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(54) **Title**: VIRUS-LIKE PARTICLES AND METHODS RELATED THERETO

(57) **Abstract**: The invention concerns virus-like particles, methods of preparing virus-like particles, immunogenic compositions that include virus-like particles, methods of eliciting an immune response using virus-like particles or immunogenic compositions that include virus-like particles, and methods of treatment using virus-like particles or immunogenic compositions that include virus-like particles. A virus-like particle (VLP) can include a viral core protein that can self-assemble into the VLP core; at least one antigenic polypeptide expressed on the surface of the VLP, which is select from among a tumor antigen, Dengue virus antigen, or malaria antigen; and at least one adjuvant molecule expressed on the surface of the VLP, such as flagellin.

DESCRIPTION

VIRUS-LIKE PARTICLES AND METHODS RELATED THERETO

5 CROSS-REFERENCE TO RELATED APPLICATION

The present application claims the benefit of U.S. Provisional Application Serial No. 61/899,783, filed November 4, 2013, which is hereby incorporated by reference herein in its entirety, including any figures, tables, nucleic acid sequences, amino acid sequences, or drawings.

10

BACKGROUND OF THE INVENTION

Virus-like particles (VLPs) closely resemble mature virions, but they do not contain viral genomic material (*e.g.*, viral genomic RNA). Therefore, VLPs are nonreplicative in nature, which make them safe for administration in the form of an immunogenic composition
15 (*e.g.*, vaccine). In addition, VLPs can express envelope glycoproteins on the surface of the VLP, which is the most physiological configuration. Moreover, since VLPs resemble intact virions and are multivalent particulate structures, VLPs may be more effective in inducing neutralizing antibodies to the envelope glycoprotein than soluble envelope antigens. Further, VLPs can be administered repeatedly to vaccinated hosts, unlike many recombinant vaccine
20 approaches.

BRIEF SUMMARY OF THE INVENTION

The present invention concerns virus-like particles (VLPs), methods of preparing virus-like particles, immunogenic compositions that include virus-like particles, methods of
25 eliciting an immune response using virus-like particles or immunogenic compositions that include virus-like particles, and methods of treatment using virus-like particles or immunogenic compositions that include virus-like particles. Without being limited by theory, the inventors propose that inclusion of adjuvant molecules such as flagellin can induce cytokine response and enhance major histocompatibility complex (MHC) presentation
30 on the surface of antigen-presenting cells, increasing the ability of a host's immune system to recognize the full repertoire of glycoproteins, including those of other serotypes of virus (*e.g.*,

serotypes of Dengue virus other than the serotype represented by the administered antigen), and shifting antigen profiles exhibited by malignancies, which tend to mutate. For example, administration of a VLP expressing an antigen of DEN1 and flagellin may provide an effective immune response against DEN2, DEN3, and DEN4. Likewise, administration of a 5 VLP expressing a tumor antigen and flagellin may provide an effective and durable immune response against malignancies exhibiting a shifting or mutating antigen profile. In this way, incorporation of adjuvant molecules such as flagellin into VLPs may further enhance the immunogenic properties of the VLPs, increasing the range of their immunological targets.

One aspect of the invention includes a virus-like particle (VLP) comprising a viral 10 core protein that can self-assemble into the VLP core; at least one antigenic polypeptide expressed on the surface of the VLP, wherein the antigenic polypeptide comprises a tumor antigen, Dengue virus antigen, or malaria antigen; and at least one adjuvant molecule expressed on the surface of the VLP, such as flagellin. Preferably, the antigenic polypeptide is a surface antigen.

15 Another aspect of the invention includes an immunogenic composition. The immunogenic composition includes a pharmacologically acceptable carrier and at least one of the VLPs described above. Another aspect of the invention includes a method of generating an immunological response in a subject by administering an effective amount of one or more of the VLPs or immunogenic compositions described herein to the subject. Furthermore, 20 another aspect of the invention includes a method of treating a condition by administering to a subject in need of treatment an effective amount of one or more of a VLP or immunogenic composition described herein.

Another aspect of the invention includes methods of determining exposure of a host to an antigen (a tumor antigen, Dengue virus antigen, or malaria antigen). An exemplary 25 method, among others, includes the steps of: contacting a biological fluid of the host with one or more of the VLPs expressing a tumor antigen, Dengue virus antigen, or malaria antigen on its surface, as discussed above, under conditions which are permissive for binding of antibodies in the biological fluid with the VLP; and detecting binding of antibodies within the biological fluid with the VLP, whereby exposure of the host to the virus is determined by the 30 detection of antibodies bound to the VLP.

Another aspect of the invention includes methods of making VLPs. In some embodiments, the method includes the steps of: providing a viral core protein expression vector; providing an antigenic polypeptide expression vector, wherein the antigenic

polypeptide is a tumor antigen, Dengue virus antigen, or malaria antigen; providing a membrane-anchored adjuvant molecule expression vector; and introducing into a cell the viral core protein expression vector, the antigenic polypeptide expression vector, and the adjuvant molecule expression vector and allowing for expression of the antigenic polypeptide
5 and the adjuvant molecule (such as flagellin), whereby the VLP is formed by the cells.

Another embodiment of a method of making VLPs includes the steps of: providing one or more expression vectors including polynucleotide sequences encoding for a viral core protein, at least one antigenic polypeptide, wherein the antigenic polypeptide is a tumor antigen, Dengue virus antigen, or malaria antigen, and at least one adjuvant molecule, such as
10 flagellin; introducing the one or more expression vectors into a host cell; and expressing the viral core protein, the at least antigenic polypeptide, and the at least one adjuvant molecule, whereby the VLP is formed by the cell.

DETAILED DESCRIPTION OF THE INVENTION

15 Methods and components for making VLPs are disclosed in U.S. Patent Publication No. 20060216702 (Compans R.W. *et al.*), entitled "Virus-like particles, methods of preparation, and immunogenic compositions, which is incorporated herein by reference in its entirety. The methods and components for VLP production described therein can be utilized to make the VLPs of the subject invention, with the differences noted herein as to the use of a
20 tumor antigen, Dengue virus antigen, or malaria antigen. In addition, VLPs of the subject invention may be produced using hollow-fiber bioreactors in accordance with methods disclosed in PCT Publication No. WO 2012/171026 (Hirschel M. *et al.*), entitled Method and Apparatus for Virus and Vaccine Production", which is incorporated herein by reference in its entirety.

25 The present invention provides virus-like particles, methods of using the virus-like particles, and methods of making virus-like particles that can be used in immunogenic compositions to treat conditions in a subject, and the immunogenic compositions that include virus-like particles. The virus-like particles can be used to enhance immune responses (*e.g.*, antibody production, cytotoxic T cell activity, and cytokine activity). In particular, virus-like
30 particles can act as a prophylactic as a vaccine to prevent or delay the onset of malignancies, Dengue virus infections, and malaria infections.

In general, the virus-like particle ("VLP") includes at least a viral core protein (hereinafter "viral protein") and at least one antigenic polypeptide selected from tumor antigen, Dengue virus antigen, and malaria antigen. Thus, the at least one antigenic polypeptide can be of viral origin (Dengue virus), plasmodium origin (malaria), or originate
5 from a malignancy (tumor antigen). Optionally, the tumor antigen can be one obtained from a subject to which the VLP or immunogenic composition is to be administered.

In addition, the VLP can include at least one adjuvant molecule. The adjuvant molecules can include more than one type of adjuvant molecule. Furthermore, the VLP can include a lipid membrane, viral glycoprotein transmembrane unit, and a matrix protein. In
10 particular, chimeric VLPs are VLPs having at least one antigenic polypeptide incorporated into the VLP, wherein the viral core protein and at least one antigenic polypeptide are from different viruses. Thus, chimeric VLPs also include VLPs wherein there are more than one type of antigenic polypeptide, and wherein one or both of are from a different virus than the viral core protein. Furthermore, a phenotypically mixed VLPs include VLPs having at least
15 one adjuvant molecule incorporated into the VLP, where at least one of the adjuvant molecule(s) have an origin different from that of the viral core protein and/or the antigenic polypeptide. Phenotypically mixed VLPs also include VLPs wherein there are more than one type of adjuvant molecule, and wherein two or more adjuvant molecules are from a different source from each other. Phenotypically mixed VLPs also include VLPs wherein the source
20 of one or more adjuvant molecules is different from the source of at least one antigen polypeptide and/or from the viral core protein.

Viral proteins include proteins that are capable of self-assembling into the VLP (Freed, E. O., *J. Virol.*, 76, 4679-87 (2002)). In pd1024articular, the viral core proteins can include, but are not limited to, a viral Gag protein, in particular, a retrovirus gag protein [*e.g.*,
25 a HIV Gag viral protein (*e.g.*, HIV-1 NL43 Gag (GenBank serial number AAA44987)), a simian immunodeficiency virus (SIV) Gag viral protein (*e.g.*, SIVmac239 Gag (GenBank serial number CAA68379)), or a murine leukemia virus (MuLV) Gag viral protein (*e.g.*, MuLV Gag (GenBank serial number S70394))], a retrovirus matrix protein, a rhabdovirus matrix protein M protein [*e.g.*, a vesicular stomatis virus (VSV) M protein (*e.g.*, VSV Matrix
30 protein (GenBank serial number NP041714))], a filovirus viral core protein (*e.g.*, an Ebola VP40 viral protein (*e.g.*, Ebola virus VP40 (GenBank serial number AAN37506))), a Rift Valley Fever virus N protein (*e.g.*, RVFV N Protein (GenBank serial number NP049344)), a coronavirus M, E and NP protein (*e.g.*, GenBank serial number NP040838 for NP protein,

NP 040835 for M protein, CAC39303 for E protein of Avian Infections Bronchitis Virus and NP828854 for E protein of the SARS virus)), a bunyavirus N protein (GenBank serial number AAA47114)), an influenza M1 protein, a paramyxovirus M protein, an arenavirus Z protein (*e.g.*, a Lassa Fever Virus Z protein), and combinations thereof. Appropriate surface glycoproteins and/or viral RNA may be included to form the VLP.

In general, the viral protein sequence and the corresponding polynucleotide sequence can be found in GenBank and the access numbers can be obtained online at National Center for Biotechnology Information (NCBI). In addition, the sequences identified for the viral proteins above are only illustrative examples of representative viral proteins. Furthermore, variants that are substantially homologous to the above referenced viral proteins and viral proteins having conservative substitutions of the above referenced viral proteins can also be incorporated into VLPs of the present disclosure to enhance the immunogenic characteristics of VLPs.

The antigenic polypeptide (tumor antigen, Dengue virus antigen, or malaria antigen, or at least an epitope thereof) is disposed (*e.g.*, expressed) on the surface of the VLP, so that it can interact with target molecules or cells to produce immunogenic responses (*e.g.*, antibody production). In general, the antigenic polypeptide sequence and the corresponding polynucleotide sequence can be found in GenBank and the access numbers can be obtained online at NCBI. In addition, the sequences identified for the antigenic polypeptides above are only illustrative examples of representative antigenic polypeptide sequences. Further, variants that are substantially homologous to the above referenced antigenic polypeptides and those having conservative substitutions of the above referenced antigenic polypeptides can also be incorporated into VLPs of the present disclosure to enhance the immunogenic characteristics of VLPs.

Any proteinaceous Dengue virus antigen, malaria antigen, or tumor antigen that is expressible on the surface of the VLP may be used. The term “polypeptide” is inclusive of peptides and glycoproteins.

Optionally, the antigenic polypeptide can be modified and/or truncated to improve the immunogenic properties of the VLP. For example, the VLP can be conformationally changed by hydrostatic pressure-induced techniques. In another embodiment, the antigenic polypeptide can be modified to expose neutralizing epitopes in the polypeptide by removing obstructing structural features such as, but not limited to, glycosylation sites. By eliminating

these obstructing features, the immunogenic properties of the VLP that includes such modified glycoproteins can be enhanced.

