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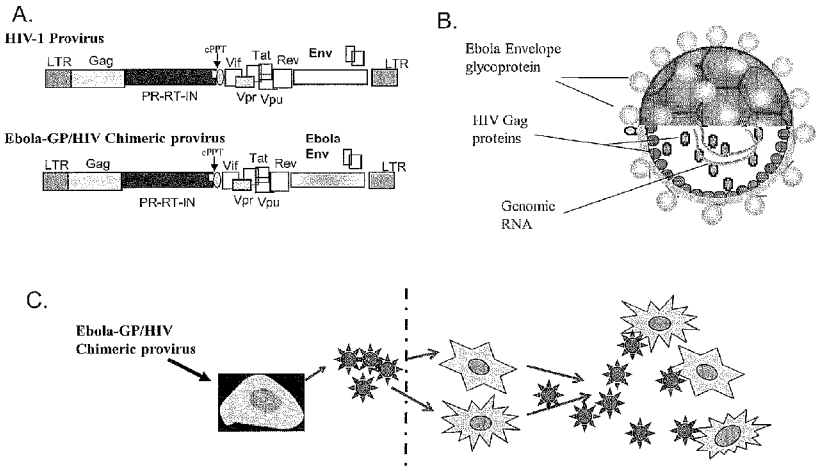
Fig. 1



(57) Abstract: Described herein are a series of Dual-Action Vaccine strategies that combine different components of HIV-1 virus and EBOV envelop glycoprotein (Ebola-GP) to generate a multiple-cycle replicating Ebola- GP/HIV chimeric virus, a single-cycle replicating Ebola-GP/HIV chimeric virus, and a non-replicating chimeric virus. All of these constructs produce GP/HIV chimeric virus-like particles (VLPs) that can efficiently target human antigen-presenting cells (APCs), including dendritic cells and monocyte-derived macrophages, and CD4 negative cells.

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DUAL-ACTION VACCINES (DAV) TO PREVENT HIV AND EBOLA VIRUS INFECTIONS

PRIOR APPLICATION INFORMATION

The instant application claims the benefit of US Provisional Patent Application serial number 62/259,194, filed November 24, 2015.

BACKGROUND OF THE INVENTION

Acquired immunodeficiency syndrome (AIDS) is a slow degenerative disease of the immune and nervous systems resulting from HIV infection. This infectious disease is the fourth leading cause of human death worldwide and global estimates of the HIV-1 pandemic indicate that there are an estimated 34 million people living with HIV-1 and 12 million cumulative AIDS related deaths so far (9). The pathogenesis of HIV-1 infection is linked closely to the replication of the virus in vivo (15, 29). The development of anti-HIV chemotherapy was a key success with a major impact on the survival of infected individuals. However, chemotherapy cannot eliminate the virus from the body and the appearance of drug-resistant HIV variants limits the application of anti-HIV chemotherapy (25, 27). In addition, cost and availability exclude a large portion of the HIV positive population in developing countries from chemotherapy, indicating that long-term intervention with anti-HIV chemotherapy is not an effective strategy for controlling the global epidemic. Certainly, the development of an efficient protective measure to prevent AIDS dissemination has become a priority.

Ebola and Marburg viruses are some of the most virulent and fatal pathogens known to humans. These viruses cause severe hemorrhagic fevers, with case fatality rates of 88%. In March 2014, the largest Ebola outbreak in history exploded across West Africa. As of February 2015, the World Health Organization has reported a total of 23,253 Ebola virus disease (EVD) cases and 9,380 deaths from this outbreak. The EVD outbreak has stimulated investigation of several different therapeutic strategies that target specific viral structures and mechanisms of EBOV. Preventing the entry of EBOV into host cells is an attractive strategy and a limited number of compounds,

chemical substances and EBOV glycoprotein-specific monoclonal antibodies (MAb) have been found to inhibit EBOV infection by blocking viral entry (7, 12, 24, 28). Some of these therapeutic agents are now entering accelerated human trials in EVD-endemic countries. However, it is still unknown when these treatments will be available for populations that are at great risk for EVD. Hence, it is also urgent to develop the promising anti-EBOV agents as well as develop vaccine(s) to combat these deadly viruses.

HIV-1 primarily targets and replicates in $CD4^+$ helper T cells and causes the loss of $CD4^+$ T cells in HIV-infected individuals. $CD4^+$ T cells have a pivotal role in orchestrating efficient immune responses against foreign pathogens (14. 18). By specifically targeting $CD4^+$ T cells and neutralizing their functions, HIV-1 facilitates viral expansion and compromises the entire immune protection of the host. This results in immunosuppression and increasing susceptibility to life-threatening opportunistic infections (22, 23). The development of an efficient vaccine has been sought since the recognition that HIV is the etiologic agent of AIDS. Several vaccine strategies have been evaluated in vivo for their potency at stimulating the immune system (8, 11, 20). Furthermore, it is well known that vaccines based on replicating viruses usually induce a stronger immune response and better protection against SIV and/or SHIV in non-human primates. Vaccines based on live attenuated SIV offer the most potent protection against wild-type SIV in macaques (17). Unfortunately, the application of hypothetical live attenuated HIV vaccines in humans raises considerable safety issues, making the development of that approach unlikely (4, 5, 16). Nevertheless, these studies suggest that an AIDS vaccine based on a replicating virus mimicking HIV viral processing, maturation and antigen presentation as in the course of natural infection would be optimal for eliciting protective immunity.

Dendritic cells (DCs) are specialized cell lineages that form a critical link between the innate and adaptive immune responses (21). After being stimulated or after uptake of antigen, DCs initiate immune responses via the secretion of chemokines and pro-inflammatory cytokines and the up-regulation of a variety of costimulatory and chemokine receptors. After maturation, they efficiently present

antigens and initiate both adaptive immune responses as well as innate immune responses (10, 13). In addition, DCs can release specific cytokines, direct the activation of and guide Th1 and/or Th2 arm(s) of T cell responses to pathogens. Thus, given their central role in the development of immunity, DCs are usually targeted by pathogens that evade host immune responses. Ebola viruses have been shown to be able to efficiently target and replicate in antigen-presenting cells (APCs), dendritic cells (DCs) and monocyte-derived macrophages (6). This is because the EBOV envelope glycoprotein (GP) has a preference for some cell types such as endothelial cells, dendritic cells, monocytes, and macrophages.

SUMMARY OF THE INVENTION

According to a first aspect of the invention, there is provided a method of preparing a recombinant virus or virus-like particle comprising:

expressing a nucleic acid molecule encoding human immunodeficiency virus (HIV) gag peptide,

expressing a nucleic acid molecule encoding Ebola virus env peptide;

providing sufficient HIV pol peptide to promote virus or virus-like particle assembly; and

recovering the assembled virus or virus-like particles.

In some embodiments, the HIV gag peptide and the Ebola virus env peptide are expressed from one promoter.

In some embodiments, the nucleic acid molecule comprising the HIV gag peptide and the Ebola virus env peptide is a provirus further comprising two HIV long terminal repeats.

In some embodiments, the HIV pol and HIV env peptides are provided by expression of the nucleic acid molecule. That is, in these embodiments, the nucleic acid molecule also encodes HIV pol peptide. In some embodiments, the HIV pol peptide is expressed between the HIV gag peptide and the Ebola virus env peptide.

In other embodiments, the HIV pol peptide is expressed separately from the nucleic acid molecule. Because the HIV pol peptide is not present on the nucleic acid

molecule, these particles are capable of only one round of infection, as discussed herein.

In yet other embodiments, the recovered assembled virus or virus-like particles are treated with DNase and/or RNase.

According to another aspect of the invention, there is provided a recombinant provirus comprising: a nucleic acid molecule encoding a first human immunodeficiency virus (HIV) long terminal repeat (LTR), an HIV gag protein, an Ebola virus env protein and a second HIV LTR.

In some embodiments, the recombinant provirus further comprises HIV pol peptide.

According to a further aspect of the invention, there is provided a purified virus or virus-like particle comprising human immunodeficiency virus (HIV) gag peptide and Ebola virus env peptide.

