



(51) International Patent Classification:

A61K 35/76 (2015.01)

A61K 39/21 (2006.01)

A61K 39/12 (2006.01)

Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

(21) International Application Number:

PCT/US2018/024758

Published:

— with international search report (Art. 21(3))

— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

— with sequence listing part of description (Rule 5.2(a))

(22) International Filing Date:

28 March 2018 (28.03.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/477,480

28 March 2017 (28.03.2017)

US

(71) Applicant: CHILDREN'S HOSPITAL MEDICAL CENTER [US/US]; 3333 Burnett Avenue, Cincinnati, OH 45229 (US).

(72) Inventor; and

(71) Applicant: CHEN, Xuemin [US/US]; 1919 Ridgemont Lane, Decatur, GA 30033 (US).

(72) Inventors: SINGH, Karnail; 7795 Golden Meadow Drive, Deerfield Township, OH 45040 (US). SPEARMAN, Paul; 946 Monastery Street, Cincinnati, OH 45202-1561 (US).

(74) Agent: TEPE, Nicole, M. et al.; Frost Brown Todd LLC, 3300 Great American Tower, 301 East Fourth Street, Cincinnati, OH 45202 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: VLP-BASED MONOVALENT EBOLA VACCINES AND METHODS OF MAKING AND USING SAME

(57) Abstract: Disclosed herein are virus-like particle (VLP)-based monovalent vaccine compositions. The compositions may comprise a spherical retroviral Group-specific Antigen ("Gag") protein core and a single Ebola glycoprotein selected from either a Zaire (EBOV) glycoprotein or a Sudan (SUDV) glycoprotein. The Ebola glycoprotein may be incorporated into the surface of the spherical Gag core, wherein said VLP-based vaccine presents a single Ebola glycoprotein antigen.



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VLP-BASED MONOVALENT EBOLA VACCINES AND METHODS OF MAKING AND USING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and benefit of 62/477,480, entitled “Development and Evaluation of a Novel Bivalent Virus Like Particle (VLP) based Ebola Vaccine” filed March 28, 2017, the contents of which are incorporated in its entirety for all purposes.

BACKGROUND

[0002] Ebolaviruses have caused epidemics in humans that are characterized by high mortality rates. The 2013-2016 Ebolavirus epidemic in West Africa resulted in more than 28,600 infections and 11,300 deaths (WHO June 2016). The extent of the outbreak coupled with a high rate of mortality emphasized the importance of developing new therapeutics and preventive vaccines against Ebolaviruses. Several candidate Ebola vaccines have shown protection in the non-human primate (NHP) model of Ebola. Three of the most promising live attenuated vaccines for Ebola are those derived from Vesicular stomatitis virus (VSV), Chimp Adenovirus Type 3 (ChAd3), and Modified Vaccinia Ankara (MVA). However, none of these approaches has proven capable of eliciting long-lasting protective immune responses.

[0003] Successful development of a vaccine will significantly help in the prevention and control of Ebola epidemics in Africa as well as protect health-care workers and researchers working with these deadly viruses. Such a vaccine will also be extremely useful in the event of a bio-terrorism attack and protecting American troops abroad. Clinical care of Ebola infected patients needs special infrastructure, restricted-access facilities and highly trained health-care workers and there is a very high cost associated with this. Thus, the economic impact of developing such a vaccine also cannot be underestimated. The instant disclosure addresses one or more of the aforementioned needs in the art.

BRIEF SUMMARY

[0004] Disclosed herein are virus-like particle (VLP)-based monovalent vaccine compositions. The compositions may comprise a spherical retroviral Group-specific Antigen

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("Gag") protein core and a single Ebola glycoprotein selected from either a Zaire (EBOV) glycoprotein or a Sudan (SUDV) glycoprotein. The Ebola glycoprotein may be incorporated into the surface of the spherical Gag core, wherein said VLP-based vaccine presents a single Ebola glycoprotein antigen.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] This application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0006] Those of skill in the art will understand that the drawings, described below, are for illustrative purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

[0007] FIG 1. Inducible expression of Ebola GPs and HIV-1 Gag in EBOVGPGag and SUDVGPGag 293F cells. (A) EBOVGPGag 293F cells and (B) SUDVGPGag 293F cells were cultured as such or induced with Dox for 24 h and cell lysates probed by western blotting for EBOV GP, HIV-1 Gag and actin (A) or SUDV GP, HIV-1 Gag and actin (B) using specific antibodies.

[0008] FIG 2. Dox induced secretion of monovalent EBOVGPGag or SUDVGPGag VLPs by stably transfected 293F cells. Supernatant from cells induced with Dox was layered on 20% sucrose cushion and subjected to ultracentrifugation. VLP pellets so obtained and the cell lysates were probed by western blotting using anti-EBOV GP and anti-HIV-1 Gag (A) or anti-SUDV GP and anti-HIV-1 Gag (B) specific antibodies.

[0009] FIG 3. Buoyance density analysis of monovalent EBOVGPGag and SUDVGPGag VLPs. Fractions collected after ultracentrifugation of monovalent Ebola VLPs over 20-60% sucrose gradient were probed by western blotting using anti-EBOV GP and anti-HIV-1 Gag (A) or anti-SUDV GP and anti-HIV-1 Gag (B) specific antibodies. Buoyance density of each fraction was determined by using a refractometer.

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[0010] FIG 4. Negative electron microscopy of monovalent EBOVGPGag and SUDVGPGag VLPs. Ebola VLPs harvested from culture supernatants after ultracentrifugation on 20% sucrose cushion were analyzed by negative electron microscopy. The analysis showed spherical particles abundantly covered with spikes of glycoproteins on their surface.

[0011] FIG 5. Immunoreactivity of rabbit anti-monovalent Ebola VLP sera with recombinant Ebola glycoproteins. Elisa plates were coated with 20 ng/well of either recombinant EBOV GP (A) or SUDV GP (B) proteins, blocked and incubated with increasing dilutions of rabbit anti-monovalent Ebola VLP sera. Protein bound antibodies were detected by incubating the wells with horse radish peroxidase (HRP) bound anti-rabbit IgG detecting antibodies followed by the addition of HRP substrate and measuring the optical density at 450 nm.

[0012] FIG 6. Rabbit anti-monovalent Ebola VLP sera strongly neutralize all four species of Ebolavirus pathogenic to humans. EBOV, SUDV, BDBV and TAFV GP containing HIV-1 Δ Env pseudovirions were incubated with different dilutions of rabbit anti-ESGPGag VLP sera for one hour and then added onto TZM-bl cells that express luciferase under the control of HIV-1 Tat. 48 hours later luciferase activity was measured and percentage neutralization calculated by comparing luciferase activity in the test wells to that in the wells that received respective pseudovirions that was not incubated with any antibody/serum.

DETAILED DESCRIPTION

[0013] DEFINITIONS

[0014] Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The

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materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

[0015] As used herein and in the appended claims, the singular forms “a,” “and,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a method” includes a plurality of such methods and reference to “a dose” includes reference to one or more doses and equivalents thereof known to those skilled in the art, and so forth.

[0016] The term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, e.g., the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, “about” can mean a range of up to 20%, or up to 10%, or up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term “about” meaning within an acceptable error range for the particular value should be assumed.