The adjuvant molecule, or at least a portion of the adjuvant molecule, is disposed (*e.g.*, expressed) on the surface of the VLP. The adjuvant molecule can interact with other molecules or cells (*e.g.*, mucosal surfaces having sialic acid residues disposed thereon and antigen-presenting cells such as dendritic cells and follicular dendritic cells).

The adjuvant molecule can include, but is not limited to, an influenza hemagglutinin (HA) molecule (GenBank access number J02090), a parainfluenza hemagglutinin-neuraminidase (HN) molecule (GenBank access number z26523 for human parainfluenza virus type 3 HN sequence information), a Venezuelan equine encephalitis (VEE) adjuvant molecule (GenBank access number nc001449), a fms-like tyrosine kinase ligand (Flt3) adjuvant molecule (GenBank access number NM013520), a C3d adjuvant molecule (GenBank access number nm009778 for mouse C3 sequence and access number nm000064 for human C3 sequence), a mannose receptor adjuvant molecule, a CD40 ligand adjuvant molecule (GenBank access number m83312 for mouse CD40), and combinations thereof. The adjuvant molecule 16 can also include membrane anchored forms of a mammalian toll-like receptor (TLR) ligand molecule, a MIP-1.alpha. molecule, a RANTES MIP-1.beta. molecule, a GM-CSF molecule, a Flt3 ligand molecule, a CD40 ligand molecule, an IL-2 molecule, an IL-10 molecule, an IL-12 molecule, an IL-15 molecule, an IL-18 molecule, and an IL-21 molecule, and combinations thereof. Examples of membrane-anchored forms of mammalian TLR ligand molecules include, but are not limited to, ligands listed in Akira, S. and Takeda, K. Toll-Like Receptor Signalling. *Nature Reviews/Immunology*, 4: 499-511 (2004), which is incorporated by reference herein. In particular, exemplary TLR ligand molecules include glycoproteins from Prevotella intermedia, Respiratory syncytial virus protein F, fibronectin A domain, fibrinogen, flagellin, a measles virus HA protein, and Pam2Cys lipoprotein/lipopeptide (MALP-2).

In general, the adjuvant molecule sequence and the corresponding polynucleotide sequence can be found in GenBank and the access numbers can be obtained online at the NCBI. In addition, the sequences identified for the adjuvant molecules above are only illustrative examples of representative adjuvant molecules. Further, variants that are substantially homologous to the above referenced adjuvant molecules and adjuvant molecules having conservative substitutions of the above referenced adjuvant molecules can also be

incorporated into VLPs of the present disclosure to enhance the immunogenic characteristics of VLPs.

Antigen presenting cells can be targeted by VLPs by including one or more of the following adjuvant molecules on the surface of the VLP: the VEE adjuvant molecule, the Flt3 5 ligand molecule, the mannose adjuvant molecule, the CD40 adjuvant molecule, and the Cd3 ligand molecule. In particular, the VEE adjuvant molecule, the Flt3 adjuvant molecule, the mannose receptor adjuvant molecule, and the CD40 adjuvant molecule can be used to target dendritic cells, while the Cd3 ligand molecule can be used to target follicular dendritic cells.

Dendritic cells (DCs) are very efficient antigen presenting cells involved in priming 10 native CD4 and CD8 T cells, thus inducing primary immune responses and permitting establishment of immunological memory (Inaba, K., *et al.*, J. Exp. Med., 166:182-194 (1987) and Inaba, K., *et al.*, J. Exp. Med., 172:631-640 (1990)). Antigens taken up by DCs are expressed at the cell surface in the form of peptides associated with MHC class II, which stimulates CD4 Th cells. For induction of CD8 T cells, MHC class 1 associated peptides are 15 derived from endogenously synthesized proteins as well as from some exogenous antigens (*e.g.*, infectious agents, dying cells, proteins associated with inert particles, and immune complexes) by DC endocytosis (Heath, W. R. and F. R. Carbone, Curr. Opin. Immunol., 11:314-318 (1999); Reimann, J. and R. Schirmbeck, Immunol. Rev., 172:131-152 (1999); Regnault, A., *et al.*, J. Exp. Med., 189:371-380 (1999); and Machy, P., *et al.*, Eur. J. 20 Immunol., 30:848-857 (2000)). DCs harboring immune complexes also stimulate naive B cells (Wykes, M., J. Immunol., 161:1313-1319 (1998) and Dubois, B., *et al.*, Biol., 70:633-641 (2001)). The highly developed Ag-presenting capacity of DCs has led to their study of cellular vaccine adjuvants for the immunotherapy of cancer (Schuler, G. and R. M. Steinman, J. Exp. Med., 1986:1183-1187 (1997) and Baggers, J., *et al.*, J. Clin. Oncol., 18:3879-3882 25 (2000)). HIV and SIV virions interact with DCs via DC-SIGN and/or CD4 receptors; however, this interaction appears to preferentially result in infection of the DCs as well as transmission to other target cells rather than potentiation of an immune response (Geijtenbeek, T. B., *et al.*, Cell, 100:587-597 (2000) and Geijtenbeek, T. B., *et al.*, Immunol. Lett. 79:101-107 (2001)). On the other hand, inert particulate antigens like VLPs are very 30 attractive target for antigen presenting cells, particularly DCs (Bachmann, M. F., *et al.*, Eur. J. Immunol., 26:2595-2600 (1996); Ruedl, C., *et al.*, Eur. J. Immunol., 32:818-825 (2002) and Da Silva, D. M., *et al.*, Int. Immunol., 13:633-641 (2001)). Therefore, the interaction of VLPs with DCs may result in potentiating DCs to initiate T cell activation.

The possible advantage of targeting vaccine antigens to DCs is indicated by the extremely small number of DCs in peripheral tissues and in blood, where DCs represent less than 1% of total cell number. Flt3 ligand (FL) adjuvant molecule (GenBank access number NM013520) is a hematopoietic growth factor that has the unique ability to expand the number of both CD8.alpha.- and CD8.alpha.+ DC subsets (Lyman, S. D., *et al.*, Cell, 75:1157-1167 (1993); Maraskovsky, E, *et al.*, J. Exp. Med., 184:1953-1962 (1996); Maraskovsky, E, *et al.*, Blood, 96:878-884 (2000) and Pulendran, B., *et al.*, J. Immunol., 159:222-2231 (1997)). Such expansion of DCs in mice resulted in dramatic increases in Ag-specific B and T cell responses (Pulendran, B., *et al.*, J. Exp. Med., 188:2075-2082 (1998)), enhanced T-cell mediated immune responses (Pisarev *et al.*, Int J Immunopharmacol, 11:865-76 (2000)), and protective immunity to Listeria monocytogenes (Gregory, S. H., *et al.*, Cytokine, 13:202-208 (2001)). It is suggested that FL treatment increases the capacity of DCs as antigen presenting cells by up-regulating MHC and costimulatory molecules (CD40, CD86), and by inducing production of cytokines (IFN-.gamma., IL-2, IL-12 or IL-4) (Pulendran, B., *et al.*, J. Exp. Med., 188:2075-2082 (1998) and Pulendran, B., *et al.*, Proc. Natl. Acad. Sci., U.S.A. 96:1036-1041 (1999)). Therefore, incorporation of FL adjuvant molecules into VLPs may enhance the immunogenic properties of the VLPs.

The VLP can be produced to include the FL adjuvant molecule by PCR-amplifying and cloning the whole FL gene including the signal sequence and transmembrane (TM) domain into rBV transfer vector pc/pS1. To construct a rBV expressing FL, Sf9 insect cells can be co-transfected with Baculogold DNA (available from PharMingen, Inc.) and the pc/pS1 transfer vector containing the FL gene.

The incorporation of the FL adjuvant molecule into VLPs can be enhanced by modifying the FL adjuvant molecule. In particular, the extracellular coding domain of the FL gene (from the end of signal peptide to the start of the TM segment) can be fused to the N-terminus of the SIV Env glycoprotein-41 TM domain and the tPA signal peptide can be fused to the N-terminus of the FL-chimeric protein. An alternative approach is to produce a glycosyl-phosphatidylinositol (GPI)-anchored form the FL adjuvant molecule (designated as FL-GPD using a pcDNA3-GPt cassette (GenBank access number x52645), which was previously used to produce GM-CSF in an active membrane-bound form (Poloso, N. J., *et al.*, Mol. Immunol., 38:803-816 (2002)). GPI-anchored proteins preferentially associate with lipid rafts, which are used as sites for virus assembly (Nguyen, D. H. and J. E. Hildreth; J.

Virol., 74:3265-3272 (2000)). These chimeric FL constructs can be cloned into pc/pS1 and used to produce rBVs expressing FL fusion proteins.

VEE is a member of the family Togaviridae and is typically transmitted by mosquitoes to humans or other animals, in which it causes fever and encephalitis. Following
5 inoculation into the footpad of mice, the virus was observed to be rapidly transported to the draining lymph nodes. Recent studies have shown that dendritic cells in the lymph nodes are the primary target of VEE infection, and VEE replicon particles were observed to be localized in Langerhans cells, dendritic cells of the skin, following subcutaneous inoculation (Macdonald, G. H., and Johnston, R. E., J. Virol., 74(2):914-22 (2000)). These investigators
10 also showed that the targeting of VEE adjuvant molecules to DCs was dependent upon the specific amino acid sequence of the viral envelope glycoprotein E2. Therefore, VLPs incorporating VEE adjuvant molecules may be used to target dendritic cells.

Dendritic cells use the mannose receptor (MR) as the major receptor for endocytosis of antigens (Sallusto, F., *et al.*, J. Exp. Med., 192(2):389-400 (1995)). This receptor is a 175
15 kD protein containing eight carbohydrate recognition domains with high affinity for mannose-rich glycoproteins (Stahl, P. D., Curr Opin Immunol., 4(1):49-52 (1992) and Ezekowitz, R. A., *et al.*, J. Exp. Med., 172(6):1785-94 (1990)). Following endocytosis, the MR releases its ligand at low pH and it recycles to the cell surface, thus having the capacity to interact with ligands in multiple rounds (Stahl, P., *et al.* Cell, 19(1):207-15 (1980)). It has
20 been suggested that the MR may provide a mechanism for distinguishing self from non-self antigens on the basis of glycosylation patterns since, in higher eukaryotes, mannose residues are usually buried within the carbohydrate moieties of envelope glycoproteins and therefore not available for binding to MR (Sallusto, F., *et al.*, J. Exp. Med. 192(2):389-400 (1995)). Thus, it may be possible to target VLPs to dendritic cells on the basis of distinct
25 oligosaccharide profiles.

Once dendritic cells take up antigens, immature dendritic cells need to differentiate into professional antigen presenting cells in response to maturation signals. As dendritic cells mature, expression of co-stimulatory molecules and MHC-peptide complexes increase and cytokines are produced (Banchereau, J. & I Steinman, R. M., Nature, 392:245-52 (1998) and
30 Pierre, P., Turley, *et al.*, Nature, 388:787-92 (1997)). Interaction between CD40 expressed on antigen presenting cells including dendritic cells and CD40L on activated Th cells is important for T cell dependent B cell activation and isotype switching (Rousset, F., *et al.*, J. Exp. Med., 173:705-10 (1991)). CD40 ligation with a cell line expressing CD40L activates

Langerhans cell-derived dendritic cells, and induces high level expression of MHC II and accessory molecules such as CD80 and CD86 (Caux, C., *et al.*, J. Exp. Med., 180:1263-1272 (1994)). Cross-linking CD40 with anti-CD40 antibodies play a role in ablating the tolerogenic potential of lymphoid dendritic cells (Grohmann U., *et al.*, J. Immunol. 166:277-83 (2001)). It is also shown that signaling through CD40 on the antigen presenting cells can replace the requirement for "help" from CD4 Th cells in inducing CTL activities (Bennett, S. R., *et al.*, Nature, 393:478-480 (1993) and Schoenberger, S. P., *et al.*, Nature, 393:480-483 (1998)). In anti-tumor pre-clinical model studies, it is indicated that the main mediator for dendritic cell activation is CD40 receptor engagement (Ribas, A., *et al.*, Cancer Res., 61:8787-8793 (2001) and Ridge, J. P., *et al.*, Nature, 393:474-478 (1998)). These studies suggest that CD40L seem to provide important maturation signals for dendritic cells. Therefore, VLPs incorporating CD40L adjuvant molecules may be used to target dendritic cells.