For example, in some embodiments, there is provided a nucleic acid molecule comprising the HIV signal peptide sequence fused to the Ebola env peptide sequence. In some of these embodiments, the EBOV signal peptide sequence has been deleted.

In some embodiments, the Ebola env peptide sequence corresponding to the mecin region has been deleted from the nucleic acid molecule.

In some embodiments, the stop codon of the Ebola env peptide sequence is linked to the RRE element of HIV, as discussed herein.

In some embodiments, the virus-like particle further comprises HIV env peptide.

In other embodiments, the virus-like particle further comprises influenza virus env peptide.

In yet other embodiments, the virus-like particle further comprises a viral glycoprotein other than Ebola env peptide or HIV env peptide.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. Production of multiple-cycle replicating Ebola-GP/HIV chimeric virus

particles to infect non lymphocytic cells. Schematic representation of A) the generation of EBOV-GP/HIV chimeric provirus and B). the progeny virus particles C). the provirus can be transfected into 293 cells to produce progeny chimeric virus stock, or directly used in vivo. The virus produced in vitro or in vivo can infect and replicate in CD4-negative cells and in APCs such as Dendritic cells and macrophages. These infected cells will present antigens to $CD4^+$ and $CD8^+$ T lymphocytes and promote strong protective immunity against a multitude of different HIV-1 variants by inducing HIV Gag immunity At the same time, anti-Ebola immunity will also be established.

Fig. 2. Expression of Ebola envelop glycoprotein in the chimeric virus and their ability to infect. A) Western blot showing the Ebola Env (GP1), and HIV core protein (P24) present in chimeric virus. B) Equal amounts of GP+ incorporated viruses, and the wild type HIV (adjusted by P24) were used to infect TZMb1 cells, After 48 hrs, the luc activity in the cells were measured. C) Equal amounts of GP+ incorporated viruses (adjusted by P24) were used to infect C8166 T cells. At 48 hrs of infection, the HIV p24 levels present the supernatants were measured. The wild type HIV pNL4.3 was used as positive control.

Fig. 3. Production of single-cycle replicating Ebola-GP/HIV chimeric virus particles and infection of APCs. A and B). Schematic representation of plasmids used to generate single-cycle replicating virus. C). the provirus is transfected into 293 or other cells that will then produce progeny chimeric viruses. These virus stocks can be administered locally and/or systemically in vivo to infect in CD4-negative cells and APCs. These infected cells will only produce non-infectious particles as antigens to $CD4^+$/$CD8^+$ T lymphocytes and elicit strong protective immunity against HIV-1 (by inducing antHIVGag immunity) and Ebola viruses.

Fig. 4. Production of DNA/RNA free Ebola-GP/HIV chimeric virus-like particles (VLPs) to target APCs. A and B). Schematic representation of plasmids used to generate Ebola-GP/HIVLPs . C). the provirus is transfected into 293 or other cells that will then produce progeny chimeric viruses. These virus stocks can be administered locally and/or systemically in vivo to infect in CD4-negative cells and APCs. These infected cells will only produce non-infectious particles as antigens to $CD4^+$/$CD8^+$ T

lymphocytes and elicit strong protective immunity against HIV-1 (by inducing anti-HIVGag immunity) and Ebola viruses. Since these VLPs only present as antigens in a native form without any genetic materials (DNA or RNA), it is a safe vaccine approach.

Fig. 5. DNA/RNA free multiple viral (Ebola/HIV) envelop glycoprotein coated virus-like particles (VLPs). A and B). the empty HIV particles (DNA/RNA-free) are engineered to simultaneously incorporate different viral envelop glycoproteins (including for example HIV/Ebola/Influ) envelop glycoprotein, on a VLP (as shown in Fig. 4A and B). The rationale of this design is to present multiple viral glycoproteins, including HIV and Ebola glycoproteins, in a natural forms on the surface of VLP (Fig. 4B), and deliver them in a natural ways to host antigen presenting cells and CD4 T lymphocytes for promoting protective immunity against different viral infections, including HIV, Ebola and influenza infections. Since these VLPs only present as antigens in a native form without any genetic materials (DNA and RNA), it is a much safer vaccine approach.

Fig. 6. (Ebola/HIV) envelop glycoprotein coated virus-Like particles (VLPs) containing Gaussia Luciferase (Gluc). A) Western blot showing the Ebola Env (GP1), HIV Env (gp120) and HIV core protein (P24) present in VLPs. B) Equal amounts of GP+, gp120+ or GP+/gp120+ coated VLPs (adjusted by P24) were incubated with HEK293T cells, or C) C8166 T cells. After 48 hrs, the Gluc activity of the supernatants were measured and normalized as a percentage of the positive control (value from VSV-G pseudotyped Gluc VLPs as 100%).

Fig. 7. Female balb/c mice (n=12) were intraperitoneally injected with 5×10^5 IFU of HIV, HIV-EBOV, HIV-EBOVd2, HIV-EBOVd3, or DMEM. At 10 days post-immunization, three mice per each group were bled and sacrificed for spleen isolation. To analyze the T cell response, splenocytes were obtained and stimulated overnight at 37C with peptide pools for EBOV GP (2.5 ug of each peptide per mL) or HIV p24 (2 ug of each peptide/mL), along with PMA/ionomycin as a positive control, and RPMI media as a negative control. IFN-gamma secreting cells were stained. Average spot-forming units per million were standardized using the unstimulated splenocytes as the

baseline measurement. HIV-EBOVd2 showed the greatest response to EBOV GP peptide pools compared to wild-type HIV, followed by HIV-EBOV. HIV-EBOVd2 also showed the greatest responses to the HIV p24 peptide pool compared to wild-type HIV, followed by HIV-EBOV.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference.

As used herein, "purified" does not necessarily refer to absolute purity but rather refers to an enrichment of the purified item relative to that found in nature, for example, 5 fold, 10 fold, 50 fold or 100 fold or more enriched or concentrated.

Described herein are a series of Dual-Action Vaccine strategies that combine different components of HIV-1 and EBOV envelop glycoprotein (Ebola-GP) to generate a multiple-cycle replicating Ebola· GP/HIV chimeric virus, a single-cycle replicating Ebola-GP/HIV chimeric virus, and non-replicating Ebola GP/HIV chimeric virus-like particles. All of these constructs produce GP/HIV chimeric viruses or virus-like particles (VLPs) that can efficiently target human antigen-presenting cells (APCs), including dendritic cells and monocyte-derived macrophages, and CD4 negative cells.

As will be appreciated by one of skill in the art, because these chimeric viruses are unable to infect $CD4^+$ T lymphocytes, these vaccines are able to elicit sufficient host immune responses to protect hosts from subsequent infection by HIV-1 or EBOV.

A chimeric viral particle that retargets HIV·1 exclusively to CD4 negative cells would combine the efficacy of live attenuated vaccines while substantially improving the safety due to the absence of direct toxicity to $CD4^+$ T cells. Thus, the helper T cells function promote aggressive immune responses against HIV-1 as well as other

pathogens. Furthermore, functional CD4+ T cells will help the host immune system prevent the establishment of chronic infection by the vaccine vector.

Shown schematically in Figure 1 is the multiple-cycle replicating Ebola-GP/HIV chimeric provirus (Fig. 1). As discussed above, this chimeric provirus can only replicate or be expressed in CD4-negative cells. The rationale of design is that by replicating and presenting the chimeric HIV/Ebola antigens in CD4-negative cells, including in APCs such as Dendritic cells and macrophages, it will promote strong protective immunity against a multitude of different HIV-1 variants by targeting HIV Gag immunity. The advantage of this strategy is that, unlike wild type HIV-1, this chimeric virus is unable to infect CD4+ helper T cells and will not cause any loss of CD4+ T cells in the body nor compromise the entire immune system of the host. The fully functional CD4+ T cells will prevent subsequent HIV and EBOV infections, and also eliminate the establishment of chronic infection by the vaccinated Ebola-GP/HIV chimeric virus.

In some embodiments, the Ebola-GP/HIV chimeric virus is further modified into a more attenuated vaccine by deleting accessary gene products such as Nef and Vpr.