[0017] As used herein, the term “effective amount” means the amount of one or more active components that is sufficient to show a desired effect. This includes both therapeutic and prophylactic effects. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

[0018] The terms “individual,” “host,” “subject,” and “patient” are used interchangeably to refer to an animal that is the object of treatment, observation and/or experiment. Generally, the term refers to a human patient, but the methods and compositions may be equally applicable to non-human subjects such as other mammals. In some embodiments, the terms refer to humans. In further embodiments, the terms may refer to children.

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[0019] Ebola virus disease (EVD) is caused by Ebola viruses that are filamentous, negative-strand RNA viruses belonging to the family Filoviridae. There are five species of the Ebola viruses; Zaire ebolavirus (EBOV), Sudan ebolavirus (SUDV), Bundibugyo ebolavirus (BDBV), Tai-forest ebolavirus (TAFV) and Reston ebolavirus (RESTV). First four have been shown to be pathogenic in humans whereas RESTV has been shown to cause disease only in non-human primates. Ebolavirus structure consists of an inner nucleocapsid made up of nucleoprotein (NP) and containing the viral RNA, along with RNA polymerase L, transcription factor VP30 and cofactor VP35. The viral nucleocapsid is surrounded by a lipid bilayer that contains the envelope protein spikes of glycoprotein (GP). Between the viral envelope and nucleocapsid are the matrix proteins VP40 and VP24 (1). Because of its integral role in the pathogenesis of EVD, GP has been the relevant target of all the candidate Ebola vaccines developed so far.

[0020] A number of candidate Ebola vaccines have been developed and evaluated in non-human primate (NHP) model of EVD as well as in clinical trials (2-11). A few of them were fast tracked to the clinical trials during the 2013-2016 Ebola epidemic. Ebola vaccines such as the vesicular stomatitis virus (VSV), adenovirus 5 (Ad5) or chimpanzee adenovirus 3 (ChAd3)-vectored vaccines appear quite promising in generating protective immune responses. However, each candidate has substantial limitations. For example, the VSV vaccine elicits a high rate of adverse events so that acceptability for licensure remains in doubt (2, 3) Recombinant human adenoviral vectored vaccines are limited by pre-existing immunity to the vector in the human populations at risk for Ebola (4). Chimpanzee adenovirus based Ebola vaccine like ChAd3-EBO and ChAd3-EBO-Z have been developed to bypass the pre-existing immunity to the vector in the humans. However, in non-human primate models of Ebola virus disease (EVD), these vaccines did not induce durable protective immunity (11). Boosting of animals after priming with this vaccine with Modified Vaccinia Ankara (MVA)-Filo vaccine that has glycoproteins (GP) from Zaire (EBOV), Sudan (SUDV) and Marburg (MARV) viruses and nucleoprotein from Tai-Forest (TAFV) virus has been shown to extend the duration of protective immune responses against lethal EBOV challenge (11). Together these studies clearly suggested that novel Ebola vaccines,

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that either alone or in combination with other vaccines, are needed that can provide long-term protective immunity against the dreadful Ebolaviruses.

[0021] Disclosed herein is the successful development and production of novel virus-like particle (VLP) based monovalent Ebola vaccines that express Ebola glycoproteins (GP) from either Zaire (EBOV) or Sudan (SUDV) species on the HIV-1 Gag core. The immunogenicity of these glycoprotein VLP Ebola vaccines and their ability to generate high titers of functional neutralizing antibodies can be tested by immunizing rabbits with these vaccines combined with adjuvants Poly (I:C) and CpG. Immunization of rabbits with EBOVGPGag VLPs produced high titered binding antibodies that neutralized all four pathogenic species of Ebola but to different degrees while immunization with SUDVGPGag VLPs produced binding antibodies that neutralized only SUDV.

[0022] The disclosed VLP platforms may be used for large-scale production of nanoparticle monovalent Ebola vaccines. The production systems may employ stable and inducible cell lines such as the EBOVGPGag 293F or SUDVGPGag cells that, upon induction with doxycycline (Dox), secrete VLPs with HIV-1 Gag core abundantly studded with Ebola glycoproteins (GPs) from either Zaire (EBOV) or Sudan (SUDV) Ebolaviruses. There are several unique aspects of this VLP approach. (1) The Gag core provides a very stable framework of ~110nm on which Ebola GPs may be incorporated in their native conformation. These stable cell production systems can be easily scaled up to produce large quantities of clinical grade Ebola VLPs. (2) The spherical Gag core exhibits enhanced stability as compared with other framework constructs such as Ebola VP40 protein. (3) The VLP-based vaccines are non-infectious, have antigen in stable and native conformation, and do not suffer from the limitation of pre-existing immunity. This Ebola vaccine may provide protection as a stand-alone vaccine, or may be even more advantageous when provided as a booster vaccine following priming with other experimental Ebola vaccines.

[0023] Disclosed herein are virus-like particle (VLP)-based monovalent vaccine compositions. The compositions may comprise a spherical retroviral Group-specific Antigen ("Gag") protein core and a single Ebola glycoprotein selected from either a Zaire (EBOV) glycoprotein or a Sudan (SUDV) glycoprotein. SUDV is described as YP_138523.1 in the

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literature, whereas an example of EBOV GP is reported as NCBI Reference Sequence: NP_066246.1, both incorporated herein in their entirety by reference. The Ebola glycoprotein may be incorporated into the surface of the spherical Gag core, wherein said VLP-based vaccine presents a single Ebola glycoprotein antigen. An exemplary Gag protein includes, but is not limited to, that of SEQ ID NO: 1. Exemplary glycoproteins include, but are not limited to, those set forth in SEQ ID NOS 2, 3, and 4. It will be understood by one of ordinary skill in the art that one may employ a sequence having at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the reference sequence, which may include the sequences disclosed herein or as otherwise known in the art. The length of comparison sequences may be at least 5 contiguous nucleotides or amino acids, or at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 contiguous nucleotides or amino acids, or the full-length nucleotide or amino acid sequence. Sequence identity may be measured using sequence analysis software on the default setting (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705). Such software may match similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications.

[0024] In one aspect, the Gag protein may be derived from a retrovirus. For example, suitable retroviruses from which the gag protein may be derived include human immunodeficiency virus (HIV), murine leukemia virus (MLV) Rous sarcoma virus (RSV), Equine infectious anemia virus (EIAV), or the like. The sequences of such will be readily appreciated by one of ordinary skill in the art.

[0025] In one aspect, it has been surprisingly found that the glycoprotein is capable of aggregating at a relatively high density at the surface of the spherical gag core. In certain aspects, the EBOV GP Concentration may be about 20 to about 200 ug/mL, or about 40 to about 150, or about 80 to 100 ug/ml, for example in one aspect, 23 to 184 ug/ml. The SUDV GP Concentration may be about 10 to about 60 ug/ml, or about 20 to about 50 ug/mL, or about 30 to about 40 ug/ml, for example, in one aspect, 13-53 ug/ml. The HIV-1 Gag Concentration may be about 1 to about 10 ug/ml, or about 3 to about 10 ug/ml. The concentrations of EBOV GP and SUDV GP, may be measured by semi-quantitative western

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blotting using known quantities of purified recombinant EBOV GP and SUDV GP respectively. Concentrations of HIV-1 Gag VLPs may be measured by an HIV-1 Gag specific ELISA using known quantities of purified HIV-1 Gag. In one aspect, the spherical Gag protein core may have a diameter of from about 100 to about 300 nanometers or about 200 to about 250 nanometers.