Follicular dendritic cells (FDCs) play an important role in germinal centers, where antibody-forming cells are generated. Recent studies have indicated that FDCs play an important co-stimulatory role in the enhancement of antibody responses (Qin, D., *et al.* J. Immunol., 161:4549-4554 (1998); Fearon, D. T. and Carroll, M. C.; Annu. Rev. Immunol., 18:393-422 (2000); Fakher, M., *et al.*, Eur. J. Immunol., 31:176-185 (2001) and Tew, J. G., *et al.*, Trends Immunol., 22:361-367 (2001)). During HIV infection, immune complexes containing virions are found in association with FDCs (Hlavacek, W. S., *et al.* Philos. Trans. R. Soc. Lond B Biol. Sci., 355:1051-1058 (2000); Rosenberg, Y. J., *et al.*, Dev. Immunol., 6:61-70 (1998); Smith, B. A. *et al.* J. Immunol., 166:690-696 (2001)), and such complexes could play a significant role in effective antigen presentation to B cells for induction of neutralizing antibody as observed during HIV infection *in vivo*. Because of their close similarity to virions, VLPs may mimic such immune complexes much more closely than soluble antigens.

The FDCs interact with components of the complement system including C3d, and it was recently demonstrated that recombinant proteins containing a segment of the C3d adjuvant molecule fused (amino acids 1024 to 1320 of SEQ ID NO: 11 of U.S. Patent Publication No. 20060216702 (Compans R.W. *et al.*), which is incorporated herein by reference) to an antigen resulted in a striking increase in the efficiency of the antibody response (Dempsey, P. W., *et al.*, Science, 271:348-350 (1996)). Complement is a plasma protein system of innate immunity that is activated by microorganisms in the absence of

antibody (Fearon, D. T. and Austen, K. F., N. Engl. J. Med., 303:259-263 (1980)). Upon activation, C3d fragment binds to its receptor, CR2 (CD21) which is primarily expressed on B cells and FDCs (Fearon, D. T. and Carter, R. H.; Annu. Rev. Immunol., 13:17-149 (1995)). The presence of C3d adjuvant molecules on the surfaces of the VLPs may result in their
5 enhanced interaction with FDCs and B cells, and thus stimulation of the antibody responses to antigenic polypeptides contained in the VLP structure.

Because of the relatively large size of the C3d adjuvant molecule fragment, which is about 300 amino acids in length, two factors may affect its function: 1) its proper exposure for interaction with CR2 on FDC; and 2) its potential interference with the proper folding of
10 the protein antigen. Two alternative approaches can be used to incorporate the C3d fragment into VLPs in order to elucidate antibody responses against viral surface glycoproteins incorporated into the VLPs.

First, the C3d adjuvant molecule fragment (amino acids 1024 to 1320 of SEQ ID NO: 11 of U.S. Patent Publication No. 20060216702 (Compans R.W. *et al.*)) can be fused to the
15 N-terminus of the selected viral surface envelope glycoprotein and a signal peptide can be introduced at the N-terminus of the viral surface envelope glycoprotein. Second, a signal peptide, such as the tPA signal peptide, can be fused to the N-terminus of the C3d adjuvant molecule and a membrane anchoring sequence (TM domain of viral glycoproteins, example SIV envelope TM, or the GPI-anchoring sequence (GenBank access number x52645,) can be
20 fused to the C-terminus of the C3d adjuvant molecule.

VLPs can be produced by *in vitro* cell culture expression systems such as, but not limited to, recombinant baculovirus expression system (BEVS) (Yamshchikov, G. V., Ritter, G. D., Vey, M., and Compans, R. W. Virology, 214:50-58 (1995). Assembly of SIV virus-like particles containing envelope proteins can be performed using expression systems, such
25 as, but not limited to, a baculovirus expression system (Yamshchikov, G. V., Ritter, G. D., Vey, M., and Compans, R. W., Virology, 214:50-58 (1995)), recombinant poxvirus expression system (MVA) (Wyatt L S, *et al.*, Vaccine, 15:1451-8 (1996)), recombinant VSV, recombinant adenovirus, and recombinant DNA expression vectors. Preferably, the VLPs are produced using recombinant BEVS and recombinant poxvirus expression systems.

30 In general, VLPs can be produced by simultaneously introducing into a cell a viral protein expression vector, an antigenic polypeptide expression vector, and/or an adjuvant molecule expression vector. The expressed viral core protein self-assembles into a VLP that incorporates the antigenic polypeptide and/or the adjuvant molecule. The antigenic

polypeptide and/or the adjuvant molecule are expressed on the VLP surface. Thereafter, the cell produces the VLP (*e.g.*, Vero cells, chimeric and/or phenotypically mixed VLPs). The cells can include, but are not limited to, insect cells (*e.g.*, spodopera frugiperda Sf 9 cells and Sf21 cells) and mammalian cells (*e.g.*, EL4 cells and HeLa cells). The elements for 5 expressing the viral core protein, antigenic polypeptide, and adjuvant molecule can also be included together in a single expression vector, or can be included in two or more expression vectors.

In general, the viral protein expression vector can be produced by operably linking a coding sequence for a viral protein of a virus to an appropriate promoter (*e.g.*, an early 10 promoter, late promoter, or hybrid late/very late promoter). The viral protein expression vector can also be modified to form a viral protein expression construct. In addition, the antigenic polypeptide expression vector can be produced by operably linking a coding sequence for an antigenic polypeptide to an appropriate promoter (*e.g.*, early promoter, late promoter, or hybrid late/very late promoter). The antigenic polypeptide expression vector can 15 be modified to form an antigenic polypeptide expression construct. Similarly, the adjuvant molecule expression vector can be produced by operably linking a coding sequence for an adjuvant molecule to an appropriate promoter (*e.g.*, early promoter, late promoter, or hybrid late/very late promoter). The adjuvant molecule expression vector can be modified to form an adjuvant molecule expression construct.

20 In other embodiments, polynucleotide sequences encoding for a viral core protein, at least one antigenic polypeptide, and at least one adjuvant molecule can be included in a single expression vector, or in two or more expression vectors. The one or more expression vectors can be introduced into a host cell, the proteins can be expressed in the cell, whereby the cell forms the VLP. In embodiments, each of the polynucleotide sequences encoding for the viral 25 core protein, the antigenic polypeptide, and the adjuvant molecule is operably linked to an appropriate promoter (*e.g.*, a baculovirus promoter, a recombinant Modified Vaccinia Ankara (MVA) promoter, a CMV promoter, an EF promoter, an adenovirus promoter, a recombinant VSV promoter, a recombinant adenovirus promoter, a recombinant alphavirus promoter, and a recombinant DNA expression vector). Appropriate promoters include, but are not limited 30 to, a constitutive or inducible promoter; an early, late or hybrid late/very late promoter.

Additional embodiments also include methods of immunizing a subject by expressing a viral core protein, at least one antigenic polypeptide selected from a tumor antigen, Dengue virus antigen, or malaria antigen, and at least one adjuvant molecule in one or more host cells

(*e.g.*, via use of one or more expression vectors). The viral core protein, at least one antigenic polypeptide, and at least one adjuvant molecule thus expressed by the subject's cell(s), assemble to form a VLP. The VLP elicits an immune response from the subject, thereby providing future protection from infection by a pathogen or malignancy corresponding to the proteins expressed by the VLP.

In the case of where the adjuvant molecule is mannose, the adjuvant molecular expression construct is not needed because the mannose molecules can be chemically added to VLPs after the VLPs are produced.

The terms "host" and "subject" are used interchangeably herein to include humans, mammals (*e.g.*, cats, dogs, horses, and cattle), and other living species that are in need of treatment. Hosts that are "predisposed to" condition(s) can be defined as hosts that do not exhibit overt symptoms of one or more of these conditions but that are genetically, physiologically, or otherwise at risk of developing one or more of these conditions.

The terms "treat", "treating", and "treatment" are an approach for obtaining beneficial or desired clinical results. For purposes of embodiments of this disclosure, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilization (*e.g.*, not worsening) of disease, preventing spread of disease, delaying or slowing of disease onset or progression, amelioration or palliation of the disease state, and remission (partial or total) whether detectable or undetectable. Accordingly, the terms are inclusive of therapy (administering to a subject having the disease) and prophylaxis (administering to a subject that does not currently have the disease). In addition, "treat", "treating", and "treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

The terms "condition" and "conditions" denote a state of health that can be related to cancer or infection by a virus. The infections and cancers that are discussed herein are to be included as conditions that can be treated by embodiments of the present disclosure.

The term "polypeptide" refers to peptides, proteins, glycoproteins, and the like, of the present disclosure comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, (*e.g.*, peptide isosteres). "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides, or oligomers, and to longer chains, generally referred to as proteins. "Polypeptides" may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification

techniques, which are well known in the art. Such modifications are described in basic texts and in more detailed monographs, as well as in a voluminous research literature.

Modifications may occur anywhere in the polypeptides of the present disclosure, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present to the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from post-translation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination (Proteins-Structure and Molecular Properties, 2nd Ed., T. E. Creighton, W.H. Freeman and Company, New York, 1993; Wold, F., Post-translational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in Post-translational Covalent Modification of Proteins, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter, *et al.*, Meth. Enzymol., 182:626-646 (1990), and Rattan, *et al.*, Ann NY Acad. Sci., 663:48-62 (1992)).

"Variant" refers to polypeptides of the present disclosure that differ from a reference polynucleotide or polypeptide, but retains essential properties. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, and deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur

naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also includes the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including, but not limited to, those described in Computational Molecular Biology, Lesk, A. M., Ed., Oxford University Press, New York, (1988); Biocomputing: Informatics and Genome Projects, Smith, D. W., Ed., Academic Press, New York, (1993); Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., Eds., Humana Press, New Jersey, (1994); Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., Eds., M Stockton Press, New York, (1991); and Carillo, H., and Lipman, D., SIAM J Applied Math., 48:1073 (1988).

Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. The percent identity between two sequences can be determined by using analysis software (*e.g.*, Sequence Analysis Software Package of the Genetics Computer Group, Madison Wis.) that incorporates the Needelman and Wunsch, (J. Mol. Biol., 48:443-453 (1970)) algorithm (*e.g.*, NBLAST, and XBLAST). The default parameters are used to determine the identity for the polynucleotides and polypeptides of the present disclosure.

By way of example, the polypeptide sequences of the present disclosure may be identical to one or more of the reference sequences described above, that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a

given % identity is determined by multiplying the total number of amino acids in the reference polypeptide by the numerical percent of the respective percent identity (divided by 100) and then subtracting that product from said total number of amino acids in the reference polypeptide.

5 The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is 10 not necessarily at the carboxyl terminus of the complete polypeptide.

The term "substantially homologous" is used herein to denote polypeptides of the present disclosure having about 50%, about 60%, about 70%, about 80%, about 90%, and preferably about 95% sequence identity to the sequences discussed above. Percent sequence identity is determined by conventional methods as discussed above.

15 In general, homologous polypeptides of the present disclosure are characterized as having one or more amino acid substitutions, deletions, and/or additions.

In addition, embodiments of the present disclosure include polynucleotides that encode polypeptides having one or more "conservative amino acid substitutions" of the wild type sequence as well as polynucleotides that encode polypeptides that are "functional 20 variants" of the wild type sequence. "Functional variants" includes polypeptides (and polynucleotides encoding such polypeptides) that may have substations, deletions or insertions of more than one amino acid (*e.g.*, substitution of an entire peptide domain or fragment thereof, such as a signal peptide domain, transmembrane domain or cytoplasmic tail domain, of a protein or peptide) but which retains the essential functions of the original, or 25 wild type, protein or peptide.