Referring to Figure 1, as can be seen, in some embodiments, the provirus comprises two HIV LTRs, HIV gag, pol, vif, vpr, tat, vpu and rev proteins and the Ebola env protein. Thus, this recombinant provirus contains all HIV structural components except its envelope glycoprotein gene which has been replaced by EBOV GP gene in the HIV genome (shown in Fig 1). As discussed herein, because this provirus lacks the HIV env peptide, any recombinant virus-like particles produced by this recombinant provirus are not able to infect CD4+ or CD8+ cells. However, by virtue of the presence of the Ebola env peptide, the recombinant virus-like particles are able to infect targets of the Ebola virus, that is, antigen-presenting cells such as for example dendritic cells and macrophages. These infected cells will present at least HIV gag peptide and/or Ebola env peptide, thereby generating host immunity against these peptides, as discussed herein. Because HIV gag peptide is highly conserved, this will provide strong protective immunity against a multitude of different HIV-1 variants, as discussed herein.

The expression of the Ebola envelope glycoprotein in the chimeric virus and the ability of the chimeric virus-like particles to infect is shown in Figure 2. Specifically, Figure 2A is a Western blot showing the Ebola Env (GP1), and HIV core protein (P24) present in chimeric virus. ZGP-HIV(P1) and ZGP-HIV(P2) are two different chimeric HIV-Ebola virus preparations while ZGP-D2-HIV has a deletion encompassing amino acid 304 to 484 of the Ebola GP peptide.

In Figure 2B, equal amounts of GP+ incorporated viruses and wild type HIV (adjusted by P24 levels) were used to infect TZMb1 cells (a human epithelial cell line expressing CD4 and co-receptors, CXCR4 and CCR5), After 48 hrs, the luc activity in the cells were measured. Interestingly, the results show that both ZGP-HIV and ZGP-D2-HIV chimeric viruses can infect TZMb1 cells more efficiently than wild type HIV (Fig 2B). This is consistent with ZGP-mediated macropinocytosis of viruses resulting in infection. However, HIV could not efficiently infect TZMb1 cells, as HIV requires DEAE-Dextran to facilitate infection of these cells (Fig. 2B, right panel).

In Figure 2C, equal amounts of GP+ pseudotyped viruses (adjusted by P24 levels) were used to infect C8166 T cells. At 48 hrs of infection, the HIV p24 levels present the supernatants were measured. The wild type HIV pNL4.3 was used as a positive control. As can be seen, the chimeric virus-like particles are 100 fold less efficient than the wild type control.

In one example, an Ebola envelope glycoprotein gene was used to replace HIV-1 envelope glycoprotein (gp160) gene in HIV provirus (Shown in Fig. 1). The intermediate plasmid was created and contains a cDNA fragment derived from pNL4.3 from the natural Sal I restriction site (position 5785) to BamHI (position 8465) with two engineered Xba I unique sites at nucleotide 6310 and 7756 (after envelop signal peptide, and before the Rev Responsive Element) in HIV-1 gp160. Then, the EBOV envelope gene was PCR amplified with Xba I mutagenic primers and cloned into the Xba I sites in frame with the leader sequence of the HIV-1 envelope. Chimeric HIV-1 molecular clones were generated by cloning the Sal 1/BamHI fragment from the intermediate back into pNL4.3 which now encode for the selected foreign envelope gene in place of the deleted half 5' section of the HIV-1 envelope gp160.

For example, in some embodiments, there is provided a nucleic acid molecule comprising the HIV signal peptide sequence fused to the Ebola env peptide sequence, as discussed herein. In some of these embodiments, the EBOV signal peptide sequence has been deleted.

In some embodiments, the Ebola env peptide sequence corresponding to the mecin region has been deleted from the nucleic acid molecule.

In some embodiments, the stop codon of the Ebola env peptide sequence is linked to the RRE element of HIV, as discussed herein.

As discussed above, the multiple-cycle replicating Ebola-GP/HIV chimeric virus contains all of the necessary components for HIV replication with the exception of the HIV env peptide, which has been replaced with the Ebola virus env peptide. As discussed herein, this results in the recombinant virus infecting for example dendritic cells and macrophages rather than CD^+ cells. Furthermore, the Ebola virus env peptide is not associated with any of the hemorrhagic fever symptomology. Consequently, the chimeric virus will replicate within the host without causing hemorrhagic fever symptoms or infecting $CD4^+$ or CD^+ cells until it is removed by the host immune system.

However, in an effort to further improve the safety of the Ebola-GP/HIV chimeric virus particles, a single-cycle replicating Ebola-GP/HIV chimeric virus particle was generated by deleting genes for two HIV enzymatic enzymes (reverse transcriptase and integrase) and supplying these enzymatic activities in trans, as discussed herein. As will be appreciated by one of skill in the art, this chimeric virus can in theory only process one-cycle replication and will express exclusively in CD4-negative cells. Furthermore, this one-cycle replication still produces non-infectious viral particles that elicit an efficient host protective immunity against challenge with wild type HIV and EBOV.

This is shown schematically in Figure 3. As can be seen, the HIV pol peptide, comprising reverse transcriptase and integrase, has been deleted from the provirus. Instead, these peptides are expressed from a plasmid lacking HIV LTRs and will not be packaged into the virus-like particles. This expression in trans can be done by a

variety of ways, for example, by fusing RT and IN genes to Vpr or Vif, or by using a gag-pol protein expressing plasmid.

When the provirus is transfected into 293 or other similar cells, progeny chimeric viruses will be produced. These virus stocks can be administered locally and/or systemically in vivo to infect CD4-negative cells and APCs. These infected cells will only produce non-infectious particles as antigens to $CD4^+/CD8^+$ T lymphocytes and elicit strong protective immunity against HIV-1 (by inducing antHIVGag immunity) and Ebola viruses, as discussed herein.

As is known to those of skill in the art, R88 is a polypeptide (amino acid 14 – 88) derived from HIV Vpr protein. This peptide is an HIV virus targeting peptide which can drive different associated peptides into HIV virions. (3)

In some embodiments, the Ebola-GP/HIV chimeric virus is further modified into a more attenuated virus by further deleting one or more accessary gene products such as Nef and Vpr.

The single-cycle Ebola-GP/HIV virus particles, with two HIV enzymatic protein genes deleted from the above Ebola-GP/HIV provirus was prepared by modifying the first two amino acids of RT into double stop codons (TGA TAG) and deleting all the RT and IN gene sequences, except the 194 bp of cPPT/CTS (3). In order to complement in trans the RT and IN enzymatic defects of the virus, a plasmid encoding a Vpr-RT-IN fusion protein (CMV-R-RT-IN) is used to cotransfect the cells. We have previously shown that a single cycle replicating virus complemented in this manner can undergo single cycle infection and express a high quantity of non-infectious particles from the infected cells (1-3). The advantage of this strategy is that, unlike wild type HIV-1, this chimeric virus is capable of only one cycle of infection and continuously expresses antigenic non-infectious particles from the infected cells., As will be apparent to one of skill in the art, this will induce adequate host immune response against subsequent HIV and EBOV infections. This immune response will in turn eliminate the vaccinated Ebola-GP/HIV chimeric virus.

The third vaccine strategy is to generate DNA/RNA-free HIV viral like particles (VLPs) incorporated with Ebola envelope glycoproteins as a vaccine preparation to

target both HIV and Ebola. This empty HIV particles (DNA/RNA-free) carry Ebola envelope glycoprotein and efficiently targets APCs, such as Dendritic cells and macrophages, as discussed above.

Specifically, as shown in Figure 4A, HIV Gag-pol and EBOV env proteins are expressed. These proteins will assemble into virus like particles comprising HIV Gag and EBOV GP (Figure 4B).

As will be apparent to one of skill in the art, the viral peptides are all supplied in trans and no HIV genomic RNA forms. Consequently, there will be no RNA and/or DNA in the particles. However, in some embodiments, these particles may be treated with DNase and/or RNase to ensure that no contaminating nucleic acid molecules are present in the particles on infection or administration.