[0026] In one aspect, the VLP composition may be sufficient to immunize an individual against a viral infection from one or more of Zaire (EBOV), Sudan (SUDV), Bundibugyo (BDBV) and Tai Forest (TAFV), or in some aspects, two or more of Zaire (EBOV), Sudan (SUDV), Bundibugyo (BDBV) and Tai Forest (TAFV), or three or more of Zaire (EBOV), Sudan (SUDV), Bundibugyo (BDBV) and Tai Forest (TAFV), or all four of Zaire (EBOV), Sudan (SUDV), Bundibugyo (BDBV) and Tai Forest (TAFV).

[0027] The disclosed compositions may further comprise one or more pharmaceutical-acceptable carriers, which may include any and all solvents, dispersion media, coatings, stabilizing agents, diluents, preservatives, antibacterial and antifungal agents, isotonic agents, adsorption delaying agents, and the like. The disclosed S particles may be provided in physiological saline. Optionally, a protectant may be included, for example, an anti-microbiological active agent, such as for example Gentamycin, Merthiolate, and the like. The compositions may further include a stabilizing agent, such as for example saccharides, trehalose, mannitol, saccharose and the like, to increase and/or maintain product shelf-life. Those of skill in the art will understand that the composition herein may incorporate known injectable, physiologically acceptable sterile solutions. For preparing a ready-to-use solution for parenteral injection or infusion, aqueous isotonic solutions, such as e.g. saline or corresponding plasma protein solutions are readily available. In addition, the immunogenic and vaccine compositions of the present invention can include diluents, isotonic agents, stabilizers, or adjuvants. Diluents can include water, saline, dextrose, ethanol, glycerol, and the like. Isotonic agents can include sodium chloride, dextrose, mannitol, sorbitol, and lactose, among others. Stabilizers include albumin and alkali salts of ethylenediaminetetracetic acid, among others. Suitable adjuvants will be appreciated by one of ordinary skill in the art.

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[0028] In one aspect, disclosed is a container comprising at least one dose of the immunogenic compositions disclosed herein. The container may comprise 1 to 250 doses of the immunogenic composition, or in other aspects, 1, 10, 25, 50, 100, 150, 200, or 250 doses of the immunogenic composition. In one aspect, each of the containers may comprise more than one dose of the immunogenic composition and may further comprises an anti-microbiological active agent. Those agents may include, for example, antibiotics such as Gentamicin and Merthiolate and the like.

[0029] A further aspect relates to a kit. The kit may comprise any of the containers described above and an instruction manual, including the information for the delivery of the immunogenic composition disclosed above. For example, instructions related to intramuscular application of at least one dose may be provided for lessening the severity of clinical symptoms associated with an infection of an antigen as disclosed here. The kits and/or compositions may further include an immune stimulant such as keyhole limpet hemocyanin (KLH), or incomplete Freund's adjuvant (KLH/ICFA). Any other immune stimulant known to a person skilled in the art may also be used.

[0030] In one aspect, a method for eliciting an immune response an individual in need thereof against an Ebola virus species selected from Zaire (EBOV), Sudan (SUDV), Bundibugyo (BDBV), Tai Forest (TAFV), and combinations thereof is disclosed. The method may comprise the step of administering a composition as disclosed herein. In this aspect, the method may include the step of administering a vaccine composition as disclosed above to an individual in need thereof. It will be readily appreciated that the disclosed compositions may be administered to an individual according to any method known in the art, and that optimal administration (including route and amounts) will not require undue experimentation. The vaccine compositions may be administered prophylactically to an individual suspected of having a future exposure to the antigen incorporated into the vaccine composition. In certain aspects, provided is a method of providing an immune response that protects an individual receiving the composition from infection, or reduces or lessens the severity of the clinical symptoms associated from an Ebola infection. Dosage regimen may be a single dose schedule or a multiple dose schedule (e.g., including booster doses) with a unit dosage form of the composition administered at different times. The term "unit dosage form," as used herein,

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refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of the antigenic compositions disclosed herein in an amount sufficient to produce the desired effect, which compositions are provided in association with a pharmaceutically acceptable excipient (e.g., pharmaceutically acceptable diluent, carrier or vehicle). The vaccine may be administered in conjunction with other immunoregulatory agents.

[0031] The dosage regimen may take a variety of different forms. For example, the individual may be administered a first dose of the disclosed composition followed by a second dose of the composition. In certain aspects, the second dose may be administered at a second point in time selected from a day after the first dose, a week after the first dose, two weeks after the first dose, three weeks after the first dose, and four weeks after the first dose.

[0032] In one aspect, a method of making the monovalent VLP compositions is disclosed. The method may comprise the steps of purifying from a cell line a VLP composition comprising a spherical retroviral Group-specific Antigen (“Gag”) protein core and an Ebola glycoprotein, wherein the Ebola glycoprotein may be incorporated into the surface of the spherical Gag protein core. The cell line may be stably transfected with a first plasmid containing a gag sequence under the control of an inducible promoter and a second plasmid containing an Ebola glycoprotein sequence under the control of an inducible promoter. The stably transfected cell line may produce the spherical Gag protein core such that the Ebola glycoprotein is located at the surface of the spherical Gag protein core. The stably transfected cell line may be such that the Gag protein core is produced upon induction with doxycycline. The first and second plasmid may be antibiotic resistant. This antibiotic resistance allows the cells stably transfected with the two plasmids to grow in a selection media containing the two antibiotics. The VLP may then be purified by ultracentrifugation, such as via a sucrose cushion, for example, a 20% sucrose cushion, or by cross-flow filtration followed by ultracentrifugation.

[0033] In one aspect, the cell line may be a human cell line modified to stably express Gag, EBOV GP or SUDV GP under inducible promoters. In one aspect, the cell line may be a 293F cell line, in a further aspect, the cell line may be a 293F 6/TR cell line

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[0034] In one aspect, a cell line comprising a first plasmid containing a sequence encoding for a gag protein under the control of an inducible promoter, and a second plasmid containing a sequence encoding for an Ebola glycoprotein under the control of an inducible promoter is disclosed. The Ebola glycoprotein may be one of EBOV or SUDV. In one aspect, the cell line may be a 293F cell line, in a further aspect, the cell line may be a 293F 6/TR cell line the cell line may be stably transfected with the plasmids. In certain aspects, the cell line may be an inducible cell line. Any of the aforementioned sequences may be codon-optimized. Codon optimization is readily understood by one of ordinary skill in the art, described, for example, at <https://www.idtdna.com/CodonOpt>.