"Conservative amino acid substitutions" can be based upon the chemical properties of the amino acids. Variants can be obtained that contain one or more amino acid substitutions of the sequences discussed above, in which an alkyl amino acid is substituted for an alkyl amino acid in a polypeptide, an aromatic amino acid is substituted for an aromatic amino acid 30 in a polypeptide, a sulfur-containing amino acid is substituted for a sulfur-containing amino acid in a polypeptide, a hydroxy-containing amino acid is substituted for a hydroxy-containing amino acid in a polypeptide, an acidic amino acid is substituted for an acidic amino acid in a polypeptide, a basic amino acid is substituted for a basic amino acid in a

polypeptide, or a dibasic monocarboxylic amino acid is substituted for a dibasic monocarboxylic amino acid in a polypeptide.

Among the common amino acids, for example, a "conservative amino acid substitution" is illustrated by a substitution among amino acids within each of the following
5 groups: (1) glycine, alanine, valine, leucine, and isoleucine, (2) phenylalanine, tyrosine, and tryptophan, (3) serine and threonine, (4) aspartate and glutamate, (5) glutamine and asparagine, and (6) lysine, arginine and histidine. Other conservative amino acid substitutions include amino acids having characteristics such as a basic pH (arginine, lysine, and histidine), an acidic pH (glutamic acid and aspartic acid), polar (glutamine and asparagine), hydrophobic
10 (leucine, isoleucine, and valine), aromatic (phenylalanine, tryptophan, and tyrosine), and small (glycine, alanine, serine, threonine, and methionine).

Polypeptides having amino acid variants can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, trans-3-methylproline, 2-4-methanoproline, cis-4-hydroxyproline, trans-4-hydroxyproline, N-
15 methyl-glycine, allo-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitro-glutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, tert-leucine, norvaline, 2-azaphenyl-alanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally
20 occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations are carried out in a cell-free system comprising an E. coli S30 extract and commercially available enzymes
25 and other reagents. Proteins are purified by chromatography. (Robertson, *et al.*, J. Am. Chem. Soc., 113:2722 (1991); Ellman, *et al.*, Methods Enzymol., 202:301 (1991); Chung, *et al.*, Science, 259:806-9 (1993); and Chung, *et al.*, Proc. Natl. Acad. Sci. USA, 90:10145-9 (1993)).

In a second method, translation is carried out in Xenopus oocytes by microinjection of
30 mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti, *et al.*, J. Biol. Chem., 271:19991-8 (1996)). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced (*e.g.*, phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (*e.g.*, 2-azaphenylalanine, 3-azaphenylalanine,

4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. (Koide, *et al.*, Biochem., 33:7470-6 (1994)). Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined
5 with site-directed mutagenesis to further expand the range of substitutions (Wynn, *et al.*, Protein Sci., 2:395-403 (1993)).

A "chimeric" VLP, as used herein, can be defined as a VLP having at least one antigenic polypeptide incorporated into the VLP, wherein the viral core protein and at least one antigenic polypeptide are from different viruses. A chimeric VLP, as used herein, may
10 include additional antigenic polypeptides that are from the same or different virus as the viral core protein, so long as at least one is different.

A "phenotypically mixed" VLP, as used herein, can be defined as a VLP having at least two different surface molecules (*e.g.*, antigenic polypeptide and/or adjuvant molecules) incorporated into the VLP. A phenotypically mixed VLP, as used herein, may include
15 additional surface molecules that are from the same or different source as the viral core protein, so long as at least one is different.

"Expressed", as used herein, can be defined as being a molecule disposed, or a portion of the molecule disposed, upon the surface of the VLP.

An "expression construct" is an expression vector containing a coding sequence for a
20 recombinant protein.

The term "recombinant" when used with reference to a cell, or nucleic acid, or vector, indicates that the cell, or nucleic acid, or vector, has been modified by the introduction of a heterologous nucleic acid or the alteration of a native nucleic acid, or that the cell is derived from a cell so modified. T hus, for example, recombinant cells express genes that are not
25 found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under-expressed or not expressed at all.

The term "operably linked" refers to the arrangement of various nucleotide sequences relative to each other such that the elements are functionally connected to and are able to interact with each other. Such elements may include, without limitation, one or more
30 promoters, enhancers, polyadenylation sequences, and transgenes. The nucleotide sequence elements, when properly oriented, or operably linked, act together to modulate the activity of one another, and ultimately may affect the level of expression of the transgene. The position of each element relative to other elements may be expressed in terms of the 5' terminus and

the 3' terminus of each element, and the distance between any particular elements may be referenced by the number of intervening nucleotides, or base pairs, between the elements.

A "vector" is a genetic unit (or replicon) to which or into which other DNA segments can be incorporated to effect replication, and optionally, expression of the attached segment. Examples include, but are not limited to, plasmids, cosmids, viruses, chromosomes and minichromosomes. Exemplary expression vectors include, but are not limited to, baculovirus vectors, modified vaccinia Ankara (MVA) vectors, plasmid DNA vectors, recombinant poxvirus vectors, bacterial vectors, recombinant baculovirus expression systems (BEVS), recombinant rhabdovirus vectors, recombinant alphavirus vectors, recombinant adenovirus expression systems, recombinant DNA expression vectors, and combinations thereof.

A "coding sequence" is a nucleotide sequence that is transcribed into mRNA and translated into a protein, *in vivo* or *in vitro*.

"Regulatory sequences" are nucleotide sequences, which control transcription and/or translation of the coding sequences, which they flank.

"Processing sites" are described in terms of nucleotide or amino acid sequences (in context of a coding sequence or a polypeptide). A processing site in a polypeptide or nascent peptide is where proteolytic cleavage occurs, where glycosylation is incorporated or where lipid groups (such as myristoylation) occurs. Proteolytic processing sites are where proteases act.

"Virus-like particles" (VLPs) are membrane-surrounded viral core structures having one or more antigenic polypeptide expressed on the VLP surface. In addition, adjuvant molecules can be expressed on the VLP. Further, viral core proteins are located within the membrane of the VLP. Additional components of VLPs, as known in the art, can be included within or disposed on the VLP. VLPs do not contain intact viral nucleic acids, and they are non-infectious. Desirably, there is sufficient antigenic polypeptide and/or adjuvant molecules expressed, at least in part, on the surface of the VLP so that when a VLP preparation is formulated into an immunogenic composition and administered to an animal or human, an immune response (cell-mediated or humoral) is raised.

A "truncated" antigenic polypeptide is one having less than a full length protein (*e.g.*, a portion of the cytoplasmic domain has been removed), which retains surface antigenic determinants against which an immune response is generated, preferably a protective immune response, and it retains sufficient antigenic polypeptide sequence for proper membrane

insertion. The skilled artisan can produce truncated antigenic polypeptides using recombinant DNA technology and virus coding sequences, which are readily available to the public.

Compositions and immunogenic preparations of the present disclosure, including vaccine compositions, comprising the VLPs of the present disclosure and capable of inducing protective immunity in a suitably treated host and a suitable carrier therefor are provided. "Immunogenic compositions" are those which result in specific antibody production or in cellular immunity when injected into a subject. Such immunogenic compositions or vaccines are useful, for example, in immunizing hosts against infection and/or damage caused by Dengue virus, malaria, and malignancies.

The vaccine preparations of the present disclosure can include an immunogenic amount of one or more VLPs, fragment(s), or subunit(s) thereof. Such vaccines can include one or more antigenic polypeptides and portions thereof, and adjuvant molecule and portions thereof on the surfaces of the VLPs, or in combination with another protein or other immunogen, such as one or more additional virus components naturally associated with viral particles or an epitopic peptide derived therefrom.

By "immunogenic amount" is meant an amount capable of eliciting the production of antibodies directed against the virus, in the host to which the vaccine has been administered. In some embodiments, the route of administration and the immunogenic composition is designed to optimize the immune response on mucosal surfaces, for example, using nasal administration (via an aerosol) of the immunogenic composition. Intravenous and intramuscular administration is also contemplated.

Immunogenic carriers can be used to enhance the immunogenicity of the VLPs from any of the viruses discussed herein. Such carriers include, but are not limited to, proteins and polysaccharides, microspheres formulated using (*e.g.*, a biodegradable polymer such as DL-lactide-coglycolide, liposomes, and bacterial cells and membranes). Protein carriers may be joined to the proteinases, or peptides derived therefrom, to form fusion proteins by recombinant or synthetic techniques or by chemical coupling. Useful carriers and ways of coupling such carriers to polypeptide antigens are known in the art.

The immunogenic compositions and/or vaccines of the present disclosure may be formulated by any of the methods known in the art. They can be typically prepared as injectables or as formulations for intranasal administration, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid prior to injection or

other administration may also be prepared. The preparation may also, for example, be emulsified, or the protein(s)/peptide(s) encapsulated in liposomes.

The active immunogenic ingredients are often mixed with excipients or carriers, which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients include but are not limited to water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. The concentration of the immunogenic polypeptide in injectable, aerosol or nasal formulations is usually in the range of about 0.2 to 5 mg/ml. Similar dosages can be administered to other mucosal surfaces.

In addition, if desired, the vaccines may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or other agents, which enhance the effectiveness of the vaccine. Examples of agents which may be effective include, but are not limited to: aluminum hydroxide; N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP); N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP); N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE); and RIBI, which contains three components extracted from bacteria: monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of the auxiliary substances may be determined by measuring the amount of antibodies (especially IgG, IgM or IgA) directed against the immunogen resulting from administration of the immunogen in vaccines which comprise the adjuvant in question. Additional formulations and modes of administration may also be used.

"Pharmaceutically acceptable salts" include, but are not limited to, the acid addition salts (formed with free amino groups of the peptide) which are formed with inorganic acids (*e.g.*, hydrochloric acid or phosphoric acids) and organic acids (*e.g.*, acetic, oxalic, tartaric, or maleic acid). Salts formed with the free carboxyl groups may also be derived from inorganic bases (*e.g.*, sodium, potassium, ammonium, calcium, or ferric hydroxides), and organic bases (*e.g.*, isopropylamine, trimethylamine, 2-ethylamino-ethanol, histidine, and procaine).

The immunogenic compositions and/or vaccines of the present disclosure can be administered in a manner compatible with the dosage formulation, and in such amount and manner as will be prophylactically and/or therapeutically effective, according to what is known to the art. The quantity to be administered, which is generally in the range of about 1 to 1,000 micrograms of antigenic polypeptide per dose and/or adjuvant molecule per dose,

more generally in the range of about 5 to 500 micrograms of glycoprotein per dose and/or adjuvant molecule per dose, depends on the subject to be treated, the capacity of the hosts immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of the active ingredient required to be administered may depend on the judgment of 5 the physician or veterinarian and may be peculiar to each individual, but such a determination is within the skill of such a practitioner.

The vaccine or immunogenic composition may be given in a single dose; two dose schedule, for example two to eight weeks apart; or a multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may include 1 to 10 or more 10 separate doses, followed by other doses administered at subsequent time intervals as required to maintain and/or reinforce the immune response (*e.g.*, at 1 to 4 months for a second dose, and if needed, a subsequent dose(s) after several months). Humans (or other animals) immunized with the VLPs of the present disclosure are protected from infection by the cognate virus.

15 It should also be noted that the vaccine or immunogenic composition can be used to boost the immunization of a host having been previously treated with a vaccine such as, but not limited to, DNA vaccine and a recombinant virus vaccine.

Subjects in need of treatment using the methods of the present invention can be identified using standard techniques known to those in the medical or veterinary professions, 20 as appropriate.

The terms “cancer” and “malignancy” are used herein interchangeably to refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. The cancer may be multi-drug resistant (MDR) or drug-sensitive. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, 25 and leukemia. More particular examples of such cancers include breast cancer, prostate cancer, colon cancer, squamous cell cancer, lung cancer (*e.g.*, small-cell lung cancer, non-small cell lung cancer), gastrointestinal cancer, pancreatic cancer, cervical cancer, ovarian cancer, peritoneal cancer, liver cancer, *e.g.*, hepatic carcinoma, bladder cancer, colorectal cancer, endometrial carcinoma, kidney cancer, and thyroid cancer. In some embodiments, 30 the cancer is multiple myeloma or another hematologic malignancy.