For example, the expression constructs engineered to express HIV gag, HIV pol and Ebola env are transfected into 293 or other suitable cells. As these peptides are expressed, the HIV gag and Ebola env peptides will form virus-like particles. However, because no HIV genomic RNA is produced, no nucleic acid will be incorporated into these virus-like particles. These virus-like particles can be administered locally and/or systemically in vivo to infect CD4-negative cells and APCs. These infected cells will only produce non-infectious particles as antigens to $CD4^+/CD8^+$ T lymphocytes and elicit strong protective immunity against HIV-1 (by inducing anti-HIVGag immunity) and Ebola viruses. Thus, these VLPs only present antigens (in native forms) without any genetic material (DNA or RNA).

As shown in Figure 5, this strategy can be extended to include additional viral glycoproteins. In the example provided herein, HIV env and influenza env peptides are also supplied; however, any suitable virus glycoprotein may be used. These additional viral glycoproteins will also be expressed on the surface of the virus-like particle. These particles will still infect the APCs which will in turn result in CD4 T lymphocytes promoting protective immunity against the viruses, for example, influenza virus, HIV and Ebola virus. Furthermore, these VLPs only present viral proteins as antigens and do not contain any genetic material.As will be appreciated by one of skill in the art, the envelop glycoprotein from any enveloped virus can be used

in the virus-like particles discussed above and shown in Figure 5.

These particles will be produced in *in vitro*, including in cell culture systems. For example, we can co-transfect an HIV Gag-pol expressor and EBOV GP expressor (or different viral GP expressor) in 293 cells or other human cells. The produced virus like particles containing HIV Gag and EBOV GP will be collected and may be treated with RNAse and DNAse. The virus-like particles are then purified and used for vaccine preparations, as discussed herein. As discussed above, treating these particles with DNase and/or RNase will ensure that there is no non-specific DNA or RNA packaging in the VLPs.

These three platforms of dual-action vaccine are applicable to various clades of HIV, including clade A, B, C, and D.

Also, this vaccine design is applicable to env peptides of Marburg virus (MARV) and different EBOV species, including Zaire Ebolavirus (ZEBOV), Sudan Ebolavirus (SEBOV), Cote d'Ivoire Ebolivirus (CIEBOV), Reston Ebolavirus (REBOV).

According to one aspect of the invention, there is provided a method of preparing a recombinant virus-like particle comprising:

expressing a nucleic acid molecule encoding human immunodeficiency virus (HIV) gag peptide,

expressing a nucleic acid molecule encoding Ebola virus env peptide;

providing sufficient HIV pol peptide to promote virus-like particle assembly; and

recovering the assembled virus-like particles.

In some embodiments, the HIV gag peptide and the Ebola virus env peptide are expressed from one promoter, that is, co-expressed.

In some embodiments, the nucleic acid molecule comprising the HIV gag peptide and the Ebola virus env peptide is a provirus further comprising two HIV long terminal repeats.

In some embodiments, the HIV pol and HIV env peptides are provided by expression of the nucleic acid molecule. That is, in these embodiments, the nucleic acid molecule also encodes HIV pol peptide. In some embodiments, the HIV pol peptide is expressed in the provirusbetween the HIV gag peptide and the Ebola virus

env peptide.

In other embodiments, the HIV pol peptide is expressed separately from the nucleic acid molecule. Because the HIV pol peptide is not present on the nucleic acid molecule, these particles are capable of only one round of infection, as discussed herein.

In yet other embodiments, the recovered assembled virus-like particles are treated with DNase and/or RNase.

According to another aspect of the invention, there is provided a recombinant provirus comprising: a nucleic acid molecule encoding a first human immunodeficiency virus (HIV) long terminal repeat (LTR), an HIV gag protein, an Ebola virus env protein and a second HIV LTR.

In some embodiments, there is provided a recombinant provirus comprising a nucleic acid molecule encoding, in sequence, a first human immunodeficiency virus (HIV) long terminal repeat (LTR), an HIV gag protein, an Ebola virus env protein and a second HIV LTR.

In some embodiments, the recombinant provirus further comprises HIV pol peptide.

According to a further aspect of the invention, there is provided a purified virus-like particle comprising human immunodeficiency virus (HIV) gag peptide and Ebola virus env peptide.

In some embodiments, the virus-like particle further comprises HIV env peptide.

In other embodiments, the virus-like particle further comprises influenza virus env peptide.

In yet other embodiments, the virus-like particle further comprises a viral glycoprotein.

The invention will now be further explained by way of examples. However, the invention is not necessarily limited to the examples.

Figure 6 shows (Ebola/HIV) envelope glycoprotein coated virus-Like particles (VLPs) containing Gaussia Luciferase (Gluc). A) Western blot showing the Ebola Env

(GP1), HIV Env (gp120) and HIV core protein (P24) present in VLPs. B) Equal amounts of GP+, gp120+ or GP+/gp120+ coated VLPs (adjusted by P24) were incubated with HEK293T cells, or C) C8166 T cells. After 48 hrs, the Gluc activity of the supernatants were measured and normalized as a percentage of the positive control (value from VSV-G pseudotyped Gluc VLPs as 100%). Results show Ebola envelop glycoprotein coated VLPs (Gp-VLPs) can only target (or infect) 293T cells (human epithelial cell line) (Fig. 6B, bar 3), but not CD4+ C8166 T cells (Fig. 6C, bar 3), while HIV envelope glycoprotein coated VLPs (Env-VLPs) can only target (or infect) CD4+ C8166 T cells (Fig. 6C, bar 2), but not 293T cells (Fig. 6B, bar 2). Interestingly, when VLPs are incorporated with HIV/EBOV-GPs, they can target both types of cells (Fig. 6B an C, bar 4), indicating the incorporated HIV/EBOV-GPs are fully functional. Therefore, immunization of VLPs incorporating both HIV-gp and EBOV-GPs would elicit an efficient host immune response against both deadly viruses. As discussed above, these viruses would be nucleic acid free.

As shown in Figure 7, Female balb/c mice (n=12) were intraperitoneally injected with 5 x 10^5 IFU of HIV, HIV-EBOV, HIV-EBOVd2, HIV-EBOVd3, or DMEM. At 10 days post-immunization, three mice per each group were bled and sacrificed for spleen isolation. To analyze the T cell response, splenocytes were obtained and stimulated overnight at 37C with peptide pools for EBOV GP (2.5 ug of each peptide per mL) or HIV p24 (2 ug of each peptide/mL), along with PMA/ionomycin as a positive control, and RPMI media as a negative control. IFN-gamma secreting cells were stained. Average spot-forming units per million were standardized using the unstimulated splenocytes as the baseline measurement. Results revealed that HIV-EBOVd2 showed the greatest response to EBOV GP peptide pools compared to wild-type HIV, followed by HIV-EBOV. HIV-EBOVd2 also showed the greatest responses to the HIV p24 peptide pool compared to wild-type HIV, followed by HIV-EBOV.

These data suggest that immunization with HIV-EBOV and HIV-EBOVd2 in mice could not only provide strong immune response to EBOV-GP, but also elicit stronger anti-HIV (specifically against Gag) immunity than immunization with wild type HIV. While not wishing to be bound to a particular theory or hypothesis, it may be that

compared to HIV, the HIV-EBOV chimeric virus has a stronger ability to target antigen-presenting cells, such as Dendritic cells and macrophages and consequently elicits a more efficient immune responses against both EBOV and HIV.

The scope of the claims should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

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CLAIMS

1. A method of preparing a recombinant virus-like particle comprising:

expressing a nucleic acid molecule encoding human immunodeficiency virus (HIV) gag peptide,

expressing a nucleic acid molecule encoding Ebola virus env peptide;

providing sufficient HIV pol peptide to promote virus-like particle assembly; and

recovering the assembled virus-like particles.

2. The method according to claim 1 wherein the HIV gag peptide and the Ebola virus env peptide are expressed from one promoter.

3. The method according to claim 2 wherein the nucleic acid molecule comprising the HIV gag peptide and the Ebola virus env peptide is a provirus further comprising two HIV long terminal repeats.