EXAMPLES

[0035] The following non-limiting examples are provided to further illustrate embodiments of the invention disclosed herein. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent approaches that have been found to function well in the practice of the invention, and thus can be considered to constitute examples of modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

[0036] Methods

[0037] Plasmids: Plasmids useful for the disclosed methods compositions are described as follows. The HIV-1 Gag gene was introduced between HindIII and BamHI sites of plasmid pcDNA4/TO (Invitrogen, Carlsbad, CA) to generate plasmid pcDNA4/TO HIV-1 Gag. pcDNA5/TO-puro and pcDNA5/TO-puro plasmids may be created by replacing hygromycin resistance gene sequence of original pcDNA5/TO vector (Invitrogen, Carlsbad, CA) with a puromycin resistance gene. Briefly, the puromycin resistance gene was amplified by polymerase chain reaction (PCR). The hygromycin resistance gene was deleted from the original pcDNA5/TO plasmid by digestion with enzyme Pml I, and the puromycin resistance gene inserted in its place. The sequence and orientation of the inserts may be verified. Codon-optimized EBOV GP Mayinga gene may be synthesized by GenScript (Piscataway, NJ) and

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placed under a tetracycline-controlled cytomegalovirus (CMV) promoter into plasmid pcDNA5/TO-puro using BamHI and EcoRI sites to generate the plasmid pcDNA5/TO-puro EBOV GP. Codon-optimized SUDV GP Gulu gene may be purchased from Sino-Biological (Beijing, China) and placed under a tetracycline-controlled cytomegalovirus (CMV) promoter into plasmid pcDNA5/TO-puro using HindIII and XbaI sites to generate the plasmid pcDNA5/TO-puro SUDV GP.

[0038] Stably transfected EBOVGPGag 293F and SUDVGPGag 293F cells with inducible expression of EBOV GP, or SUDV GP and HIV-1 Gag: 293F cells, cultured in 293 Expression Media supplemented with 5 µg/ml blasticidin (Invivogen, San Diego, CA), may be transfected with pcDNA4/TO HIV-1 Gag and pcDNA5/TO-puro EBOV GP or with pcDNA4/TO HIV-1 Gag and pcDNA5/TO-puro SUDV GP plasmids together using Lipofectamine 2000. Forty-eight hours later, the cells may be washed and transferred into the selection media (293 Expression Media supplemented with 5 µg/ml blasticidin, 10 µg/ml Zeocin (Invivogen) and 1.0 µg/ml puromycin (Invivogen)). Cells may be selected and expanded over the coming weeks till their number reached $\sim 10 \times 10^6$. A part of the cells may be harvested and aliquots may be cryopreserved in liquid nitrogen. Remaining cells may be continued in the cultured and then either left untreated or induced with Dox (2 µg/ml) (Sigma-Aldrich) for 24 hours, cells harvested and cell lysates tested for EBOV GP or SUDV GP and HIV-1 Gag expression by western blotting using specific antibodies. Cell cultures with high EBOV GP or SUDV GP and HIV-1 Gag expression may be selected and labeled EBOVGPGag and SUDVGPGag 293F cells respectively, expanded and selected for further work. Multiple aliquots of these cells may be cryopreserved in liquid nitrogen.

[0039] Analysis of EBOVGPGag and SUDVGPGag VLPs secreted by 293F cells stably transfected with Ebola GPs:

[0040] EBOVGPGag and SUDVGPGag 293F cells may be cultured in 293 Expression Media in Erlenmeyer flasks to a density of 1.5×10^6 cells per ml and cultures induced with Dox (2 µg/ml). 40 hours later, cells and supernatants may be separated by high-speed centrifugation. Harvested cells may be lysed and cell lysates frozen in -80°C freezer. The supernatant was cleared by centrifugation and by passing through 0.45 micron filtration

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unit. Cleared supernatant was loaded on a 20% sucrose cushion in ultracentrifuge tubes and tubes centrifuged at 30,000 rpm for 90 minutes at 4°C in a Beckman Coulter Optima XPN90 ultracentrifuge. VLP pellets may be re-suspended in PBS. Cell lysates and VLPs may be resolved on 10% polyacrylamide gels, proteins transferred onto nitrocellulose membranes and the membranes probed by western blotting for the presence EBOV GP, or SUDV GP and HIV-1 Gag using specific antibodies.

[0041] Production and characterization of EBOVGPGag and SUDVGPGag VLPs:

[0042] Once confirmed for EBOVGPGag or SUDVGPGag VLP secretion, stably transfected 293F cell cultures may be scaled up, VLP pellets may be collected as described above and layered on a 20-60% sucrose gradient in ultracentrifuge tubes. Tubes may be centrifuged at 35,000 rpm at 4°C for 16 hours in an ultracentrifuge. After discarding the top 1.0 ml, twelve 900 µl fractions may be collected in clean tubes. Buoyance density of each fraction may be determined by using a refractometer. Fractions may be probed by western blotting using anti-EBOV GP, or anti-SUDV GP and anti-HIV-1 Gag specific antibodies. In separate experiments, VLPs collected after centrifugation on 20% sucrose cushion may be analyzed by negative-stain electron microscopy for their shape and size. VLPs from multiple runs may be pooled and quantified for EBOV GP or SUDV GP contents by quantitative western blotting using known amounts of recombinant EBOV GP protein or SUDV GP protein as the standards and specific antibodies.

[0043] Immunization of rabbits with EBOVGPGag or SUDVGPGag VLPs:

[0044] Raising of rabbit sera against EBOVGPGag or SUDVGPGag VLPs may be out-sourced to Cocalico Biologicals, Reamstown, PA. Briefly, two rabbits may be primed intramuscularly with VLPs (equivalent to 10 ug of either of the two Ebola GP) mixed with 500 ug of CpG and 200 ug of Poly(I:C) adjuvants. This may be followed by booster doses on day 21, 42 and 70 and animals may be bled on day 77. Blood samples for serum may be collected at pre-bleed and at days 49 and 77.

[0045] Binding antibodies:

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[0046] ELISA binding antibody titers against EBOV GP or SUDV GP may be quantified by ELISA using purified recombinant GPs as the coating antigens. Briefly, ELISA plates may be coated with 100 ul/well of 200 ng/ml of recombinant EBOV GP or SUDV GP. Wells may be blocked and incubated for 2 hours at 37°C with increasing dilutions of rabbit anti- EBOVGPGag or SUDVGPGag VLP sera. Protein bound antibodies may be detected by incubating the wells with optimally diluted horse radish peroxidase (HRP) bound anti-rabbit IgG detecting antibodies followed by the addition of TMB substrate, stopping the reaction with H₂SO₄ and measuring the optical density at 450 nm.

[0047] Neutralizing antibody titers:

[0048] Neutralizing antibody titers may be evaluated by using rHIV-1ΔEnv pseudovirions expressing EBOV or SUDV GP in a TZM-bl pseudovirus reporter cell method. rHIV-1ΔEnv pseudovirions expressing BDBV or TAFV GP may be included to study the ability of anti-ESGPGag VLP sera to cross-neutralize other pathogenic Ebolaviruses as well as MARV. Pseudovirions may be incubated with different dilutions of rabbit anti- EBOVGPGag or SUDVGPGag VLP sera for one hour at 37°C and then added onto confluent TZM-bl cells that express luciferase under the control of an HIV-1 Tat protein. 48 hours later luciferase activity may be measured after the addition of the luciferase substrate and percentage neutralization calculated by comparing luciferase number in test wells with those in the wells that received the respective pseudovirion that was not incubated with any antibody/serum. Serum antibody titers giving 50% neutralization may be calculated.