Other non-limiting examples of cancers that may be treated are basal cell carcinoma, biliary tract cancer; bone cancer; brain and CNS cancer; choriocarcinoma; connective tissue cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer; intra-

epithelial neoplasm; larynx cancer; lymphoma including Hodgkin's and Non-Hodgkin's lymphoma; melanoma; myeloma; neuroblastoma; oral cavity cancer (*e.g.*, lip, tongue, mouth, and pharynx); retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; sarcoma; skin cancer; stomach cancer; testicular cancer; uterine cancer; cancer of the urinary system, as well as other carcinomas and sarcomas. Examples of cancer types that may potentially be evaluated and/or treated using the methods of the present invention are also listed in Table 1.

As used herein, the term "tumor" refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. For example, a particular cancer may be characterized by a solid mass tumor or non-solid tumor. The solid tumor mass, if present, may be a primary tumor mass. A primary tumor mass refers to a growth of cancer cells in a tissue resulting from the transformation of a normal cell of that tissue. In most cases, the primary tumor mass is identified by the presence of a cyst, which can be found through visual or palpation methods, or by irregularity in shape, texture or weight of the tissue. However, some primary tumors are not palpable and can be detected only through medical imaging techniques such as X-rays (*e.g.*, mammography) or magnetic resonance imaging (MRI), or by needle aspirations. The use of these latter techniques is more common in early detection. Molecular and phenotypic analysis of cancer cells within a tissue can usually be used to confirm if the cancer is endogenous to the tissue or if the lesion is due to metastasis from another site. The treatment methods of the invention can be utilized for early, middle, or late stage disease, and acute or chronic disease.

Table 1. Examples of Cancer Types

Acute Lymphoblastic Leukemia, Adult Acute Lymphoblastic Leukemia, Childhood Acute Myeloid Leukemia, Adult Acute Myeloid Leukemia, Childhood Adrenocortical Carcinoma Adrenocortical Carcinoma, Childhood AIDS-Related Cancers AIDS-Related Lymphoma Anal Cancer Astrocytoma, Childhood Cerebellar Astrocytoma, Childhood Cerebral Basal Cell Carcinoma	Hairy Cell Leukemia Head and Neck Cancer Hepatocellular (Liver) Cancer, Adult (Primary) Hepatocellular (Liver) Cancer, Childhood (Primary) Hodgkin's Lymphoma, Adult Hodgkin's Lymphoma, Childhood Hodgkin's Lymphoma During Pregnancy Hypopharyngeal Cancer Hypothalamic and Visual Pathway Glioma, Childhood Intraocular Melanoma Islet Cell Carcinoma (Endocrine Pancreas)

Bile Duct Cancer, Extrahepatic Bladder Cancer Bladder Cancer, Childhood Bone Cancer, Osteosarcoma/Malignant Fibrous Histiocytoma Brain Stem Glioma, Childhood Brain Tumor, Adult Brain Tumor, Brain Stem Glioma, Childhood Brain Tumor, Cerebellar Astrocytoma, Childhood Brain Tumor, Cerebral Astrocytoma/Malignant Glioma, Childhood Brain Tumor, Ependymoma, Childhood Brain Tumor, Medulloblastoma, Childhood Brain Tumor, Supratentorial Primitive Neuroectodermal Tumors, Childhood Brain Tumor, Visual Pathway and Hypothalamic Glioma, Childhood Brain Tumor, Childhood Breast Cancer Breast Cancer, Childhood Breast Cancer, Male Bronchial Adenomas/Carcinoids, Childhood Burkitt's Lymphoma Carcinoid Tumor, Childhood Carcinoid Tumor, Gastrointestinal Carcinoma of Unknown Primary Central Nervous System Lymphoma, Primary Cerebellar Astrocytoma, Childhood Cerebral Astrocytoma/Malignant Glioma, Childhood Cervical Cancer Childhood Cancers Chronic Lymphocytic Leukemia Chronic Myelogenous Leukemia Chronic Myeloproliferative Disorders	Kaposi's Sarcoma Kidney (Renal Cell) Cancer Kidney Cancer, Childhood Laryngeal Cancer Laryngeal Cancer, Childhood Leukemia, Acute Lymphoblastic, Adult Leukemia, Acute Lymphoblastic, Childhood Leukemia, Acute Myeloid, Adult Leukemia, Acute Myeloid, Childhood Leukemia, Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Hairy Cell Lip and Oral Cavity Cancer Liver Cancer, Adult (Primary) Liver Cancer, Childhood (Primary) Lung Cancer, Non-Small Cell Lung Cancer, Small Cell Lymphoma, AIDS-Related Lymphoma, Burkitt's Lymphoma, Cutaneous T-Cell, see Mycosis Fungoides and Sézary Syndrome Lymphoma, Hodgkin's, Adult Lymphoma, Hodgkin's, Childhood Lymphoma, Hodgkin's During Pregnancy Lymphoma, Non-Hodgkin's, Adult Lymphoma, Non-Hodgkin's, Childhood Lymphoma, Non-Hodgkin's During Pregnancy Lymphoma, Primary Central Nervous System Macroglobulinemia, Waldenström's Malignant Fibrous Histiocytoma of Bone/Osteosarcoma Medulloblastoma, Childhood Melanoma Melanoma, Intraocular (Eye) Merkel Cell Carcinoma Mesothelioma, Adult Malignant Mesothelioma, Childhood Metastatic Squamous Neck Cancer with Occult Primary Multiple Endocrine Neoplasia Syndrome, Childhood Multiple Myeloma/Plasma Cell Neoplasm Mycosis Fungoides Myelodysplastic Syndromes Myelodysplastic/Myeloproliferative Diseases Myelogenous Leukemia, Chronic Myeloid Leukemia, Adult Acute Myeloid Leukemia, Childhood Acute

Colon Cancer Colorectal Cancer, Childhood Cutaneous T-Cell Lymphoma, see Mycosis Fungoides and Sézary Syndrome Endometrial Cancer Ependymoma, Childhood Esophageal Cancer Esophageal Cancer, Childhood Ewing's Family of Tumors Extracranial Germ Cell Tumor, Childhood Extragonadal Germ Cell Tumor Extrahepatic Bile Duct Cancer Eye Cancer, Intraocular Melanoma Eye Cancer, Retinoblastoma Gallbladder Cancer Gastric (Stomach) Cancer Gastric (Stomach) Cancer, Childhood Gastrointestinal Carcinoid Tumor Germ Cell Tumor, Extracranial, Childhood Germ Cell Tumor, Extragonadal Germ Cell Tumor, Ovarian Gestational Trophoblastic Tumor Glioma, Adult Glioma, Childhood Brain Stem Glioma, Childhood Cerebral Astrocytoma Glioma, Childhood Visual Pathway and Hypothalamic Skin Cancer (Melanoma) Skin Carcinoma, Merkel Cell Small Cell Lung Cancer Small Intestine Cancer Soft Tissue Sarcoma, Adult Soft Tissue Sarcoma, Childhood Squamous Cell Carcinoma, see Skin Cancer (non-Melanoma) Squamous Neck Cancer with Occult Primary, Metastatic Stomach (Gastric) Cancer Stomach (Gastric) Cancer, Childhood Supratentorial Primitive Neuroectodermal Tumors, Childhood	Myeloma, Multiple Myeloproliferative Disorders, Chronic Nasal Cavity and Paranasal Sinus Cancer Nasopharyngeal Cancer Nasopharyngeal Cancer, Childhood Neuroblastoma Non-Hodgkin's Lymphoma, Adult Non-Hodgkin's Lymphoma, Childhood Non-Hodgkin's Lymphoma During Pregnancy Non-Small Cell Lung Cancer Oral Cancer, Childhood Oral Cavity Cancer, Lip and Oropharyngeal Cancer Osteosarcoma/Malignant Fibrous Histiocytoma of Bone Ovarian Cancer, Childhood Ovarian Epithelial Cancer Ovarian Germ Cell Tumor Ovarian Low Malignant Potential Tumor Pancreatic Cancer Pancreatic Cancer, Childhood Pancreatic Cancer, Islet Cell Paranasal Sinus and Nasal Cavity Cancer Parathyroid Cancer Penile Cancer Pheochromocytoma Pineoblastoma and Supratentorial Primitive Neuroectodermal Tumors, Childhood Pituitary Tumor Plasma Cell Neoplasm/Multiple Myeloma Pleuropulmonary Blastoma Pregnancy and Breast Cancer Pregnancy and Hodgkin's Lymphoma Pregnancy and Non-Hodgkin's Lymphoma Primary Central Nervous System Lymphoma Prostate Cancer Rectal Cancer Renal Cell (Kidney) Cancer Renal Cell (Kidney) Cancer, Childhood Renal Pelvis and Ureter, Transitional Cell Cancer Retinoblastoma Rhabdomyosarcoma, Childhood Salivary Gland Cancer Salivary Gland Cancer, Childhood Sarcoma, Ewing's Family of Tumors Sarcoma, Kaposi's

T-Cell Lymphoma, Cutaneous, see Mycosis Fungoides and Sézary Syndrome Testicular Cancer Thymoma, Childhood Thymoma and Thymic Carcinoma Thyroid Cancer Thyroid Cancer, Childhood Transitional Cell Cancer of the Renal Pelvis and Ureter Trophoblastic Tumor, Gestational Unknown Primary Site, Carcinoma of, Adult Unknown Primary Site, Cancer of, Childhood Unusual Cancers of Childhood Ureter and Renal Pelvis, Transitional Cell Cancer Urethral Cancer Uterine Cancer, Endometrial Uterine Sarcoma Vaginal Cancer Visual Pathway and Hypothalamic Glioma, Childhood Vulvar Cancer Waldenström's Macroglobulinemia Wilms' Tumor	Sarcoma, Soft Tissue, Adult Sarcoma, Soft Tissue, Childhood Sarcoma, Uterine Sezary Syndrome Skin Cancer (non-Melanoma) Skin Cancer, Childhood

In some embodiments, the VLP expresses one or more tumor antigens. For example, the tumor antigen may be an antigen overexpressed in tumors, hematopoietic differentiation antigen, cell surface differentiation antigen, growth factor receptor, angiogenesis or stromal antigen, *etc.* Non-limiting examples of antigens that may be used are disclosed in Carter P. *et al.*, "Identification and validation of cell surface antigens for antibody targeting in oncology", *Endocrine-Related Cancer*, 2004, 11:659-687; Alonso-Camino V. *et al.*, "CARbodies: Human Antibodies Against Cell Surface Tumor Antigens Selected from Repertoires Displayed on T Cell Chimeric Antigen Receptors", Molecular Therapy-Nucleic Acids, 2013:2:e93; Scott A.M. *et al.*, "Tumour Antigens Recognized by Antibodies," Encyclopedia of Life Sciences, 2001, pp. 1-7; Part One: Tumor-associated Antigens (TAAs): Subclasses of TAAS, in Tumor-Associated Antigens, Edited by Olivier Gires and Barbara Seliger, 2009; Kiessling A. *et al.*, "Tumor-Associated Antigens for Specific Immunotherapy of Prostate

Cancer," *Cancers*, 2012, 4:193-217, which are incorporated herein by reference in their entirety. In some embodiments, the tumor antigen is a tumor-specific antigen (TSA), which is present only on tumor cells and not on any other cell. In some embodiments, the tumor antigen is tumor-associated antigen (TAA), which is present on some tumor cells and also
5 some normal cells. It should be understood, however, that many antigens thought to be tumor-specific have been subsequently determined to be expressed in some normal cells as well. In some embodiments, the tumor antigen is overexpressed in cancer cells relative to the corresponding normal, non-cancerous cell type.