4. The method according to claim 3 wherein the HIV pol and HIV env peptides are provided by expression of the nucleic acid molecule.

5. The method according to claim 4 wherein the HIV pol peptide is expressed between the HIV gag peptide and the Ebola virus env peptide.

6. The method according to claim 1 wherein the recovered assembled virus-like particles are treated with DNase and/or RNase.

7. A recombinant provirus comprising: a nucleic acid molecule encoding a first human immunodeficiency virus (HIV) long terminal repeat (LTR), an HIV gag protein, an Ebola virus env protein and a second HIV LTR.

8. The recombinant provirus according to claim 7 further comprising HIV pol peptide.

9. A purified virus-like particle comprising human immunodeficiency virus (HIV) gag peptide and Ebola virus env peptide.

10. The purified virus-like particle according to claim 9 further comprising HIV env peptide.

11. The virus-like particle according to claim 9 further comprising influenza virus env peptide.

12. The virus-like particle according to claim 9 further comprising a viral

glycoprotein.

Fig. 1

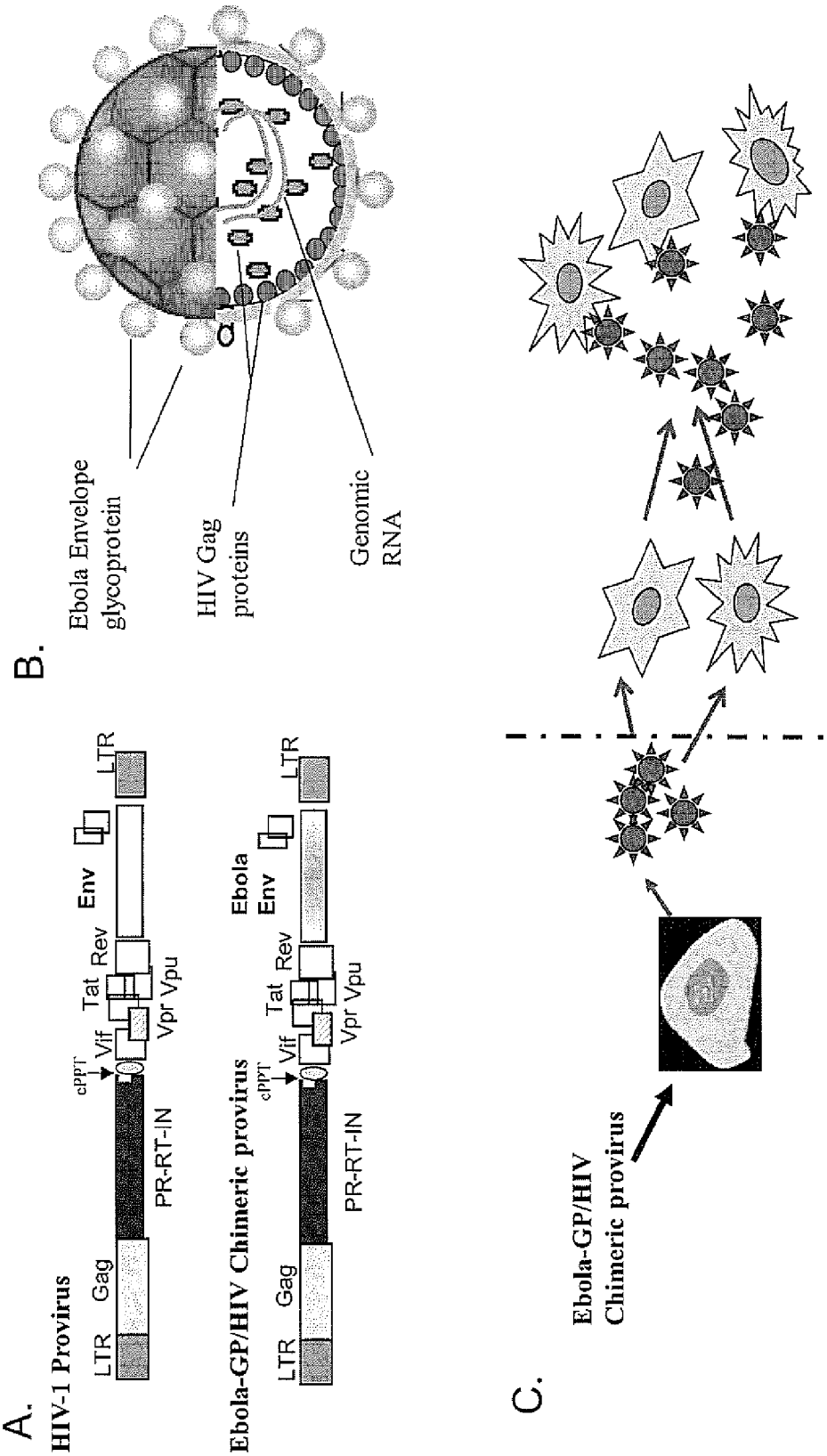

A.
HIV-1 Provirus
LTR
Gag
PR-RT-IN
Vif
Vpr
Tat
Rev
Vpu
Env
LTR
cPPT
Ebola-GP/HIV Chimeric provirus
LTR
Gag
PR-RT-IN
Vif
Vpr
Tat
Rev
Vpu
Ebola
Env
LTR
cPPT
B.
Ebola Envelope glycoprotein
HIV Gag proteins
Genomic RNA
C.
Ebola-GP/HIV
Chimeric provirus

Fig. 2

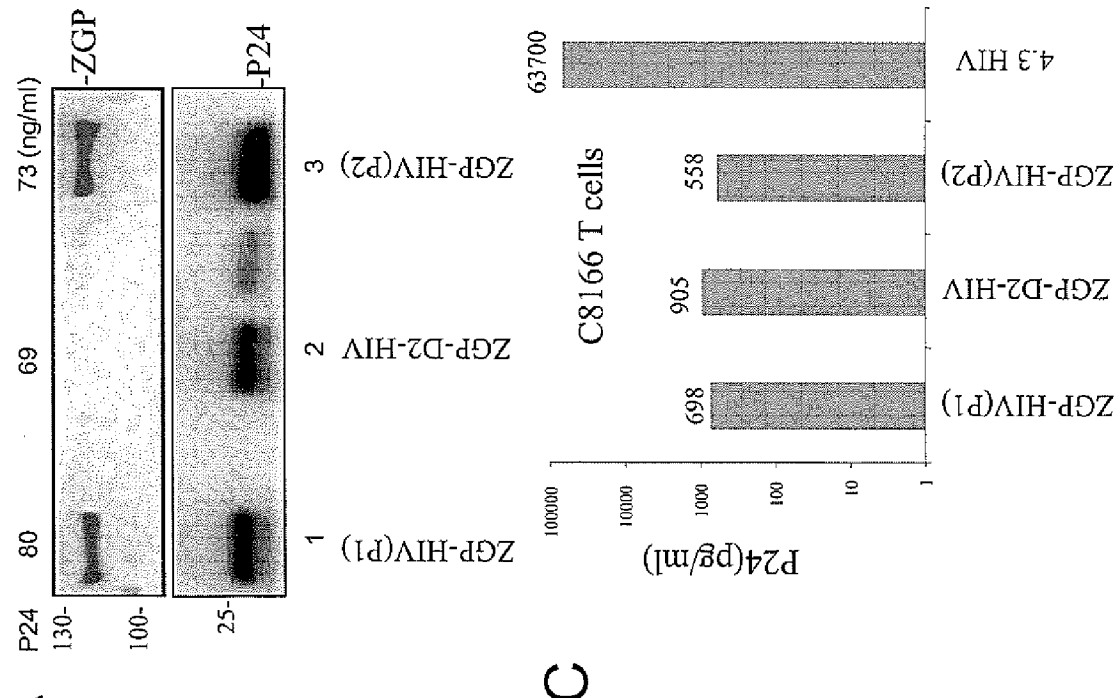

A
P24
130-
100-
25-
80
69
73 (ng/ml)
-ZGP
-P24
1 ZGP-HIV(P1)
2 ZGP-D2-HIV
3 ZGP-HIV(P2)
B
Luc activity (RLU)
25000
20000
15000
10000
5000
0
48h
72
ZGP-HIV(P1)
ZGP-D2-HIV
ZGP-HIV(P2)
Mock
25000
20000
15000
10000
5000
0
48h
Mock
HIV-1
HIV-1
+DEAE-Dextran
C
P24(pg/ml)
100000
10000
1000
100
10
1
C8166 T cells
698
905
558
63700
ZGP-HIV(P1)
ZGP-D2-HIV
ZGP-HIV(P2)
4.3 HIV