[0049] Results and Discussion

[0050] Development of stably transfected 293F cells producing monovalent Ebola VLPs:

[0051] Transfection of 293F with pcDNA4 TO-HIV-1 Gag and pcDNA5 TO-EBOV GP or pcDNA4 TO-HIV-1 Gag and pcDNA5 TO-SUDV GP plasmids followed by their selection in antibiotic containing media over a four-week period resulted in the development of cells that upon induction with Dox produced HIV-1 Gag and EBOV GP or SUDV GP respectively (Figure 1A and 1B) indicating that these gene pairs have been stably integrated

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into the genome of these cells and can be turned on with the addition of Dox. Applicant labeled these stably transfected cells as EBOVGPGag 293F cells or SUDVGPGag 293F cells respectively. Experiments may be conducted to determine if EBOV GP and HIV-1 Gag or SUDV GP and HIV-1 Gag synthesized in these cells, after Dox induction, could spontaneously assemble into VLPs and be secreted into the medium. VLPs may be isolated from the cell supernatant collected after 40 hours of Dox induction and may be analyzed, along with the corresponding cell lysates, by western blotting for the presence either EBOV GP or SUDV GP along with HIV-1 Gag proteins using specific antibodies. As shown in Figure 2, full length EBOV GP (2A) or SUDV GP (2B) and HIV-1 Gag may be detected in the cell lysate and in the VLPs indicating that either of the two Ebola GPs and HIV-1 Gag, synthesized upon induction of stably transfected 293F cells with Dox spontaneously assemble into VLPs that are secreted out into the medium.

[0052] Characterization of monovalent Ebola VLPs:

[0053] Buoyance density analysis of monovalent Ebola VLP fractions collected over 20-60% sucrose density gradient showed that these particles range in density from 1.148-1.168 (Figure 3). Electron microscopic analysis of the particles showed fine, spherical particles with a size of ~ 110 nm with EBOV GP or SUDV GP abundantly studded on the Gag matrix (Figure 4A and B). The particle density, shape and size observed in this study is in agreement with other VLPs and immature HIV-1 virions where Gag constitutes the spherical immature shell.

[0054] Immunogenicity of monovalent Ebola VLPs: Immunogenicity of monovalent Ebola VLPs may be tested by their ability to induce high-titered binding anti-EBOV GP or SUDV GP antibody responses in rabbits. Rabbit serum samples collected one week after the last booster dose may be heat inactivated and binding titers determined by analyzing the binding of antibodies present in increasing dilutions of the anti-sera to either EBOV GP or SUDV GP coated ELISA plates. As shown in Figure 5A and 5B, monovalent Ebola VLPs may be highly immunogenic in rabbits and anti-sera collected had anti-EBOV GP or SUDV GP end point antibody titers approaching 10^7 and 10^6 respectively. Specificity of these antibodies may be confirmed by their reactivity with recombinant EBOV GP or SUDV GP in

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a western blot system (data not shown). Functionality of these serum antibodies may be investigated by their ability to neutralize EBOV GP or SUDV GP containing HIV-1 Δ Env pseudovirions in a luciferase based neutralization assay using TZM-bl cells. Luciferase gene in these cells is under the control of HIV-1 Tat protein. Additionally, BDBV GP, or TAF GP containing HIV-1 Δ Env pseudovirions may be included in these assays to study the ability of either of the two anti-Ebola VLP sera to cross-neutralize other pathogenic members of this species. Figure 6 summarizes the results of neutralization experiments using these two kinds of sera. Anti-EBOVGPGag VLP serum strongly neutralized EBOV with 50% neutralization titers of $> 1: 960$ while that against BDBV and TAFV $> 1:480$. 50% neutralization titer for SUDV was significantly lower ($1: 120$) (Fig. 6A). Anti-SUDVGPGag VLP serum neutralized only SUDV with 50% neutralization titers $> 1: 1920$ while no significant neutralization was observed against EBOV, BDBV and TAFV (Fig. 6B).

[0055] Ebola VLP-based vaccine on Ebola VP40 core has been used in the past to immunize non-human primates that were subsequently challenged with lethal doses of Ebola virus. This vaccine induced humoral and cellular immune responses in animals that protected these animals against the Ebola challenge (12, 13). Because of the filamentous nature of the VP40 core, these VLPs were found to be unstable. Moreover, they were produced after the transient transfection of the cells and therefore are not readily amenable to large-scale production. The disclosed VLPs are produced, in an inducible fashion, from the stably transfected 293F cells. This production system may be easily adapted to large scale Ebola VLPs production, if needed. Being smaller in size and spherical in shape they are likely to be a stable and homogenous product.

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[0061] 5. Kibuuka H, Berkowitz NM, Millard M, Enama ME, Tindikahwa A, Sekiziyivu AB, et al. Safety and immunogenicity of Ebola virus and Marburg virus glycoprotein DNA vaccines assessed separately and concomitantly in healthy Ugandan adults: a phase 1b, randomised, double-blind, placebo-controlled clinical trial. *Lancet.* 2015;385(9977):1545-54.

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[0063] 7. Tapia MD, Sow SO, Lyke KE, Haidara FC, Diallo F, Doumbia M, et al. Use of ChAd3-EBO-Z Ebola virus vaccine in Malian and US adults, and boosting of Malian adults with MVA-BN-Filo: a phase 1, single-blind, randomised trial, a phase 1b, open-label and double-blind, dose-escalation trial, and a nested, randomised, double-blind, placebo-controlled trial. *Lancet Infect Dis.* 2016;16(1):31-42.

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[0065] 9. Henao-Restrepo AM, Longini IM, Egger M, Dean NE, Edmunds WJ, Camacho A, et al. Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial. *Lancet.* 2015;386(9996):857-66.

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[0066] 10. Ledgerwood JE, DeZure AD, Stanley DA, Coates EE, Novik L, Enama ME, et al. Chimpanzee Adenovirus Vector Ebola Vaccine. *N Engl J Med*. 2017;376(10):928-38.

[0067] 11. Stanley DA, Honko AN, Asiedu C, Trefry JC, Lau-Kilby AW, Johnson JC, et al. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. *Nature medicine*. 2014;20(10):1126-9.

[0068] 12. Warfield KL, Dye JM, Wells JB, Unfer RC, Holtsberg FW, Shulenin S, et al. Homologous and heterologous protection of nonhuman primates by Ebola and Sudan virus-like particles. *PloS one*. 2015;10(3):e0118881.

[0069] 13. Warfield KL, Swenson DL, Olinger GG, Kalina WV, Aman MJ, Bavari S. Ebola virus-like particle-based vaccine protects nonhuman primates against lethal Ebola virus challenge. *The Journal of infectious diseases*. 2007;196 Suppl 2:S430-7.

[0070] All percentages and ratios are calculated by weight unless otherwise indicated.

[0071] All percentages and ratios are calculated based on the total composition unless otherwise indicated.

[0072] It should be understood that every maximum numerical limitation given throughout this specification includes every lower numerical limitation, as if such lower numerical limitations were expressly written herein. Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

[0073] The dimensions and values disclosed herein are not to be understood as being strictly limited to the exact numerical values recited. Instead, unless otherwise specified, each such dimension is intended to mean both the recited value and a functionally equivalent range

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surrounding that value. For example, a dimension disclosed as “20 mm” is intended to mean “about 20 mm.”