In some embodiments, the tumor antigen(s) is overexpressed in cancer cells (relative
10 to the corresponding normal, non-cancerous cells) in a cancer of the subject to which the VLP is to be administered. In some embodiments, the tumor antigen is a tumor antigen obtained from the subject to which the VLP is to be administered.

In some embodiments, the tumor antigen is selected from among CD138, CD44, alpha4 integrin, alpha3 integrin, alpha6 integrin, EGFR, tumor suppressor cell antigen,
15 cytotoxic T cell antigen, 17-1A, 707-AP, AFP, Annexin II, ART-4, BAGE, BAGE-1, β-catenin, BCG, bcr/abl, Bcr/abl e14a2 fusion junction, bcr-abl (b3a2), bcr-abl (b3a2), bcr-abl p190 (e1a2), bcr-abl p210 (b2a2), bcr-abl p210 (b3a2), bcr-abl p210 (b3a2), bullous pemphigoid antigen-1, CA19-9, CA125, CA215, CAG-3, CAMEL, Cancer-testis antigen, Caspase-8, CCL3, CCL4, CD16, CD20, CD3, CD30, CD55, CD63, CDC27, CDK-4, CDR3,
20 CEA, cluster 5, cluster-5A, cyclin-dependent kinase-4, Cyp-B, DAM-10, DAM-6, Dek-cain, E7, EGFRvIII, EGP40, ELF2 M, EpCAM, FucGM1, G250, GA733, GAGE, GAGE-1-8, gastrin cancer associated antigen, GD2, GD3, globoH, glycophorin, GM1, GM2, GM3, GnTV, Gn-T-V, gp100, Her-2/neu, HERV-K-ME, high molecular weight-associated antigen, high molecular weight proteo-glycan (HMPG), HPV-16 E6, HPV-16 E7, HPVE6, HSP70-
25 2M, HST-2, hTERT, human chorionic gonadotropin (HCG), Human milk fat globule (HMFG), iCE, KIAA0205, KK-LC-1, KM-HN-1, L6, LAGE-1, Lcose4Cer, LDLR/FUT, Lewis A, Lewis v/b, M protein, MAGE-1, MVC, MAGE-A1-12, MAGE-C2, MAHGE-3, MART-1/Melan-A, MC1R, ME491, MUC1, MUC2, mucin, MUM-1, MUM-2, MUM-3, mutated p53, Myosin, MZ2-E, N9 neuraminidase, NA88, NA88-A, nasopharyngeal
30 carcinoma antigen, NGA, NK1/c-3, Novel bcr/ablk fusion BCR exons 1, 13, 14 with ABL exons 4, NY-ESO-1/LAGE-2, NY-ESO-1b, OC125, osteosarcoma associated antigen-1, P15, p190 mimor bcr-abl (e1a2), p53, Pml/RARα, Polysialic acid, PRAME, PSA, PSM, RU1, RU2, SAGE, SART-1, SART-2, SART-3, Sialyl LeA, Sp17, SSX-2, SSX-4, surface

immunoglobulin, TAG-1, TAG-2, TEL/AML1, TPI, TRAG-3, TRP-1(gp75), TRP-2, TRP2-INT2, hTRT, tumor associated glycoprotein-72 (TAG-72), tyrosinase, u-PA, WT1, and XAGE-1b, or an immunogenic fragment of any of the foregoing antigens.

Examples of tumor antigens and their associated cancers include alphafetoprotein (AFP) and germ cell tumors or hepatocellular carcinomas; carcinoembryonic antigen (CEA) and bowel cancers (occasionally lung or breast cancer); CA-125 and ovarian cancer; MUC-1 and breast cancer; epithelial tumor antigen (ETA) and breast cancer; tyrosinase and malignant melanoma (normally present in minute quantities); melanoma-associated antigen (MAGE) (also normally present in testis); and abnormal products of ras and p53 and various tumors. Thus, for treatment of cancer, an appropriate tumor antigen(s) for the VLP can be selected on the basis of the cancer(s) with which the tumor antigen(s) is associated.

In some embodiments, the tumor antigen is identified by the SEREX (serological analysis of recombinant expression cloning) approach or based on the serological screening of cDNA expression library generated from tumor tissues of various origin or cancer cell lines, and identifying immunogenic tumor proteins based on their reactivity with autologous patient sera.

In some embodiments, the Dengue virus antigen is selected from the group consisting of C, prM, E, NS1, NS2a, NS3, NS4a, NS4b, and NS5 of serotype 1, 2, 3, or 4.

The malaria antigen may occur in one or more stages of the parasite life cycle. In some embodiments, the malaria antigen is selected from a Plasmodium from *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, or *P. knowlesi*. In some embodiments, the antigen is selected from the group consisting of CSP-1, STARP, SALSA, SSP-2, LSA-1, EXP-1, LSA-3, Pfs24, Pfs230, Pfg27, Pfg27, Pfs45/48, Pfs16, Pfs28, RAP-1, SERA-1, MSP-2, MSP-3, AMA-1, EBA-175, MSP-1, MSP-5, Pf55, RAP-2, RESA, GLURP, EMP-1, MSP-4, and Pf35.

Except as noted hereafter, standard techniques for peptide synthesis, cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook *et al.* (1989) Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory, Plainview, N.Y.; Maniatis *et al.* (1982) Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, N.Y.; Wu (ed.) (1993) Meth. Enzymol. 218, Part I; Wu (ed.) (1979) Meth. Enzymol. 68; Wu *et al.* (eds.) (1983) Meth. Enzymol. 100 and 101;

Grossman and Moldave (eds.) Meth. Enzymol. 65; Miller (ed.) (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., Old Primrose (1981) Principles of Gene Manipulation, University of California Press, Berkeley; Schleif and Wensink (1982) Practical Methods in Molecular Biology; Glover (ed.) (1985)
5 DNA Cloning Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) Nucleic Acid Hybridization, IRL Press, Oxford, UK; Setlow and Hollaender (1979) Genetic Engineering: Principles and Methods, Vols. 1-4, Plenum Press, N.Y.

Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

10 The term "immune response" is used herein to mean the activation of a host's immune system, *e.g.*, that of a mammal, such as a human, in response to the introduction of an antigen (*e.g.*, an antigenic polypeptide such Dengue antigen or tumor antigen), via the provided VLPs, compositions containing them, and methods described herein. The immune response can be in the form of a cellular or humoral response, or both.

15

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

20 It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with
25 any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

Exemplified embodiments of the invention include, but are not limited to:

30

Embodiment 1. A virus-like particle (VLP), comprising: a viral core protein that can self-assemble into a VLP core; at least one tumor antigen expressed on the surface of the VLP; and at least one adjuvant molecule expressed on the surface of the VLP.

Embodiment 2. A virus-like particle (VLP), comprising: a viral core protein that can self-assemble into a VLP core; at least one Dengue virus antigen expressed on the surface of the VLP; and at least one adjuvant molecule expressed on the surface of the VLP.

Embodiment 3. A virus-like particle (VLP), comprising: a viral core protein that can self-assemble into a VLP core; at least one malaria antigen expressed on the surface of the VLP; and at least one adjuvant molecule expressed on the surface of the VLP.

Embodiment 4. The VLP of embodiment 1, wherein the at least one tumor antigen is selected from the group consisting of CD138, CD44, alpha4 integrin, alpha3 integrin, alpha6 integrin, EGFR, tumor suppressor cell antigen, cytotoxic T cell antigen, 17-1A, 707-AP, AFP, Annexin II, ART-4, BAGE, BAGE-1, β-catenin, BCG, bcr/abl, Bcr/abl e14a2 fusion junction, bcr-abl (b3a2), bcr-abl (b3a2), bcr-abl p190 (e1a2), bcr-abl p210 (b2a2), bcr-abl p210 (b3a2), bcr-abl p210 (b3a2), bullous pemphigoid antigen-1, CA19-9, CA125, CA215, CAG-3, CAMEL, Cancer-testis antigen, Caspase-8, CCL3, CCL4, CD16, CD20, CD3, CD30, CD55, CD63, CDC27, CDK-4, CDR3, CEA, cluster 5, cluster-5A, cyclin-dependent kinase-4, Cyp-B, DAM-10, DAM-6, Dek-cain, E7, EGFRvIII, EGP40, ELF2 M, EpCAM, FucGM1, G250, GA733, GAGE, GAGE-1-8, gastrin cancer associated antigen, GD2, GD3, globoH, glycophorin, GM1, GM2, GM3, GnTV, Gn-T-V, gp100, Her-2/neu, HERV-K-ME, high molecular weight-associated antigen, high molecular weight proteo-glycan (HMPG), HPV-16 E6, HPV-16 E7, HPVE6, HSP70-2M, HST-2, hTERT, human chorionic gonadotropin (HCG), Human milk fat globule (HMFG), iCE, KIAA0205, KK-LC-1, KM-HN-1, L6, LAGE-1, Lcose4Cer, LDLR/FUT, Lewis A, Lewis v/b, M protein, MAGE-1, MVC, MAGE-A1-12, MAGE-C2, MAHGE-3, MART-1/Melan-A, MC1R, ME491, MUC1, MUC2, mucin, MUM-1, MUM-2, MUM-3, mutated p53, Myosin, MZ2-E, N9 neuraminidase, NA88, NA88-A, nasopharyngeal carcinoma antigen, NGA, NK1/c-3, Novel bcr/ablk fusion BCR exons 1, 13, 14 with ABL exons 4, NY-ESO-1/LAGE-2, NY-ESO-1b, OC125, osteosarcoma associated antigen-1, P15, p190 mimor bcr-abl (e1a2), p53, Pml/RARα, Polysialic acid, PRAME, PSA, PSM, RU1, RU2, SAGE, SART-1, SART-2, SART-3, Sialyl LeA, Sp17, SSX-2, SSX-4, surface immunoglobulin, TAG-1, TAG-2, TEL/AML1, TPI, TRAG-3, TRP-1(gp75), TRP-2, TRP2-INT2, hTRT, tumor associated glycoprotein-72 (TAG-72), tyrosinase, u-PA, WT1, and XAGE-1b, or an immunogenic fragment of any of the foregoing antigens.

Embodiment 5. The VLP of embodiment 1 or 4, wherein the at least one tumor antigen is an antigen associated with a hematologic malignancy.

Embodiment 6. The VLP of embodiment 2, wherein the at least one Dengue virus antigen is selected from the group consisting of C, prM, E, NS1, NS2a, NS3, NS4a, NS4b, and NS5 of serotype 1, 2, 3, or 4.

Embodiment 7. The VLP of embodiment 3, wherein the at least one malaria antigen is selected from a Plasmodium from *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, or *P. knowlesi*.

Embodiment 8. The VLP of any one of embodiments 1 to 7, wherein the viral core protein is selected from: a retrovirus Gag protein, a retrovirus matrix protein, a rhabdovirus M protein, a filovirus viral core protein, a corona virus M protein, a corona virus E protein, a corona virus NP protein, a bunyavirus N protein, an influenza M1 protein, a paramyxovirus M protein, an arenavirus Z protein, and combinations thereof.

Embodiment 9. The VLP of embodiment 8, wherein the viral core protein comprises a retrovirus Gag protein is selected from: a Human Immunodeficiency Virus (HIV) Gag protein, a Simian Immunodeficiency Virus (SIV) Gag protein, and a Murine Leukemia Virus (MuLV) Gag protein.

Embodiment 10. The VLP of embodiment 8, wherein the viral core protein is selected from: a Vesicular Stomatitis Virus (VSV) M protein, an Ebola Virus VP40 protein, a Lassa Fever Virus Z protein, and combinations thereof.

Embodiment 11. The VLP of any preceding embodiment, wherein the at least one adjuvant molecule is selected from: an influenza HA adjuvant molecule; a parainfluenza HN adjuvant molecule; a Venezuelan equine encephalitis (VEE) glycoprotein adjuvant molecule; a C3d adjuvant molecule; a mannose receptor adjuvant molecule; a membrane-anchored form of a molecule selected from: a mammalian toll-like receptor (TLR) ligand molecule, a MIP-1 alpha molecule, a RANTES MIP-1 beta molecule, a GM-CSF molecule, a Flt3 ligand molecule, a CD40 ligand molecule, an IL-2 molecule, an IL-10 molecule, an IL-12 molecule, an IL-15 molecule, an IL-18 molecule, and an IL-21 molecule; or any combination of two or more of the foregoing.

