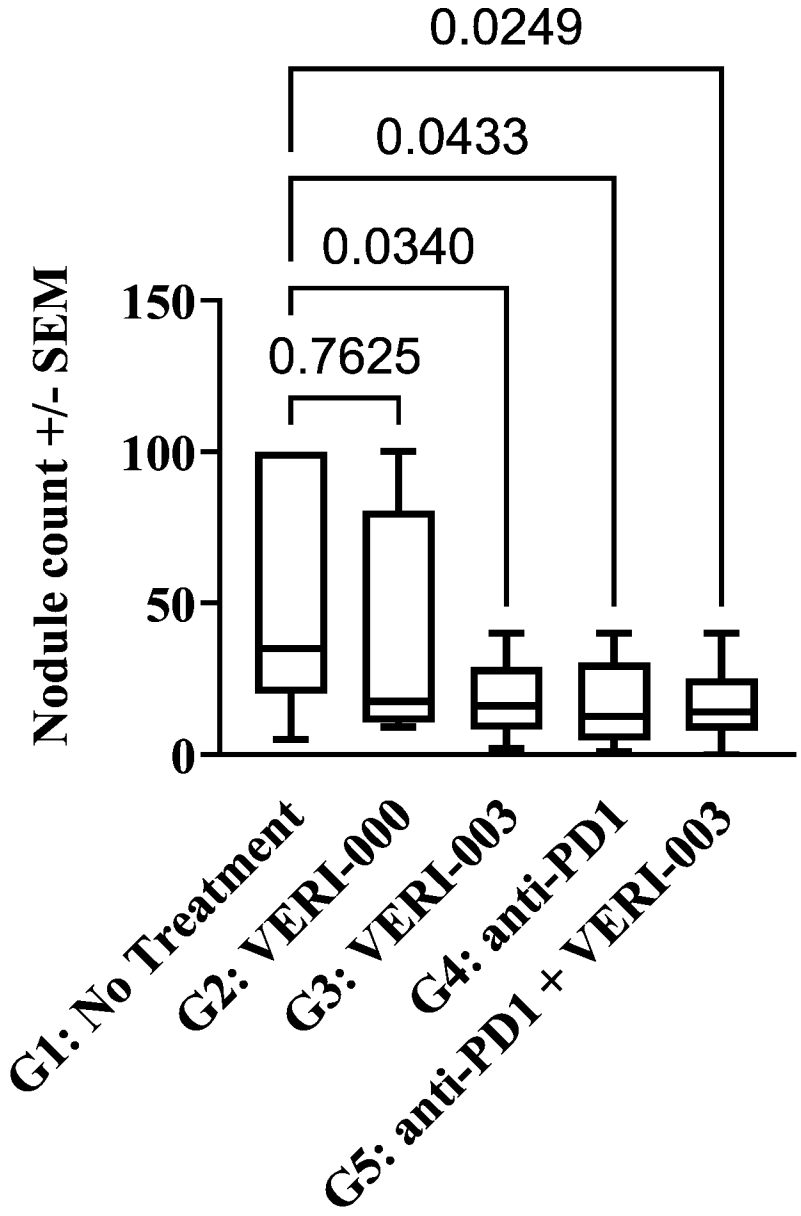
0.0249
0.0433
0.0340
0.7625
150
100
50
0
Nodule count +/- SEM
G1: No Treatment
G2: VERI-000
G3: VERI-003
G4: anti-PD1
G5: anti-PD1 + VERI-003

FIG. 30