Fig. 3

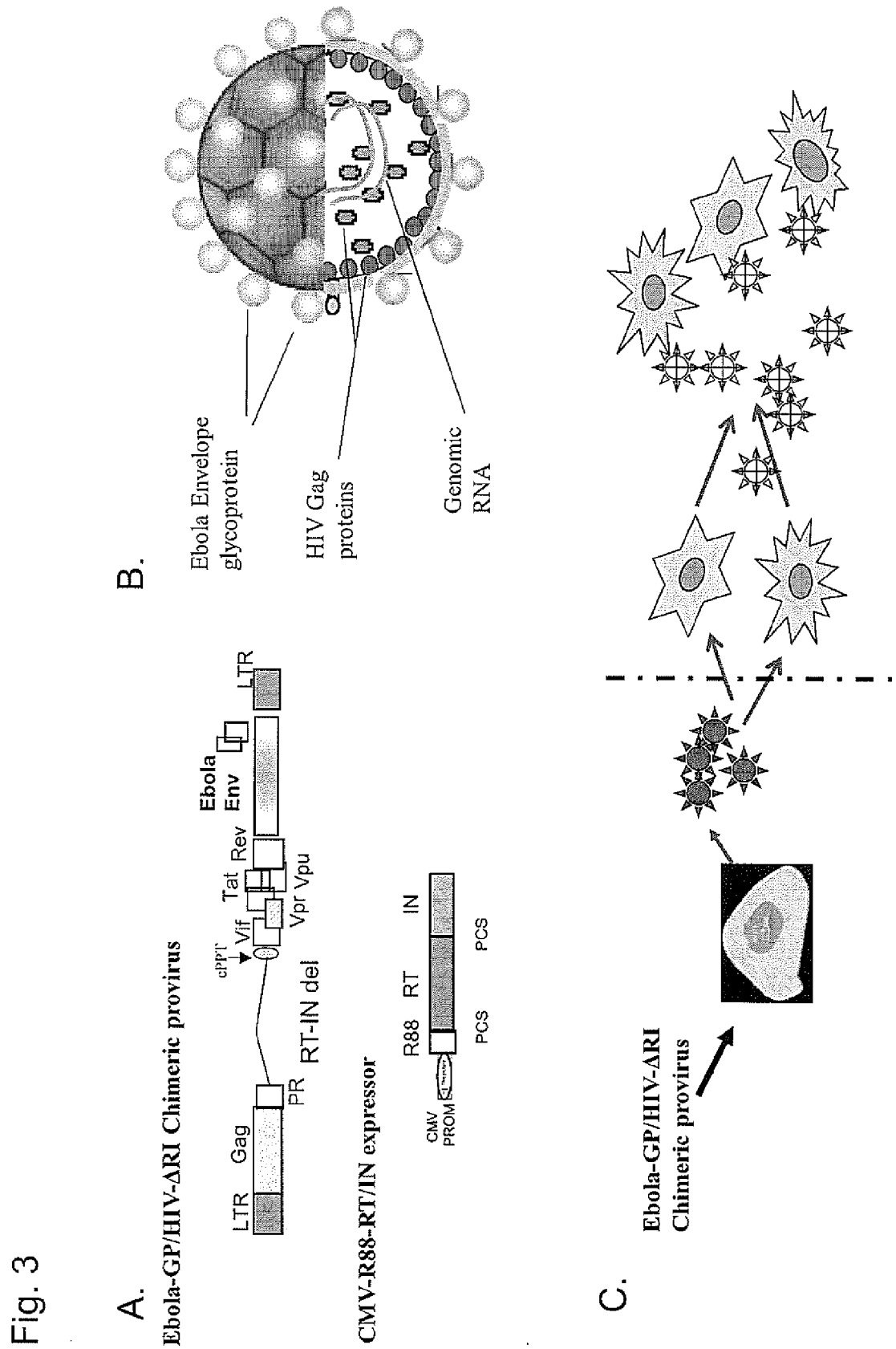

A.
Ebola-GP/HIV-ΔRI Chimeric provirus
LTR
Gag
PR
RT-IN del
cPPT
Vif
Tat
Rev
Vpr
Vpu
Ebola Env
LTR
CMV-R88-RT/IN expressor
CMV PROM
R88
RT
IN
PCS
PCS
B.
Ebola Envelope glycoprotein
HIV Gag proteins
Genomic RNA
C.
Ebola-GP/HIV-ΔRI Chimeric provirus