Embodiment 12. The VLP of embodiment 11, wherein the membrane-anchored form of a mammalian TLR ligand molecule is selected from: a Prevotella intermedia glycoprotein, a respiratory synctial virus protein F, a fibronectin A domain, fibrinogen, flagellin, a measles virus HA protein, and Pam2Cys lipoprotein/lipopeptide (MALP-2).

Embodiment 13. The VLP of embodiment 11, wherein the at least one adjuvant molecule comprises flagellin.

Embodiment 14. An immunogenic composition, comprising the VLP of any one of embodiments 1 to 13; and a pharmacologically acceptable carrier.

Embodiment 15. A method of generating an immunological response in a subject, comprising administering an effective amount of a VLP or immunogenic composition of any 5 one of embodiments 1 to 14 to the subject.

Embodiment 16. A method of treating a malignancy, comprising administering to a subject in need of treatment an effective amount of a VLP or immunogenic composition of embodiment 1, embodiment 4, embodiment 5, one of embodiments 8 to 13, or embodiment 14.

10 Embodiment 17. The method of embodiment 16, wherein the subject has the malignancy, and wherein the VLP or immunogenic composition is administered for therapy.

Embodiment 18. The method of embodiment 16, wherein the subject does not have the malignancy, and wherein the VLP or immunogenic composition is administered for prophylaxis.

15 Embodiment 19. A method of treating Dengue virus infection, comprising administering to a subject in need of treatment an effective amount of a VLP or immunogenic composition of embodiment 2, embodiment 6, one of embodiments 8 to 13, or embodiment 14.

Embodiment 20. The method of embodiment 19, wherein the subject has the Dengue 20 virus infection, and wherein the VLP or immunogenic composition is administered for therapy.

Embodiment 21. The method of embodiment 19, wherein the subject does not have the Dengue virus infection, and wherein the VLP or immunogenic composition is administered for prophylaxis.

25 Embodiment 22. A method of treating malaria, comprising administering to a subject in need of treatment an effective amount of a VLP or immunogenic composition of embodiment 3, embodiment 7, one of embodiments 8 to 13, or embodiment 14.

Embodiment 23. The method of embodiment 22, wherein the subject has a malarial infection, and wherein the VLP or immunogenic composition is administered for therapy.

30 Embodiment 24. The method of embodiment 22, wherein the subject does not have a malarial infection, and wherein the VLP or immunogenic composition is administered for prophylaxis.

Embodiment 25. A method of determining exposure of a host to an antigen, comprising the steps of: contacting a biological fluid of the subject with a virus-like particle (VLP) comprising: a viral core protein that can self-assemble into the VLP core; and at least one antigenic polypeptide expressed on the surface of the VLP, wherein the at least one antigenic polypeptide is selected from a tumor antigen, Dengue virus antigen, malaria antigen, under conditions which are permissive for binding of antibodies in the biological fluid with the VLP; and detecting binding of antibodies within the biological fluid with the VLP, wherein exposure of the subject to the virus is determined by the detection of antibodies bound to the VLP.

Embodiment 26. The method of embodiment 25, wherein detecting includes the use of a labeled second antibody which is specific to antibodies in the biological fluid being tested.

Embodiment 27. The method of embodiment 25, wherein the VLP further comprises at least one adjuvant molecule expressed on the surface of the VLP.

Embodiment 28. The method of embodiment 27, wherein the at least one adjuvant molecule is selected from: an influenza HA adjuvant molecule; a parainfluenza HN adjuvant molecule; a Venezuelan equine encephalitis (VEE) glycoprotein adjuvant molecule; a C3d adjuvant molecule; a mannose receptor adjuvant molecule; a membrane-anchored form of a molecule selected from: a mammalian toll-like receptor (TLR) ligand molecule, a MIP-1 alpha molecule, a RANTES MIP-1 beta molecule, a GM-CSF molecule, a Flt3 ligand molecule, a CD40 ligand molecule, an IL-2 molecule, an IL-10 molecule, an IL-12 molecule, an IL-15 molecule, an IL-18 molecule, and an IL-21 molecule; or any combination of two or more of the foregoing.

Embodiment 29. The method of embodiment 28, wherein the membrane-anchored form of a mammalian TLR ligand molecule is selected from: a Prevotella intermedia glycoprotein, a respiratory synctial virus protein F, a fibronectin A domain, fibrinogen, flagellin, a measles virus HA protein, and Pam2Cys lipoprotein/lipopeptide (MALP-2).

Embodiment 30. The method of embodiment 28, wherein the at least one adjuvant molecule comprises flagellin.

Embodiment 31. A method of producing a virus-like particle (VLP) in a host cell, comprising: providing one or more expression vectors, wherein the one or more expression vectors comprise polynucleotide sequences encoding for a viral core protein, at least one antigenic polypeptide selected from a tumor antigen, Dengue virus antigen, or malaria

antigen, and at least one adjuvant molecule; introducing the one or more expression vectors into a host cell; and expressing the viral core protein, at least one antigenic polypeptide, and at least one adjuvant molecule, whereby the VLP is formed by the cell.

Embodiment 32. The method of embodiment 31, wherein the one or more vectors are
5 selected from: plasmids, cosmids, viral vectors, chromosomes, minichromosomes, baculovirus vectors, modified vaccinia Ankara (MVA) vectors, plasmid DNA vectors, recombinant poxvirus vectors, bacterial vectors, recombinant baculovirus expression systems (BEVS), recombinant VSV vectors, recombinant adenovirus expression systems, recombinant DNA expression vectors, and combinations thereof.

10 Embodiment 33. The method of embodiment 32, wherein the polynucleotide sequences encoding for the viral core protein, the at least one antigenic polypeptide, and the at least one adjuvant molecule are each operably linked to a promoter.

Embodiment 34. The method of embodiment 33, wherein the promoter is selected from a constitutive promoter and an inducible promoter.

15 Embodiment 35. The method of embodiment 34, wherein the promoter is selcted from: a baculovirus promoter, a recombinant Modified Vaccinia Ankara (MVA) promoter, a CMV promoter, an EF promoter, an adenovirus promoter, a recombinant VSV promoter, a recombinant adenovirus promoter, a recombinant alphavirus promoter, and combinations thereof.

20 Embodiment 36. The method of embodiment 35, wherein the VLP is selected from: human immunodeficiency virus (HIV) VLP, a simian-human immunodeficiency virus (SHIV) VLP, a feline immunodeficiency virus (FIV) VLP, a feline leukemia virus VLP, a bovine immunodeficiency virus VLP, a bovine leukemia virus VLP, a equine infectious anemia virus VLP, a human T-cell leukemia virus VLP, a Bunya Virus VLP, a Lassa fever
25 virus VLP, an Ebola virus VLP, a corona virus VLP, an Arena virus VLP, a Filovirus VLP, an influenza virus VLP, a paramyxovirus VLP, a rhabdovirus VLP, an alphavirus VLP, and a flavivirus VLP.

Embodiment 37. A method of immunizing a host comprising: co-expressing a viral core protein, at least one antigenic polypeptide selected from a tumor antigen, Dengue virus
30 antigen, and malaria antigen, and at least one adjuvant molecule in one or more host cells; whereby the viral core protein, the least one antigenic polypeptide, and the at least one adjuvant molecule assemble to form a Virus Like Particle (VLP).

CLAIMS

We claim:

1. A virus-like particle (VLP), comprising: a viral core protein that can self-assemble into a VLP core; at least one tumor antigen expressed on the surface of the VLP; and at least one adjuvant molecule expressed on the surface of the VLP.

2. A virus-like particle (VLP), comprising: a viral core protein that can self-assemble into a VLP core; at least one Dengue virus antigen expressed on the surface of the VLP; and at least one adjuvant molecule expressed on the surface of the VLP.

3. A virus-like particle (VLP), comprising: a viral core protein that can self-assemble into a VLP core; at least one malaria antigen expressed on the surface of the VLP; and at least one adjuvant molecule expressed on the surface of the VLP.

4. The VLP of claim 1, wherein the at least one tumor antigen is selected from the group consisting of CD138, CD44, alpha4 integrin, alpha3 integrin, alpha6 integrin, EGFR, tumor suppressor cell antigen, cytotoxic T cell antigen, 17-1A, 707-AP, AFP, Annexin II, ART-4, BAGE, BAGE-1, β-catenin, BCG, bcr/abl, Bcr/abl e14a2 fusion junction, bcr-abl (b3a2), bcr-abl (b3a2), bcr-abl p190 (e1a2), bcr-abl p210 (b2a2), bcr-abl p210 (b3a2), bcr-abl p210 (b3a2), bullous pemphigoid antigen-1, CA19-9, CA125, CA215, CAG-3, CAMEL, Cancer-testis antigen, Caspase-8, CCL3, CCL4, CD16, CD20, CD3, CD30, CD55, CD63, CDC27, CDK-4, CDR3, CEA, cluster 5, cluster-5A, cyclin-dependent kinase-4, Cyp-B, DAM-10, DAM-6, Dek-cain, E7, EGFRvIII, EGP40, ELF2 M, EpCAM, FucGM1, G250, GA733, GAGE, GAGE-1-8, gastrin cancer associated antigen, GD2, GD3, globoH, glycophorin, GM1, GM2, GM3, GnTV, Gn-T-V, gp100, Her-2/neu, HERV-K-ME, high molecular weight-associated antigen, high molecular weight proteo-glycan (HMPG), HPV-16 E6, HPV-16 E7, HPVE6, HSP70-2M, HST-2, hTERT, human chorionic gonadotropin (HCG), Human milk fat globule (HMFG), iCE, KIAA0205, KK-LC-1, KM-HN-1, L6, LAGE-1, Lcose4Cer, LDLR/FUT, Lewis A, Lewis v/b, M protein, MAGE-1, MVC, MAGE-A1-12, MAGE-C2, MAHGE-3, MART-1/Melan-A, MC1R, ME491, MUC1, MUC2, mucin,

MUM-1, MUM-2, MUM-3, mutated p53, Myosin, MZ2-E, N9 neuraminidase, NA88, NA88-A, nasopharyngeal carcinoma antigen, NGA, NK1/c-3, Novel bcr/ablk fusion BCR exons 1, 13, 14 with ABL exons 4, NY-ESO-1/LAGE-2, NY-ESO-1b, OC125, osteosarcoma associated antigen-1, P15, p190 mimor bcr-abl (e1a2), p53, Pml/RARα, Polysialic acid, PRAME, PSA, PSM, RU1, RU2, SAGE, SART-1, SART-2, SART-3, Sialyl LeA, Sp17, SSX-2, SSX-4, surface immunoglobulin, TAG-1, TAG-2, TEL/AML1, TPI, TRAG-3, TRP-1(gp75), TRP-2, TRP2-INT2, hTRT, tumor associated glycoprotein-72 (TAG-72), tyrosinase, u-PA, WT1, and XAGE-1b, or an immunogenic fragment of any of the foregoing antigens.

5. The VLP of claim 4, wherein the at least one tumor antigen is an antigen associated with a hematologic malignancy.

6. The VLP of claim 2, wherein the at least one Dengue virus antigen is selected from the group consisting of C, prM, E, NS1, NS2a, NS3, NS4a, NS4b, and NS5 of serotype 1, 2, 3, or 4.

7. The VLP of claim 3, wherein the at least one malaria antigen is selected from a Plasmodium from *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, or *P. knowlesi*.

8. The VLP of any one of claims 1, 4, or 5, wherein the viral core protein is selected from: a retrovirus Gag protein, a retrovirus matrix protein, a rhabdovirus M protein, a filovirus viral core protein, a corona virus M protein, a corona virus E protein, a corona virus NP protein, a bunyavirus N protein, an influenza M1 protein, a paramyxovirus M protein, an arenavirus Z protein, and combinations thereof.