[0074] Every document cited herein, including any cross referenced or related patent or application, is hereby incorporated herein by reference in its entirety unless expressly excluded or otherwise limited. The citation of any document is not an admission that it is prior art with respect to any invention disclosed or claimed herein or that it alone, or in any combination with any other reference or references, teaches, suggests or discloses any such invention. Further, to the extent that any meaning or definition of a term in this document conflicts with any meaning or definition of the same term in a document incorporated by reference, the meaning or definition assigned to that term in this document shall govern.

[0075] While particular embodiments of the present invention have been illustrated and described, it would be obvious to those skilled in the art that various other changes and modifications can be made without departing from the spirit and scope of the invention. It is therefore intended to cover in the appended claims all such changes and modifications that are within the scope of this invention.

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CLAIMS

What is claimed is:

1. A virus-like particle (VLP)-based monovalent vaccine composition comprising a spherical retroviral Group-specific Antigen (“Gag”) protein core and a single Ebola glycoprotein selected from either a Zaire (EBOV) glycoprotein or a Sudan (SUDV) glycoprotein, wherein said Ebola glycoprotein is incorporated into the surface of said spherical Gag core, wherein said VLP-based vaccine presents a single Ebola glycoprotein antigen.
2. The VLP composition of claim 1, wherein said Gag protein is derived from a retrovirus selected from human immunodeficiency virus (HIV), murine leukemia virus (MLV) Rous sarcoma virus (RSV), or Equine infectious anemia virus (EIAV).
3. The VLP composition of claim 1 or 2, wherein said single Ebola glycoprotein is located at the exterior surface of said spherical Gag protein core.
4. The VLP composition of any preceding claim, wherein said composition is capable of immunizing an individual against a viral infection from one or more of Zaire (EBOV), Sudan (SUDV), Bundibugyo (BDBV) and Tai Forest (TAFV).
5. The VLP composition of any preceding claim, wherein said spherical Gag protein core is ranges from approximately 100-300 nanometers in diameter.
6. The composition of any preceding claim, further comprising an adjuvant.
7. The composition of any preceding claim, further comprising a pharmaceutically acceptable carrier.
8. A kit comprising the composition of any preceding claim, wherein said composition is provided in a unit dose.

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9. The kit of claim 8, further comprising a second unit dose.
10. The kit of claim 8 or 9, further comprising a delivery vehicle for said composition.
11. A method for protecting an individual in need thereof against an Ebola virus species selected from Zaire (EBOV), Sudan (SUDV), Bundibugyo (BDBV), Tai Forest (TAFV), and combinations thereof, comprising administering the composition of any of claims 1 through 7.
12. The method of claim 11, wherein said individual is administered a second dose of the composition of claim 1.
13. The method of claim 12, wherein said second dose is administered at a second point in time selected from a day after the first dose, a week after the first dose, two weeks after the first dose, three weeks after the first dose, and four weeks after the first dose.
14. A method of making the monovalent VLP composition of claim 1, comprising the steps of
 - a. purifying from a cell line a VLP composition comprising a spherical retroviral Group-specific Antigen ("Gag") protein core and an Ebola glycoprotein, wherein said Ebola glycoprotein is incorporated into the surface of said spherical Gag protein core;wherein said cell line is stably transfected with a first plasmid containing a gag sequence under the control of an inducible promoter and a second plasmid containing an Ebola glycoprotein sequence under the control of an inducible promoter.

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15. The method of claim 14, wherein said stably transfected cell line produces said spherical Gag protein core, and wherein said Ebola glycoprotein is located at the surface of said spherical Gag protein core.
16. The method of claim 14 or 15, wherein said stably transfected cell line produces said Gag protein core upon induction with doxycycline.
17. The method of any of claims 14 through 16, wherein said VLP is purified by ultracentrifugation, wherein said purification is through a sucrose cushion, preferably a 20% sucrose cushion, or by cross-flow filtration followed by ultracentrifugation.
18. The method of any of claims 14 through 17, wherein said first and second plasmid are antibiotic resistant.
19. The method of any of claims 14 through 18, wherein said cell line is a human cell line modified to stably express Gag, EBOV GP or SUDV GP under inducible promoters.
20. The method of any of claims 14 through 16, wherein said cell line is a 293F cell line.
21. The method of any of claims 14 through 17, wherein said cell line is a 293F 6/TR cell line
22. A composition comprising a cell line comprising a first plasmid containing a sequence encoding for a gag protein under the control of an inducible promoter, and a second plasmid containing a sequence encoding for an Ebola glycoprotein under the control of an inducible promoter.
23. The composition of claim 22, wherein said Ebola glycoprotein is selected from EBOV or SUDV.
24. The composition of claim 22 or 23, wherein said cell line is 293F

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25. The composition of any of claims 22 through 24, wherein said cell line is stably transfected with said plasmids.
26. The composition of any of claims 22 through 25, wherein said cell line is inducible.