Fig. 4

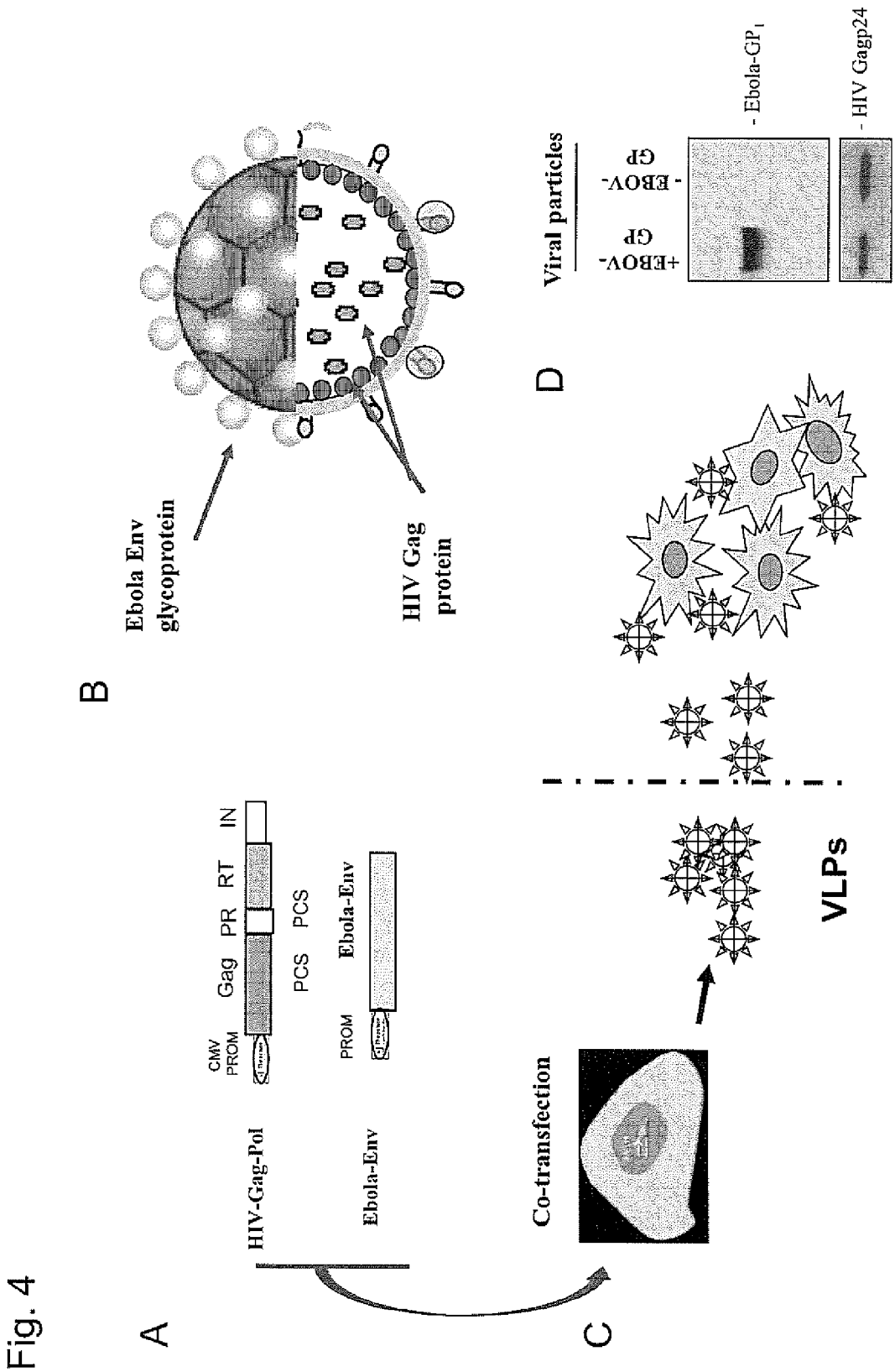

A
HIV-Gag-Pol
CMV PROM
Gag
PR
RT
IN
PCS
PCS
Ebola-Env
PROM
Ebola-Env
B
Ebola Env glycoprotein
HIV Gag protein
C
Co-transfection
VLPs
D
Viral particles
+EBOV-GP
- EBOV-GP
- Ebola-GP1
- HIV Gagp24

Fig. 5

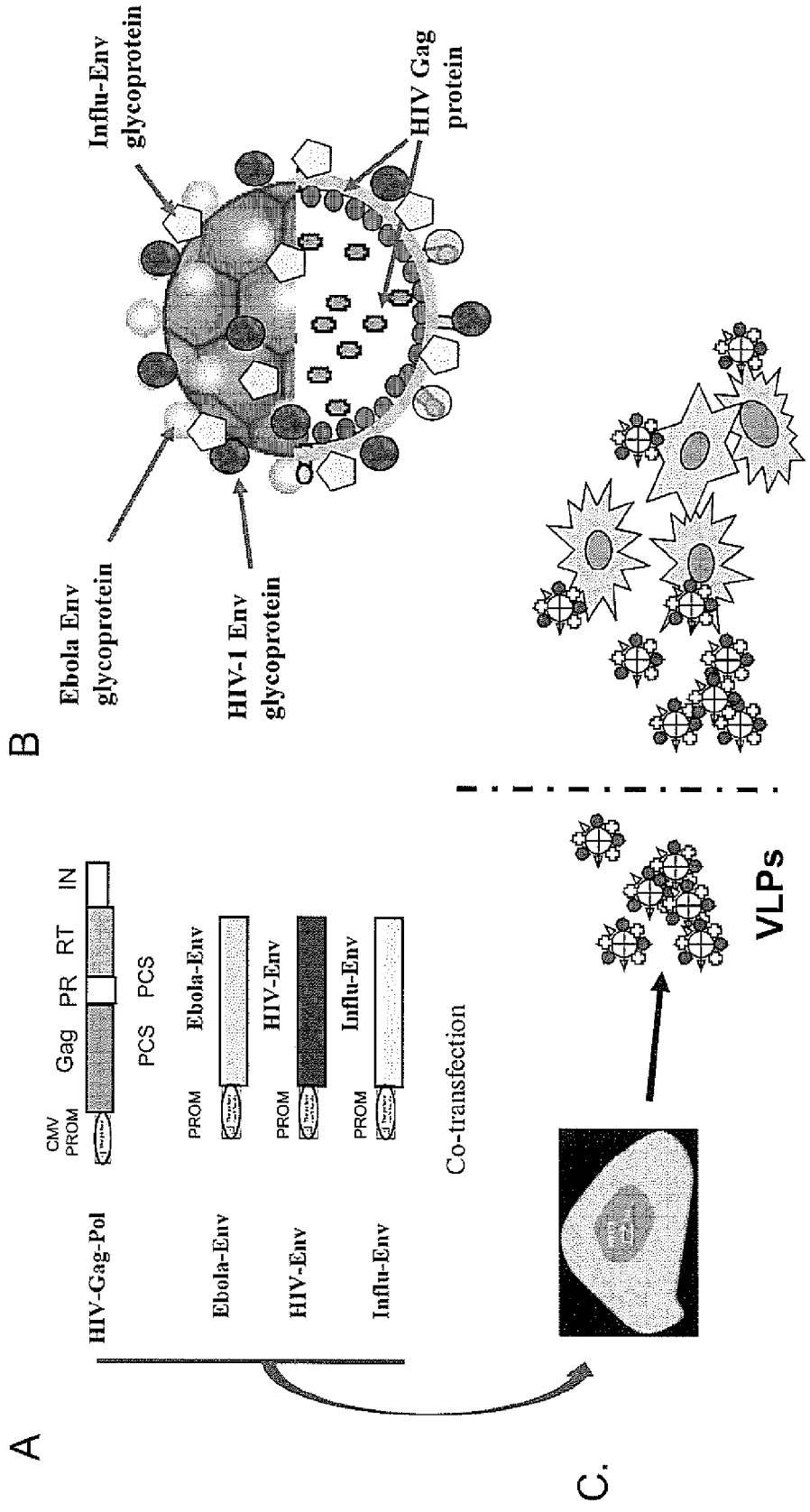
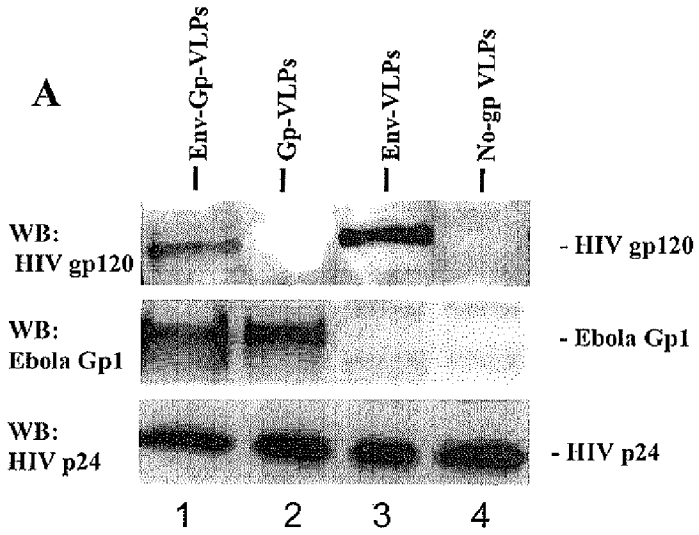
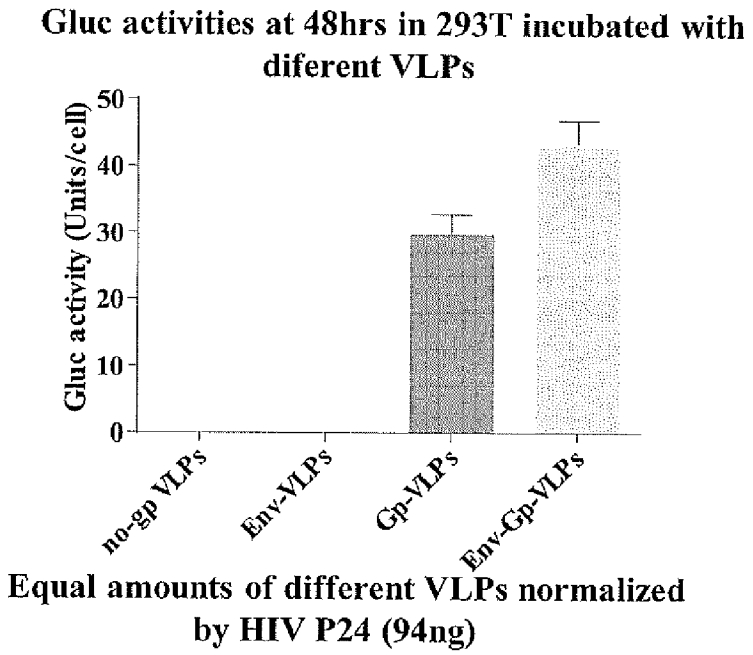