9. The VLP of claim 8, wherein the viral core protein comprises a retrovirus Gag protein is selected from: a Human Immunodeficiency Virus (HIV) Gag protein, a Simian Immunodeficiency Virus (SIV) Gag protein, and a Murine Leukemia Virus (MuLV) Gag protein.

10. The VLP of claim 8, wherein the viral core protein is selected from: a Vesicular Stomatitis Virus (VSV) M protein, an Ebola Virus VP40 protein, a Lassa Fever Virus Z protein, and combinations thereof.

11. The VLP of claim 2 or 6, wherein the viral core protein is selected from: a retrovirus Gag protein, a retrovirus matrix protein, a rhabdovirus M protein, a filovirus viral core protein, a corona virus M protein, a corona virus E protein, a corona virus NP protein, a bunyavirus N protein, an influenza M1 protein, a paramyxovirus M protein, an arenavirus Z protein, and combinations thereof.

12. The VLP of claim 11, wherein the viral core protein comprises a retrovirus Gag protein is selected from: a Human Immunodeficiency Virus (HIV) Gag protein, a Simian Immunodeficiency Virus (SIV) Gag protein, and a Murine Leukemia Virus (MuLV) Gag protein.

13. The VLP of claim 11, wherein the viral core protein is selected from: a Vesicular Stomatitis Virus (VSV) M protein, an Ebola Virus VP40 protein, a Lassa Fever Virus Z protein, and combinations thereof.

14. The VLP of claim 3 or 7, wherein the viral core protein is selected from: a retrovirus Gag protein, a retrovirus matrix protein, a rhabdovirus M protein, a filovirus viral core protein, a corona virus M protein, a corona virus E protein, a corona virus NP protein, a bunyavirus N protein, an influenza M1 protein, a paramyxovirus M protein, an arenavirus Z protein, and combinations thereof.

15. The VLP of claim 14, wherein the viral core protein comprises a retrovirus Gag protein is selected from: a Human Immunodeficiency Virus (HIV) Gag protein, a Simian Immunodeficiency Virus (SIV) Gag protein, and a Murine Leukemia Virus (MuLV) Gag protein.

16. The VLP of claim 14, wherein the viral core protein is selected from: a Vesicular Stomatitis Virus (VSV) M protein, an Ebola Virus VP40 protein, a Lassa Fever Virus Z protein, and combinations thereof.

17. The VLP of any one of claims 1-3, wherein the at least one adjuvant molecule is selected from: an influenza HA adjuvant molecule; a parainfluenza HN adjuvant molecule; a

Venezuelan equine encephalitis (VEE) glycoprotein adjuvant molecule; a C3d adjuvant molecule; a mannose receptor adjuvant molecule; a membrane-anchored form of a molecule selected from: a mammalian toll-like receptor (TLR) ligand molecule, a MIP-1 alpha molecule, a RANTES MIP-1 beta molecule, a GM-CSF molecule, a Flt3 ligand molecule, a CD40 ligand molecule, an IL-2 molecule, an IL-10 molecule, an IL-12 molecule, an IL-15 molecule, an IL-18 molecule, and an IL-21 molecule; or any combination of two or more of the foregoing.

18. The VLP of claim 17, wherein the at least one adjuvant molecule is a membrane-anchored form of a mammalian TLR ligand molecule selected from: a Prevotella intermedia glycoprotein, a respiratory synctial virus protein F, a fibronectin A domain, fibrinogen, flagellin, a measles virus HA protein, and Pam2Cys lipoprotein/lipopeptide (MALP-2).

19. The VLP of claim 17, wherein the at least one adjuvant molecule comprises flagellin.

20. An immunogenic composition, comprising the VLP of claim 1; and a pharmacologically acceptable carrier.

21. An immunogenic composition, comprising the VLP of claim 2; and a pharmacologically acceptable carrier.

22. An immunogenic composition, comprising the VLP of claim 3; and a pharmacologically acceptable carrier.

23. A method of generating an immunological response in a subject, comprising administering an effective amount of a VLP or immunogenic composition of any one of claims 1-3 or 20-22 to the subject.

24. A method of treating a malignancy, comprising administering to a subject in need of treatment an effective amount of a VLP or immunogenic composition of claim 1 or 20.

25. The method of claim 24, wherein the subject has the malignancy, and wherein the VLP or immunogenic composition is administered for therapy.

26. The method of claim 24, wherein the subject does not have the malignancy, and wherein the VLP or immunogenic composition is administered for prophylaxis.

27. A method of treating Dengue virus infection, comprising administering to a subject in need of treatment an effective amount of a VLP or immunogenic composition of claim 2 or 21.

28. The method of claim 27, wherein the subject has the Dengue virus infection, and wherein the VLP or immunogenic composition is administered for therapy.

29. The method of claim 27, wherein the subject does not have the Dengue virus infection, and wherein the VLP or immunogenic composition is administered for prophylaxis.

30. A method of treating malaria, comprising administering to a subject in need of treatment an effective amount of a VLP or immunogenic composition of claim 3 or 22.

31. The method of claim 30, wherein the subject has a malarial infection, and wherein the VLP or immunogenic composition is administered for therapy.

32. The method of claim 30, wherein the subject does not have a malarial infection, and wherein the VLP or immunogenic composition is administered for prophylaxis.

33. A method of determining exposure of a host to an antigen, comprising the steps of: contacting a biological fluid of the subject with a virus-like particle (VLP) comprising: a viral core protein that can self-assemble into the VLP core; and at least one antigenic polypeptide expressed on the surface of the VLP, wherein the at least one antigenic polypeptide is selected from a tumor antigen, Dengue virus antigen, malaria antigen, under conditions which are permissive for binding of antibodies in the biological fluid with the VLP; and detecting binding of antibodies within the biological fluid with the VLP, wherein

exposure of the subject to the virus is determined by the detection of antibodies bound to the VLP.

34. The method of claim 33, wherein detecting includes the use of a labeled second antibody which is specific to antibodies in the biological fluid being tested.

35. The method of claim 33, wherein the VLP further comprises at least one adjuvant molecule expressed on the surface of the VLP.

36. The method of claim 35, wherein the at least one adjuvant molecule is selected from: an influenza HA adjuvant molecule; a parainfluenza HN adjuvant molecule; a Venezuelan equine encephalitis (VEE) glycoprotein adjuvant molecule; a C3d adjuvant molecule; a mannose receptor adjuvant molecule; a membrane-anchored form of a molecule selected from: a mammalian toll-like receptor (TLR) ligand molecule, a MIP-1 alpha molecule, a RANTES MIP-1 beta molecule, a GM-CSF molecule, a Flt3 ligand molecule, a CD40 ligand molecule, an IL-2 molecule, an IL-10 molecule, an IL-12 molecule, an IL-15 molecule, an IL-18 molecule, and an IL-21 molecule; or any combination of two or more of the foregoing.

37. The method of claim 36, wherein the membrane-anchored form of a mammalian TLR ligand molecule is selected from: a Prevotella intermedia glycoprotein, a respiratory synctial virus protein F, a fibronectin A domain, fibrinogen, flagellin, a measles virus HA protein, and Pam2Cys lipoprotein/lipopeptide (MALP-2).

38. The method of claim 36, wherein the at least one adjuvant molecule comprises flagellin.

39. A method of producing a virus-like particle (VLP) in a host cell, comprising: providing one or more expression vectors, wherein the one or more expression vectors comprise polynucleotide sequences encoding for a viral core protein, at least one antigenic polypeptide selected from a tumor antigen, Dengue virus antigen, or malaria antigen, and at least one adjuvant molecule; introducing the one or more expression vectors into a host cell;

and expressing the viral core protein, at least one antigenic polypeptide, and at least one adjuvant molecule, whereby the VLP is formed by the cell.

40. The method of claim 39, wherein the one or more vectors are selected from: plasmids, cosmids, viral vectors, chromosomes, minichromosomes, baculovirus vectors, modified vaccinia Ankara (MVA) vectors, plasmid DNA vectors, recombinant poxvirus vectors, bacterial vectors, recombinant baculovirus expression systems (BEVS), recombinant VSV vectors, recombinant adenovirus expression systems, recombinant DNA expression vectors, and combinations thereof.

41. The method of claim 39, wherein the polynucleotide sequences encoding for the viral core protein, the at least one antigenic polypeptide, and the at least one adjuvant molecule are each operably linked to a promoter.

42. The method of claim 41, wherein the promoter is selected from a constitutive promoter and an inducible promoter.

43. The method of claim 42, wherein the promoter is selected from: a baculovirus promoter, a recombinant Modified Vaccinia Ankara (MVA) promoter, a CMV promoter, an EF promoter, an adenovirus promoter, a recombinant VSV promoter, a recombinant adenovirus promoter, a recombinant alphavirus promoter, and combinations thereof.

44. The method of claim 39, wherein the VLP is selected from: human immunodeficiency virus (HIV) VLP, a simian-human immunodeficiency virus (SHIV) VLP, a feline immunodeficiency virus (FIV) VLP, a feline leukemia virus VLP, a bovine immunodeficiency virus VLP, a bovine leukemia virus VLP, a equine infectious anemia virus VLP, a human T-cell leukemia virus VLP, a Bunya Virus VLP, a Lassa fever virus VLP, an Ebola virus VLP, a corona virus VLP, an Arena virus VLP, a Filovirus VLP, an influenza virus VLP, a paramyxovirus VLP, a rhabdovirus VLP, an alphavirus VLP, and a flavivirus VLP.

45. A method of immunizing a host comprising: co-expressing a viral core protein, at least one antigenic polypeptide selected from a tumor antigen, Dengue virus antigen, and

malaria antigen, and at least one adjuvant molecule in one or more host cells; whereby the viral core protein, the least one antigenic polypeptide, and the at least one adjuvant molecule assemble to form a Virus Like Particle (VLP).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/063937

A. CLASSIFICATION OF SUBJECT MATTER

C07K 14/005(2006.01)i, C12Q 1/04(2006.01)i, C12Q 1/70(2006.01)i, G01N 33/53(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K 14/005; C07K 19/00; A61K 39/385; C12N 7/00; C12Q 1/70; C12N 15/62; C12Q 1/04; G01N 33/53

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: virus-like particle, core protein, tumor antigen, Dengue virus antigen, malaria antigen, adjuvant, surface, flagellin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011-056899 A2 (LIGOCYTE PHARMACEUTICALS, INC.) 12 May 2011 See abstract; claims 1-34; and paragraphs [0006]-[0018], [0067]-[0077], [0094]-[0115], [0120]-[0142], [0149]-[0152], [0164]-[0171].	1-22,39-44
Y		33-38
Y	US 2006-0216702 A1 (COMPANS et al.) 28 September 2006 See abstract; claims 1-49; paragraphs [0033]-[0041]; and figure 1.	33-38
A	WO 2011-150249 A1 (SELECTA BIOSCIENCES, INC.) 01 December 2011 See abstract; claims 1-131; and pages 6-7, 26-27.	1-22,33-44
A	PEJAWAR-GADDY et al., `Generation of a tumor vaccine candidate based on conjugation of a MUC1 peptide to polyionic papillomavirus virus-like particles` Cancer Immunology, Immunotherapy, Vol. 59, Issue 11, pp. 1685-1696 (2010) See the whole document.	1-22,33-44
A	MIZEL et al., `Flagellin as an adjuvant: cellular mechanisms and potential` The Journal of Immunology, Vol. 185, No. 10, pp. 5677-5682 (2010) See the whole document.	1-22,33-44

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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

Date of the actual completion of the international search	Date of mailing of the international search report
03 February 2015 (03.02.2015)	**04 February 2015 (04.02.2015)**

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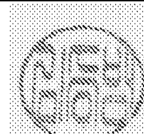

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/063937

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

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

1. [X] Claims Nos.: 23-32,45
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 23-32 and 45 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.

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

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

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

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

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

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

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

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

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2014/063937

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