FIG. 1

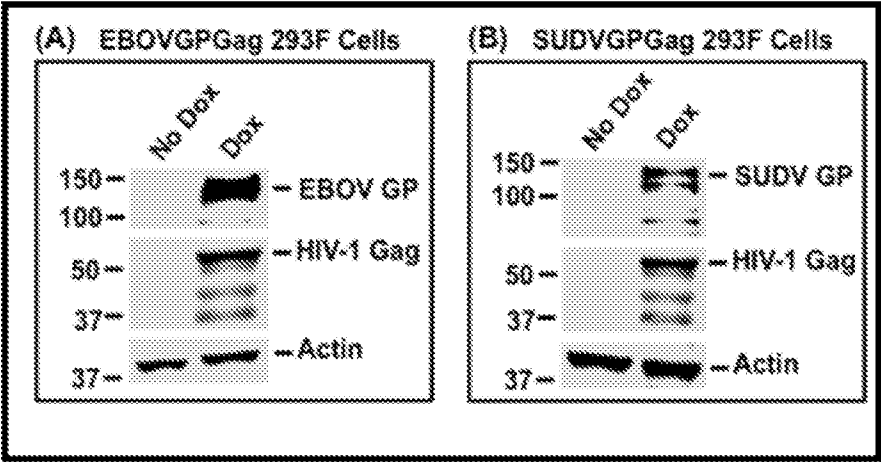


FIG. 2

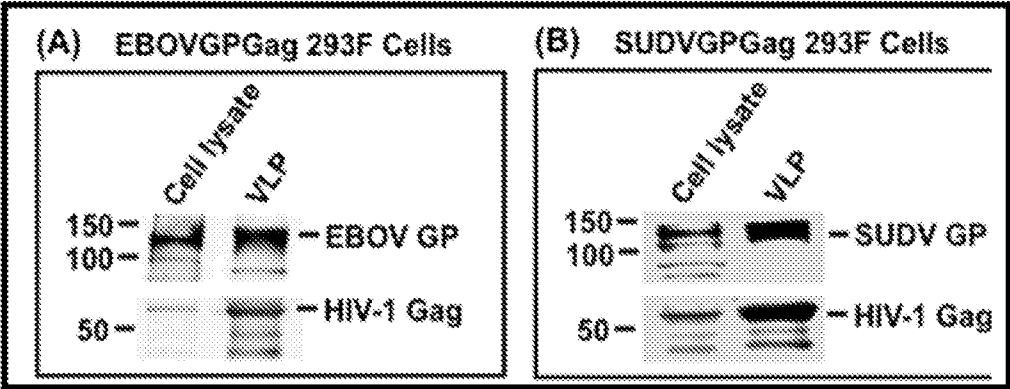
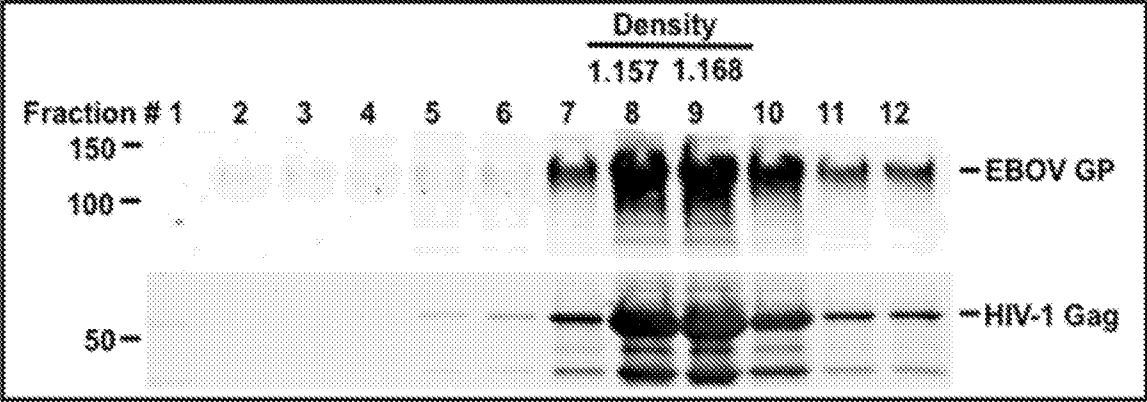


FIG. 3

(A) EBOVGPGag VLPs



(B) SUDVGPGag VLPs

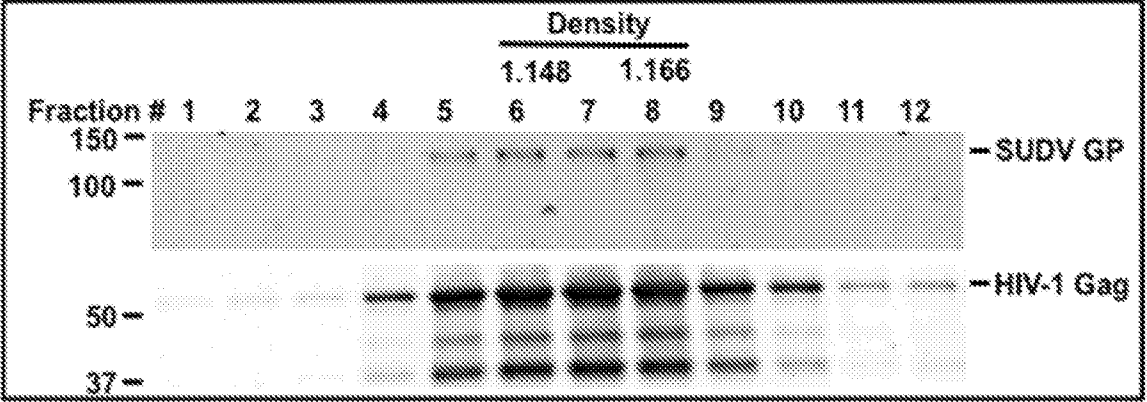


FIG. 4

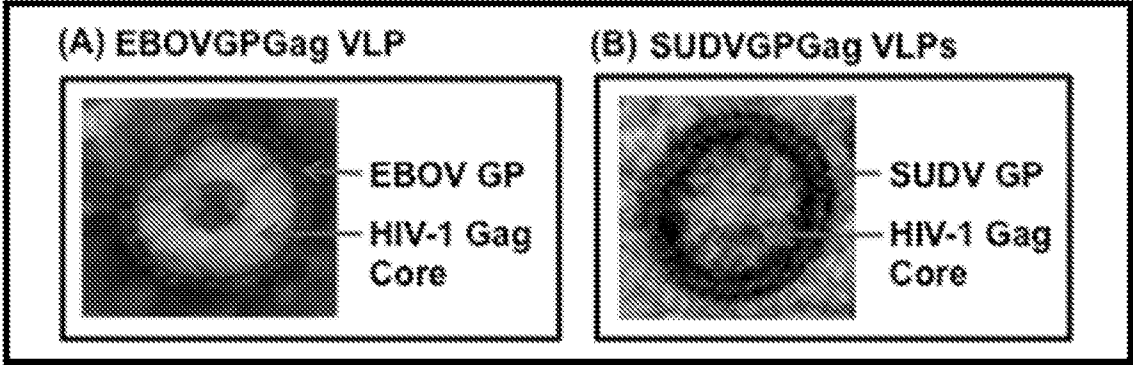


FIG. 5

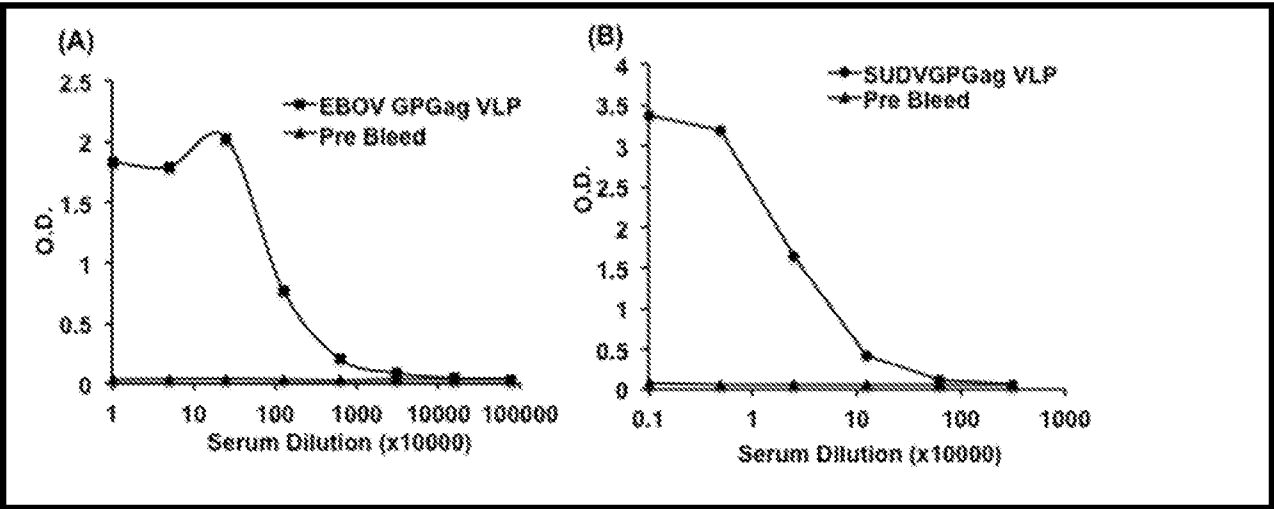
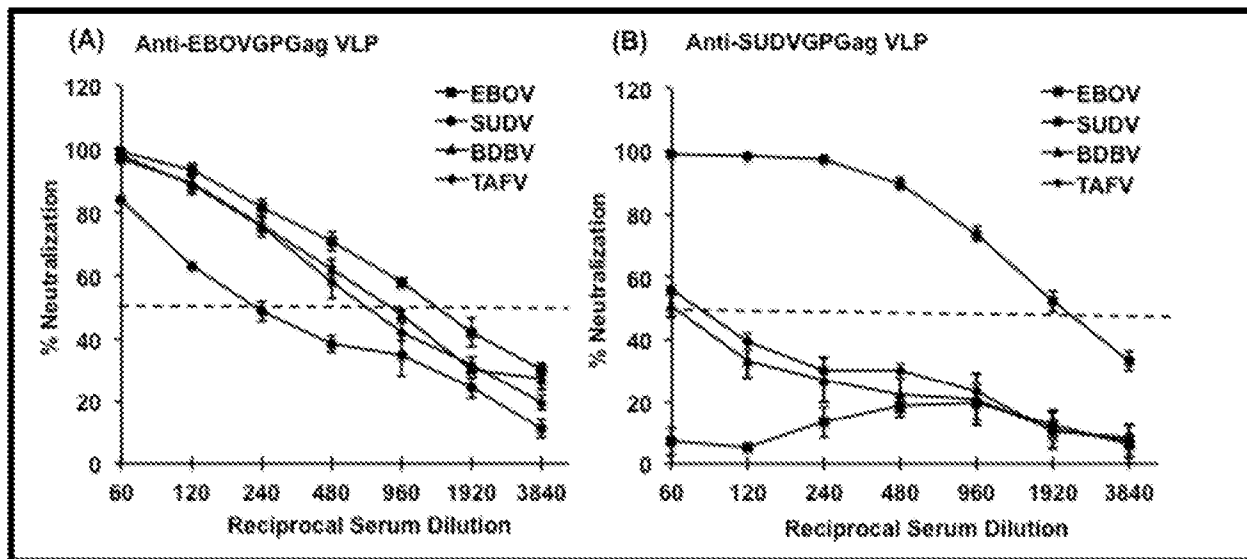