A
HIV-Gag-Pol
CMV PROM
Gag
PR
RT
IN
PCS
PCS
Ebola-Env
PROM
Ebola-Env
HIV-Env
PROM
HIV-Env
Influ-Env
PROM
Influ-Env
Co-transfection
B
Influ-Env glycoprotein
HIV Gag protein
Ebola Env glycoprotein
HIV-1 Env glycoprotein
C.
VLPs

Fig. 6


A
Env-Gp-VLPs
Gp-VLPs
Env-VLPs
No-gp VLPs
WB:
HIV gp120
- HIV gp120
WB:
Ebola Gp1
- Ebola Gp1
WB:
HIV p24
- HIV p24
1
2
3
4


B
Gluc activities at 48hrs in 293T incubated with diferent VLPs
Gluc activity (Units/cell)
50
40
30
20
10
0
no-gp VLPs
Env-VLPs
Gp-VLPs
Env-Gp-VLPs
Equal amounts of different VLPs normalized by HIV P24 (94ng)

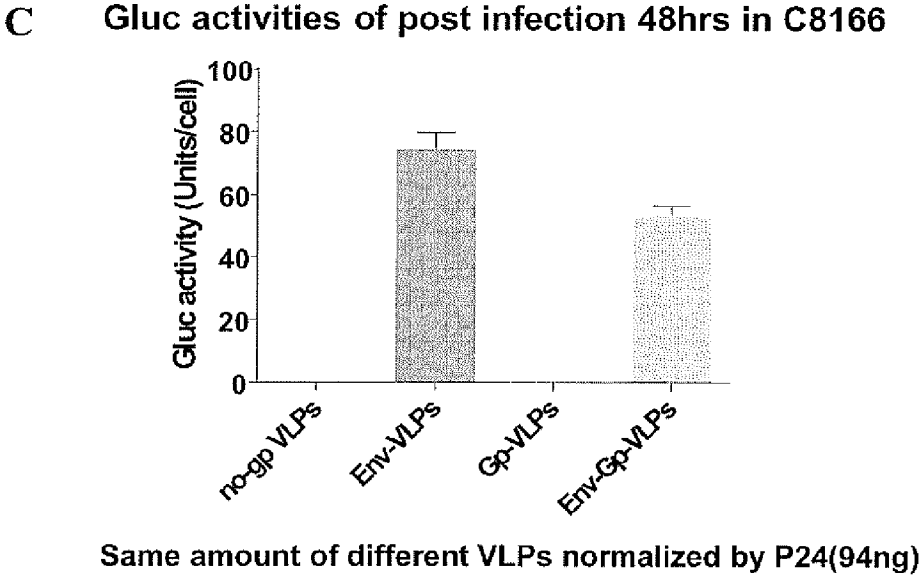
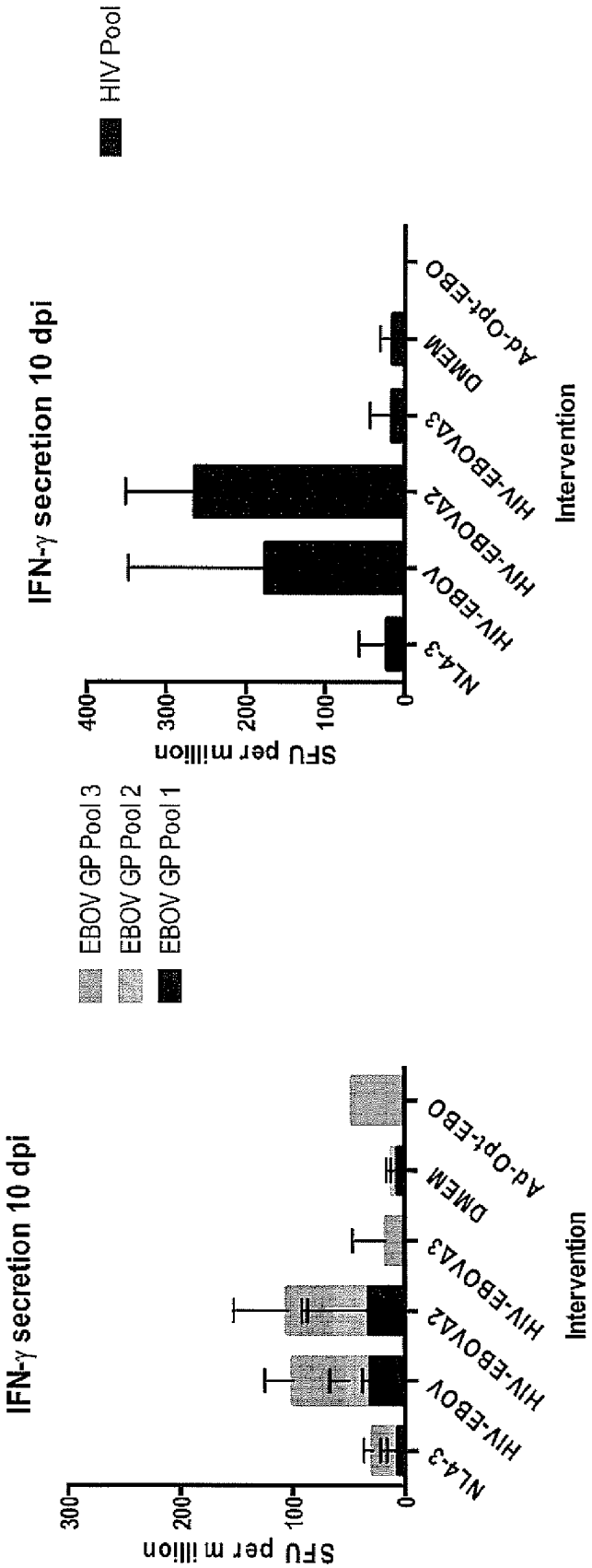

C
Gluc activities of post infection 48hrs in C8166
Gluc activity (Units/cell)
100
80
60
40
20
0
no-gp VLPs
Env-VLPs
Gp-VLPs
Env-Gp-VLPs
Same amount of different VLPs normalized by P24(94ng)

Fig 7


IFN-γ secretion 10 dpi
SFU per million
300
200
100
0
NL4-3
HIV-EBOV
HIV-EBOVA2
HIV-EBOVA3
DMEM
Ad-Opt-EBO
Intervention
EBOV GP Pool 3
EBOV GP Pool 2
EBOV GP Pool 1
HIV Pool

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2016/051374

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C12N 7/01*** (2006.01) , ***A61K 39/12*** (2006.01) , ***A61K 39/21*** (2006.01) , ***A61K 39/295*** (2006.01) , ***A61P 31/14*** (2006.01) , ***A61P 37/04*** (2006.01) (more IPCs on the last page)

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)
IPC: ALL

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Questel orbit, Google, Canadian Patent Database,
Keywords: vaccine, HIV env, pop, gag, Ebola, filovirus, RNA, authors names

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO2003092582 (WILSON et AL) 13 November 2003 (13-11-2003)	1 – 12
A	WO2001083730 (KOBINGER et AL) 8 November 2001 (08-11-2001)	1 - 12

☐ 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 18 February 2017 (18-02-2017)	Date of mailing of the international search report 06 March 2017 (06-03-2017)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Henrietta Bor (819) 639-7761

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2016/051374

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO03092582A2	13 November 2003 (13-11-2003)	WO03092582A2	13 November 2003 (13-11-2003)
		WO03092582A3	01 July 2004 (01-07-2004)
		WO03092582B1	16 December 2004 (16-12-2004)
		AU2003232004A1	17 November 2003 (17-11-2003)
		AU2003232004A8	17 November 2003 (17-11-2003)
		US2005255123A1	17 November 2005 (17-11-2005)
WO01083730A2	08 November 2001 (08-11-2001)	WO01083730A2	08 November 2001 (08-11-2001)
		WO01083730A3	23 May 2002 (23-05-2002)

C07K 14/08 (2006.01) , ***C07K 14/16*** (2006.01) , ***C12N 15/40*** (2006.01) , ***C12N 15/49*** (2006.01) , ***C12N 15/86*** (2006.01) , ***C12N 7/00*** (2006.01)