FIG. 6



0010872.0656611 4821-8651-5296v1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/24758

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 35/76, A61K 39/12, A61K 39/21 (2018.01)

CPC - A61K 2039/5258, C12N 2740/10023, A61K 39/12, A61K 39/21

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015/0266929 A1 (EMORY UNIVERSITY) 24 September 2015 (24.09.2015) para [0013], [0060], [0061], [0063], [0111], [0154], [0155]; Fig. 1	1-3
A	US 2010/0143406 A1 (SMITH et al.) 10 June 2010 (10.06.2010) para [0009], [0043], [0046], [0058]; claims 12, 16, 26	1-3

☐ 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

09 August 2018

Date of mailing of the international search report

28 AUG 2018

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

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Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/24758

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☐ forming part of the international application as filed:
☐ in the form of an Annex C/ST.25 text file.
☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

The electronic sequence listing submitted on 20 April 2018 in response to the PCT/ISA/225 issued on 11 April 2018 is acknowledged; however, no required late furnishing fee as set forth in 37 CFR 1.445(a)(5) was not provided. Therefore, the international search was not carried out on the basis of that non-compliant electronic sequence listing.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/24758

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-13, 17-21, 25-26
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:

----- please see extra sheet -----

1. ☐ As all required additional 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 additional fees, this Authority did not invite payment of 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.: 1-3

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/24758

Continuation of: Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-3, directed to a virus-like particle (VLP)-based monovalent vaccine composition.

Group II, claims 14-16, directed to a method of making the monovalent VLP composition.

Group III, claims 22-24, directed to a composition comprising a cell line.

The inventions listed as Groups I-III do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features:

Group I has the special technical feature of a VLP vaccine composition, that is not required by Group II or III.

Group II has the special technical feature of purifying from a cell line a VLP composition, that is not required by Group I or III.

Group III has the special technical feature of a composition comprising a cell line comprising a first and second plasmids, that is not required by Group I or II.

Common technical features:

Groups I-II share the common technical feature of a monovalent VLP composition comprising a spherical retroviral Gag protein core and a single Ebola glycoprotein, wherein said Ebola glycoprotein is incorporated into the surface of said spherical Gag core.

Groups II-III further share the common technical feature of a cell line comprising a first plasmid containing a sequence encoding for a gag protein under the control of an inducible promoter, and a second plasmid containing a sequence encoding for an Ebola glycoprotein under the control of an inducible promoter.

However, these shared technical features do not represent a contribution over prior art, because these shared technical features are made obvious by US 2010/0143406 A1 to Smith et al., (hereinafter Smith).

Smith teaches a VLP composition comprising a retroviral Gag protein core and a single Ebola glycoprotein, wherein said Ebola glycoprotein is incorporated into the surface of said Gag core (claim 12 "A virus like particle (VLP) comprising at least one chimeric glycoprotein on the surface of the VLP and a non-influenza viral core, wherein said chimeric glycoprotein comprises...at least a portion of a viral protein derived from a non-influenza source"; claim 16 "wherein said viral protein is...an ebola virus glycoprotein"; claim 21 "wherein said non-influenza viral core is HIV p55 Gag"; claim 26 "A vaccine comprising the VLP of claim 12"), yet does not specifically teach the vaccine is monovalent, or the gag protein core is spherical. However, spherical viral core shape is well known in the art of virology, as it is a common natural particle shape. It would have been obvious to one of ordinary skill in the art to use a spherical gag core during the ordinary course of experimentation, as it is a common natural particle shape. Further, since Smith teaches use of a VLP comprising a single Ebola glycoprotein (claim 12 "A virus like particle (VLP) comprising at least one chimeric glycoprotein on the surface of the VLP"), it would have been obvious to one of ordinary skill in the art that the vaccine could be monovalent by targeting a single viral species.

Smith further teaches a cell line comprising a plasmid containing a sequence encoding for a gag protein under the control of an inducible promoter, and containing a sequence encoding for an Ebola glycoprotein under the control of an inducible promoter (para [0043] "The invention also comprises nucleotides that encode chimeric glycoproteins cloned into an expression vector that can be expressed in a cell that induces the formation of VLPs. An "expression vector" is a vector, such as a plasmid that is capable of promoting expression, as well as replication of a nucleic acid incorporated therein...nucleotides encode for a chimeric glycoprotein...In another embodiment, said vector comprises nucleotides that encode the influenza p55 Gag protein"; [0046] "The invention also provides for constructs and/or vectors that comprise chimeric glycoproteins of the invention...The constructs and/or vectors that encode said chimeric glycoproteins should be operatively linked to an appropriate promoter, such as the AcMNPV polyhedrin promoter (or other baculovirus), phage lambda PL promoter, the E. coli lac"; note, lac is an inducible promoter), yet does not specifically teach the sequences are on different plasmids under the control of different inducible promoters. However, it was well known in the art of molecular biology to use more than one expression vector when expressing multiple transgenes in a cell, during the ordinary course of experimentation, in order to optimize expression of each transgene for a given purpose. Thus it would have been obvious to one of ordinary skill in the art to have put the gag and glycoprotein encoding sequences in separate plasmids with promoters, in order to optimize expression.

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I-III inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, continuation of number 4 above: claims 4-13, 17-21, 25, 26 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).